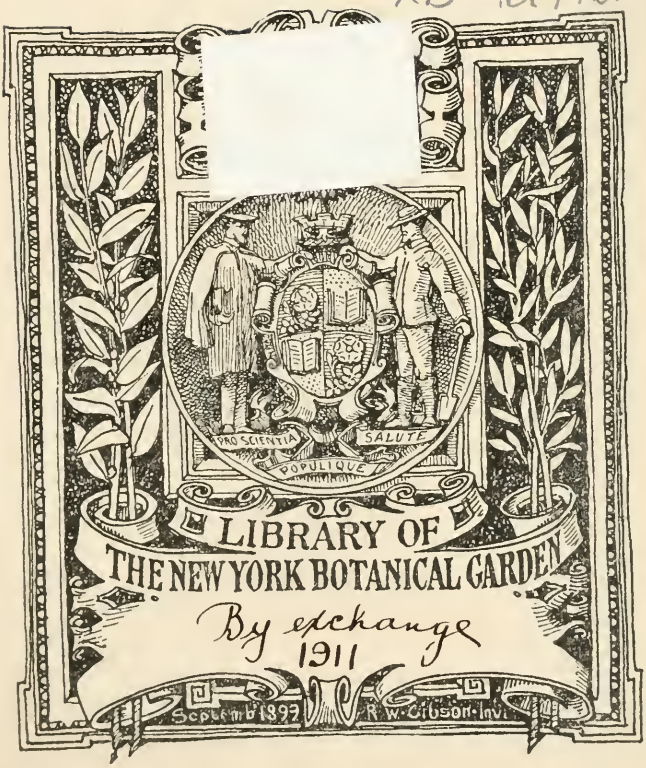
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U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 210.
B. T. GALLOWAY, *Chief of Bureau.*

HINDI COTTON IN EGYPT.

BY

O. F. COOK,
Bionomist in Charge of Crop Acclimatization and Adaptation Investigations.

ISSUED MAY 11, 1911.

WASHINGTON:
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BUREAU OF PLANT INDUSTRY.

Chief of Bureau, Beverly T. Galloway.
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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., January 13, 1911.

Sir: I have the honor to transmit herewith a paper entitled "Hindi Cotton in Egypt," by Mr. O. F. Cook, of this Bureau, and to recommend its publication as Bulletin No. 210 of the Bureau series.

This paper reports the results of a visit to the cotton-growing districts of Egypt in June and July, 1910. It shows that the admixture of inferior Hindi cotton is a serious burden upon the Egyptian industry and that our more intelligent farmers can secure an important advantage through the improved system of selection that has been developed by experiments in Arizona. A careful comparison of the results of the Arizona experiments with the conditions actually existing in Egypt became necessary in order to determine whether a satisfactory degree of uniformity has been attained in our acclimatized strains of Egyptian cotton. A previous study of the problem of diversity of the Egyptian cotton had been made in Arizona, as reported in Bulletins Nos. 147 and 156 of this series.

Respectfully,

Wm. A. Taylor,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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HINDI COTTON IN EGYPT.

INTRODUCTION.

Inspection of many cotton fields in different parts of Egypt shows that the so-called Hindi cotton is a general contamination of the Egyptian stock, responsible for a large amount of diversity and degeneration. Expression of inferior Hindi characters renders many of the plants not only worthless from the standpoint of production, but dangerous to future crops. The establishment of a profitable culture of Egyptian cotton in Arizona and southern California depends largely on the exclusion of the Hindi contamination.[a]

The Hindi cotton complicates the problem of acclimatizing and adapting the Egyptian cotton to the cultural conditions found in the United States. In this case a problem of heredity had to be studied. Instead of the physical factors alone, it has been necessary to analyze the characters of the plants in order to determine the causes of impurity and find means of elimination.

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[a] "Hindi is the name applied in Egypt to an undesirable type of cotton with a short, weak fiber, that injures the high-grade Egyptian varieties by infesting them with hybrids. The skill and cheapness of the native Egyptian labor enable the exporters to have the cotton sorted by hand in their baling establishments, so that a high reputation for uniformity has been secured in spite of the Hindi admixture.

"The introduction of the Egyptian cotton into the United States brings also the problem of the Hindi cotton, but without the resource of cheap labor which enables the difficulty to be surmounted in Egypt. The practicability of establishing a commercial culture of the Egyptian cotton in the United States depends largely upon the elimination of the Hindi contamination and other forms of diversity, so that the fiber may be produced in a satisfactory condition of uniformity. The Hindi cotton problem might be compared to that of the red rice that mixes with the white and depreciates the value of the crop. In the case of the cotton, there is a better prospect that adequate knowledge of the vegetative characters may enable the undesirable plants to be removed from the fields without too seriously increasing the cost of production." (See Circular 42, Bureau of Plant Industry, U. S. Dept. of Agriculture, entitled "Origin of the Hindi Cotton," 1909, p. 3. This circular contains the results of a previous study of the Hindi cotton made in connection with experiments in Arizona. It will be sent free on application to the Secretary of Agriculture.)

During the first years of its cultivation in Arizona the Egyptian cotton produced only small yields and rather inferior fiber. After the yield and quality began to improve, an undesirable amount of diversity appeared. A study of this diversity showed that it was due in part to hybridization with the common American Upland cotton, and that this danger was unusually serious in Arizona when the two kinds of cotton are grown in the same locality, owing to an unusual abundance of wild bees. The Hindi cotton is an additional factor of diversity inherent in the imported Egyptian stock, more difficult to understand because not previously known in the United States.

Experiments show that both of these sources of diversity can be eliminated by a more careful system of field selection, applied early in the season before the inferior plants have begun to flower, and hence before they have cross-fertilized the neighboring plants. The value of the Arizona Egyptian cotton and the prospects of cultivating this crop on a commercial scale in the United States depend largely on the degree of uniformity that can be attained in the fiber, in comparison with that of the Egyptian product. Hence, the necessity for an inspection of the cotton fields of Egypt in order to determine the extent of diversity in the crop as raised in that country.

The high cost of labor in the Southwestern States forbids any direct imitation of Egyptian methods, either in raising the crop or in preparing it for market. Other solutions of the problems of production have to be sought. The requirement of uniformity has been met in Egypt by a system of careful grading of the cotton after picking that would be very difficult to establish in the United States, and too expensive to leave any assurance of profit for the farmer even if it were established.

The Egyptian cotton trade is organized on an entirely different basis from the American. Instead of merely ginning and baling the farmer's cotton as he brings it from the fields, it is the regular practice of the Egyptian ginning establishments to buy the seed cotton from the farmer and prepare it for the market by sorting, grading, and blending. Instead of depending entirely on samples, as with American cotton, Egyptian cotton is sold largely by the marks or brands that are placed on the bales by the ginning establishments. Cotton of the same mark is supposed to represent a definite uniform quality. This is much more practicable in Egypt than it would be in most parts of the United States because of the much greater uniformity of climate and soil in Egypt.

In comparison with the wide range of soils, climates, and seasonal vicissitudes in the cotton-producing districts of the United States, the Egyptian cotton industry gives at first an impression of complete uniformity. Although people in Egypt supposed that cotton would be more advanced in Upper Egypt than about Cairo, this did

not prove to be the case. It is quite possible that the crop of Upper Egypt comes to maturity earlier in the fall, owing to hotter weather in the summer, but there was very little difference at the middle of June. The effect that would naturally be expected from higher day temperatures in Upper Egypt may be neutralized in the early part of the season by cooler nights, due to the greater radiation allowed by the drier air. In any event the cotton was found at nearly the same stage of development about Beni-Suef as about Cairo and Tanta. (See Pl. I, fig. 1.) Even at the middle of July much of the cotton in Upper Egypt, between Beni-Suef and Minieh, was still quite small, having scarcely reached the flowering stage. In some fields the plants were only 6 or 8 inches high. The same was true of many fields in Lower Egypt in the region of Mansurah. (See Pl. II, figs. 1 and 2.) To what extent the later planting was responsible for the more backward state of the cotton in these districts was not learned, nor the reasons that may exist for later planting.

The most important local differences perceptible in Egypt were not those of the external conditions or of the methods of cultivation. The superiority of the cotton raised in the Delta region may be due in part to superior conditions, as generally assumed, but better knowledge of the Hindi cotton among the native cultivators is another factor of great importance, since it determines whether the inferior Hindi cotton shall be rogued out or left to mature in the fields. Many native cultivators at Beni-Suef pay no attention to the Hindi cotton, while about Mansurah it seems to be known to everybody. But even about Mansurah the human factor is by no means uniform, as shown by widely varying proportions of Hindi cotton in the different fields.

IMPORTANCE OF UNIFORMITY IN EGYPTIAN COTTON.

The requirement of uniformity increases with the presence of other good qualities of cotton. A long, strong cotton commands higher prices, because it can be spun into stronger or finer thread and used to make stronger or finer fabrics. An admixture of short, weak fibers not only reduces the strength of the threads and impairs the quality or durability of the fabric; it interferes also with the work of the spinning and weaving machinery by the more frequent breaking of the threads.

The superiority of the Sea Island cotton does not consist alone in its length and strength, but in its extreme uniformity. This is maintained by a highly developed system of selection, well recognized among the Sea Island planters but not yet applied to any other commercial type of cotton. The seed for each season's crop is raised by itself, apart from the general planting, and traces its ancestry

back to a single superior individual of two or three generations before.[a]

In the Egyptian system of cotton culture no attempt seems to be made to imitate the methods of the Sea Island planters. Even less consideration is given to selection than in the Upland-cotton industry of the United States. While very few planters of Upland cotton have been accustomed to select their own seed, it has at least been possible for them to buy seed of selected stocks of many of the Upland varieties, whereas planters in Egypt do not appear to have any recognized source of supply from which to secure uniform stocks of seed of the Egyptian varieties free from the Hindi contamination. Differences between the seeds of the Hindi and the Egyptian cotton enable a selection to be made, even after ginning, but it seems evident from the condition of the fields in Egypt that a considerable quantity of Hindi seed must be planted and that many Hindi plants are allowed to grow to maturity and so to maintain the contamination.[b]

The advantage that the individual planter might gain by a careful and persistent selection of his own seed is difficult to realize under the Egyptian system of selling the seed cotton to the ginner. There is also a custom of exchanging seed between different villages on the theory that better yields can be obtained in this way. Thus growers of Mit Afifi cotton near Mansurah obtain their seed from Kefir Zeyat, between Tanta and Alexandria, a place that is commonly supposed to produce seed of a superior quality. Such exchanges of seed are

[a] Webber, H. J. Improvement of Cotton by Seed Selection. Yearbook of the Department of Agriculture for 1902, p. 374.

[b] "The seed reserved for sowing is passed through special riddles, which remove small and dead seed; purity can not be obtained by this means, but merely a better looking sample; that is to say, as far as general appearance is concerned, the sample may be excellent, but closer examination reveals the presence of seed not true to variety. Small cultivators do not, as a rule, trouble even to secure the best seed which is procurable, but content themselves with the employment of that resulting from the ginning of common qualities of all pickings, regardless of origin and purity. Were this seed purchased at a low price it would provide no excuse for such a short-sighted policy, but even this is not the case, the price paid to the village merchant being, as a rule, considerably higher than that for which the better qualities could be obtained.

"In order to overcome this difficulty, the Khedivial Agricultural Society, in conjunction with the Agricultural Bank, distributes annually to small cultivators the best seed obtainable at cost price, the value of which is collected at the end of the following cotton season.

"It must be remarked, however, that the seed so distributed is merely the best that can be procured.

"That it is vastly superior to that which in the absence of such a system of distributing would be employed is without doubt. At the same time this system does nothing to actually improve the seed." (See Foaden, G. P., "The Selection of Seed Cotton," Yearbook of the Khedivial Agricultural Society, 1905, p. 122.)

well calculated to preserve and distribute the Hindi contamination. Even the introduction of new, carefully selected varieties could be expected to give only temporary improvement unless the whole system were changed. The process of deterioration would be resumed at once as a result of the crossing between adjacent fields of different varieties and the exchange of seed between different localities.

After selection is relaxed the rapidity of deterioration of a variety of cotton depends on two cooperating factors, variation and crossing. Both of these factors must vary in different places, for they are influenced by external conditions. When cotton is grown under new or unfavorable conditions, more numerous variations appear. Abundance of bees or other cross-fertilizing insects causes a more rapid spreading of variations through the stock. Relatively uniform conditions and apparent scarcity of insects may give longer life to varieties in Egypt than in the United States, but the general tendencies and results of deterioration seem to be quite the same.[a]

The history of cotton culture in Egypt shows that a succession of new varieties has replaced the old at intervals of a few decades. The modern Egyptian cotton industry began with the variety discovered and popularized by Jumel, a French engineer, about 1820. The Jumel cotton was replaced by the Ashmuni after 1860, the Ashmuni by the Mit Afifi about 1890, and more recent varieties, such as the Jannovitch and Nubari, are now replacing the Mit Afifi. Other varieties, such as the Bamieh, Gallini, Zafiri, Abbasi, Sultani, etc., have either failed to gain any general popularity or have aroused only temporary interest.

LINT AND SEED CHARACTERS OF HINDI COTTON.

The character that renders the Hindi cotton so unwelcome as an element of admixture in the Egyptian stock is the much shorter and coarser fiber. The Hindi fiber is also pure white in color, whereas in the more popular Egyptian varieties the lint is a somewhat creamy white, tinged with buff or brown. White-linted varieties of Egyptian cotton have been cultivated to a small extent, but have never become popular in Egypt.

The difference in the color of the lint is of much assistance in the work of sorting out the Hindi admixture after the fiber has been picked and brought to the ginning establishment. Any thorough separation of the inferior Hindi fiber from a white variety must be

[a] Though very few insects were noticed in the Egyptian fields in June and July, they may be more abundant later in the season. Balls reports between 5 and 10 per cent of crossing, and even 25 per cent in one of his experiments. (See Balls, W. L., "Cross-Fertilization in Cotton," Cairo Scientific Journal, vol. 2, 1908, p. 405.)

much more difficult, if not entirely impracticable. From this point of view it is easy to understand why the culture of Sea Island cotton or of the superior white varieties of Egyptian, such as Abbasi, has not become more extensive.

The superiority claimed for the lint of the white varieties, such as Abbasi, is in accordance with other indications of a general correlation between the color and the length of the lint. Study of the lint characters of many variations and hybrids seems to indicate a general tendency in brown fibers to be shorter and coarser than white fibers. Thus the Jannovitch variety has lint longer and whiter than the Mit Afifi, though still with a very slight tinge of brown. The Abbasi lint is still longer, but is pure white in color.[a]

If the need of sorting the fiber were removed by more effective methods of eliminating the Hindi variations, the way would be open to a larger use of white-linted varieties. Though brownish lint is preferred for a few purposes, the color seems to be valuable chiefly for the aid it gives in sorting out the inferior fiber that results from the Hindi contamination. If American growers are sufficiently careful to keep out the Hindi contamination, they may be able to grow white varieties that have longer and stronger fibers than the brown-linted varieties now popular in Egypt.

In addition to the long fibers that compose the lint, the seeds of typical Egyptian plants are always provided with short fibers, or "fuzz," that continue to adhere to the seed after the lint has been removed by ginning. The fuzz may be confined to small tufts at the ends of the seed or may extend down one side, or may be more widely spread over the surface. The seeds of the typical Hindi cotton, on the other hand, are entirely without fuzz. The black surface is left entirely naked after the lint has been removed. The absence of fuzz makes the small, sharp-pointed, black stalk or funiculus at the base of the seed much more conspicuous in the Hindi cotton, though it is present in other varieties.

The seeds of the Hindi cotton are more angular in shape than those of the Egyptian cotton. Though not adhering like the seeds of kidney cotton, they seem to be more closely crowded together in the boll than the seeds of the Egyptian cotton, and this mutual pressure tends

[a] The production of Abbasi cotton is said to be irregular because the price fluctuates with Sea Island cotton. When Sea Island cotton is cheap there is small demand for the Abbasi. Another variety that gave very promising results in an experiment in Arizona in 1909, the Nubari, is said to be not very highly appreciated in Egypt because of a tendency to produce small bolls. While many small-bolled plants were found in the Nubari field in Arizona, there was less diversity in this and other respects than in any other lot of plants grown from imported seed.

to make the Hindi seeds longer and more angular. Fully developed Egyptian seeds are usually plump, with all the sides distinctly convex and with a larger diameter than the Hindi seeds.

The smooth surface and narrower shape of Hindi seeds make it possible to separate most of them by sifting, as the Egyptian ginning establishments are said to do. Nevertheless, it is not to be expected that any complete elimination of the Hindi cotton can be accomplished in this way, for Hindi plants are occasionally found with fuzzy seeds much like the seeds of American Upland cotton. The seeds of Hindi hybrids are also somewhat fuzzy, often in the same way as the Egyptian seeds.

Hand selection of seed intended for planting is said to be done in Egypt, though it does not seem to be a regular practice. Experiments carried on by Mr. Argyle McLachlan in Arizona indicate that Hindi variations and other aberrant tendencies can usually be detected if the seeds are studied with sufficient care and discrimination.

The sorting out of the Hindi cotton is also assisted by the fact that the Hindi lint is very lightly attached, allowing the black surfaces of the seeds to be very readily seen. Even before the cotton is picked from the plants this difference is often very apparent.

In addition to being short and coarse, the fibers of the Hindi cotton are relatively straight and have very little tendency to cling together, like the longer and more abundant fibers of Egyptian and Upland varieties. After the Hindi bolls are open the seeds soon begin to separate and fall out, especially if they have a little assistance from wind or rain. In other words, the Hindi cotton is conspicuously lacking in storm-proof qualities.

The naked surfaces of the Hindi seeds may be responsible for the fact that young plants of the Hindi cotton often appear to make more rapid growth than adjacent Egyptian plants. Experiments have shown that the germination of fuzzy-seeded varieties may be seriously delayed in dry weather, while seeds without fuzz may germinate promptly in the same soil. Obviously, too, a Hindi seedling that had germinated promptly and had sent out roots to absorb water would retard the germination of other seeds in the same hill. The cotton is planted in Egypt in relatively dry soil, the young plants being easily destroyed by any excess of water. Under such conditions there is usually a very unequal development of the young plants. Two or three plants in each hill, or perhaps only a single one, may develop several leaves and attain a height of 8 or 10 inches, while the other seedlings of the same hill remain with only the cotyledons expanded.

DISTINCTIVE CHARACTERS OF HINDI PLANTS.

HABITS OF GROWTH OF HINDI COTTON.

If the Hindi cotton could be recognized only by the characters of the lint and seeds, it might be impossible to effect a complete elimination of the Hindi characters by selection. As long as Hindi plants are allowed to flower in the fields with the Egyptian plants and cross-fertilize them the undesirable Hindi characteristics may be expected to reappear. Even if no seeds of the Hindi form are planted, some of the apparently normal Egyptian seeds are likely to contain Hindi hybrid embryos, and these in turn can grow to maturity and produce pollen for continuing the Hindi infection to further generations. It is fortunate, therefore, that the Hindi cotton has several very definite differences in the vegetative parts, so that all Hindi plants can be recognized and rogued out of a field or a seed plat before the age of blooming and cross-fertilization is reached.

The general form or habit of growth of the Hindi plants is different from that of the Egyptian cotton, though this is not so apparent in the Egyptian fields, where the plants are crowded closely together, as in experimental plantings, where more space is allowed the individual plants. The tendency of the Hindi cotton is to produce a broader and more bushy plant, more like the Upland than the Egyptian cotton. (See Pl. I, fig. 2.)

There is a general impression that the Hindi cotton is larger and more luxuriant than the Egyptian, but this may relate to the Hindi hybrids rather than to the genuine Hindi individuals. The Hindi plants may appear larger early in the season, perhaps as a result of more prompt germination, but they are usually outgrown by the neighboring Egyptian plants by the time the fruiting stage is reached.

The Egyptian cotton, as well as the Hindi, shows different habits of growth under different conditions. In the cooler climate of Lower Egypt there is no such luxuriance of vegetative growth as in Arizona, but the branches are more spreading and the foliage more open. The habit of the Egyptian cotton in Egypt is more like that of Upland cotton in our Southern States. The similarity was especially strong in the Fayum Oasis, where some of the cotton is planted on rather poor land. It flowers and fruits when only 8 or 10 inches high, maturing small, bushy plants, like Upland cotton on poor soil in the South. Something of the exuberant tendency was shown in an experimental planting of Egyptian cotton at Siut (Assiut), in Upper Egypt.

The habits of branching of the Hindi cotton are also different from those of the Egyptian. The fertile branches are less definitely specialized than in the Egyptian cotton and have a stronger tendency

to grow in upright or oblique positions and to assume the functions of vegetative branches, the flower buds being often aborted.[a]

LEAF CHARACTERS OF HINDI COTTON.

The leaves of the Hindi cotton are characterized by thinner texture and lighter color, a fresh, bright green that forms quite a definite contrast with the duller grayish or bluish green of the Egyptian leaves. The surfaces of the leaves of the Egyptian cotton are somewhat duller and more hairy in Egypt than in Arizona, though not so grayish as when the Egyptian cotton is grown in the cool climate of the Pacific coast, near Los Angeles. The color is usually darker before the fruiting stage of the Egyptian cotton is reached, when the foliage usually takes on a lighter and more yellowish tone. The dark foliage of the vegetative phase may be retained under conditions of abnormal luxuriance, or the change to the yellower shade of green may occur prematurely if the plants are affected by some unfavorable condition, such as too much water or too little.

The veins of the leaves of the Hindi cotton are usually reddish, and the red color becomes very pronounced at the pulvinus or cushion-like thickening at the bases of the veins, where they pass into the petiole or stem of the leaf. The two large veins on each side of the midrib are particularly likely to be grown together at the base, giving the pulvinus of the Hindi cotton an oblong shape. The leaves of the Egyptian cotton do not have an enlarged pulvinus, the veins passing more directly into the petiole without becoming much swollen or united at the base. The surface of the pulvinus of the Hindi cotton is naked, or with only a few scattering hairs, while the corresponding part of the Egyptian cotton is usually quite hairy.

The lack of specialization of the bases of the veins in the Egyptian cotton seems to render the leaves less capable of movement. They do not appear to change their positions to face the sun in the morning and afternoon as much as the leaves of the Hindi cotton. The turning of the leaves to the sun renders the Hindi plants more conspicuous in the morning and afternoon than in the middle of the day, when the leaves have a horizontal position. Advantage was taken of this fact in making inspections of fields from moving trains, as will be explained later.

Even in the first leaves or cotyledons of the young seedlings the reddening of the veins and the basal spot enables the Hindi cotton to be recognized and separated from the Egyptian. The difference of coloration is not so obvious in the first few leaves that appear after the cotyledons, for even in the Egyptian cotton these are likely

[a] Dimorphic Branches in Tropical Crop Plants, Bulletin 198, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1910.

to have a somewhat reddish spot at the base, especially if the conditions are not favorable for rapid growth. The differences become more obvious as the plants grow, until the flowering stage is reached, but they may lessen or disappear at maturity. In adult Egyptian plants the veins of the leaves often become reddish, while those of adult Hindi plants may become pale.[a]

After the color contrasts have disappeared, the recognition of the Hindi plants requires notice of other less obvious details of the leaves, flowers, and bolls. Thus the leaves of the Hindi cotton have the lobes broader, more abruptly narrowed toward the apex, and usually produced into longer terminal points. In Hindi hybrids there are often 5 to 7 lobes which are often somewhat folded or plicate, as in the Egyptian cotton, the true Hindi plants having the leaves nearly flat. The rounded basal lobes of the leaf are broader in the Hindi cotton, so that the leaf as a whole is more nearly square or oblong in shape. The corresponding margins of the Egyptian leaves are likely to converge or slope backward toward the stem.[b]

The sinus or notch at the base of the leaf, where the petiole is inserted, is usually much broader in the Hindi cotton, exposing the upper surface of the end of the petiole. In the Egyptian leaves the sinus is generally very narrow or completely closed by the contact or overlapping of the margins of the lobes. The wider separation of the lobes of the Hindi cotton may be considered as a consequence of the thickening of the veins and the enlargement of the end of the petiole

FLORAL CHARACTERS OF HINDI COTTON.

The involucre that incloses the bud of the cotton plant is composed of three bracts, small leaf-like organs, each margined with a fringe of narrow teeth. The bracts of the Hindi cotton are more broadly rounded at the base and have longer and more numerous teeth than those of the Egyptian cotton. Comparison of the Hindi bracts shown in Plate III with the Egyptian bracts at the top of Plate IV will enable these differences to be understood. Another diagnostic feature of the Hindi bracts is that the teeth run down nearer to the base, a tendency that is shared by the Hindi hybrids. Three hybrid bracts are shown at the bottom of Plate IV. The bracts of the Egyptian cotton seemed to be somewhat more cordate in Egypt than in the United States, but the narrowly triangular form, straight sides, and small teeth, remote from the base, generally render them

[a] Mutative Reversions in Cotton, Circular No. 53, Bureau of Plant Industry, U. S. Dept. of Agriculture, March, 1910, pp. 10–11.

[b] For natural-size illustrations of leaves of Egyptian and Hindi cotton, see Circular No. 42, Bureau of Plant Industry, December, 1909, pp. 4 and 5.

quite different from the Hindi bracts, in spite of endless variations in the minor details.

The calyx of the Hindi cotton has five distinctly prominent triangular lobes, one or two of which are often produced into a narrow needle-like point. In the Egyptian cotton the lobes of the calyx are very short and broadly rounded, never produced into long points. Three examples of the toothed calyx of the Hindi cotton are shown in Plate III; an Egyptian calyx and the calyx of a hybrid in Plate IV.

The fresh, newly opened flowers of the Hindi cotton have pale creamy-white petals like those of Upland cotton instead of lemon-yellow petals like Egyptian cotton. In the afternoon the flowers of both sorts change to a reddish pink, but the Hindi flowers attain a much deeper shade than the Egyptian.

The petals of the Hindi cotton are shorter than those of the Egyptian and open more widely. The Hindi flower may be described as cup-shaped, the Egyptian as tubular.

The purple spot found at the base of each petal in Egyptian flowers is lacking or only faintly indicated in typical Hindi flowers, though often quite pronounced in Hindi hybrids.

The pollen of the Hindi cotton is of a much paler yellow and the individual pollen grains are much smaller than those of the Egyptian cotton.

FRUIT CHARACTERS OF HINDI COTTON.

The bolls of the Hindi cotton have a rounded conic shape, broadest near the base, and taper abruptly to a short point. Egyptian bolls are more fusiform, narrower at the base than near the middle, and taper less abruptly to a rather blunt apex. The shape differs appreciably with the conditions, the less luxuriant plants in Egypt having a broader and more conic form than is usual in Arizona, more like the bolls produced by the Egyptian cotton in the vicinity of Los Angeles. (See Pl. VI.)

The surface of the Hindi bolls has a rather dull pale pea-green color, with only slight indications of the deeply buried oil glands. Egyptian bolls, on the contrary, have a fresher, darker color, with the surface smooth and shining, but rather deeply pitted around the numerous superficial oil glands, each of which appears as a distinct black dot. These differences appear somewhat less pronounced in Egypt than in Arizona. Pale-green bolls were found on many plants that seemed in all other respects to represent true Egyptian cotton. The darker color of the bolls in Arizona may be connected with the greater luxuriance of the plants.

The number of carpels, or "locks," varies in the Hindi cotton from 3 to 5, the majority of bolls having 4 locks. In the Egyptian cotton

the locks range from 2 to 4, with 3 as the prevailing number. Very few 4-locked bolls could be found in the Egyptian fields, but they are somewhat more numerous on the larger and more luxuriant plants grown in Arizona.

PREVALENCE OF HINDI COTTON IN EGYPT.

Familiarity with the vegetative characters of the Hindi cotton made it possible to secure definite information regarding the prevalence of this type of cotton in Egypt and thus obtain a basis of judgment regarding the value of the methods of selection that are being applied to the Egyptian cotton in Arizona. In attempting to judge of the practicability of establishing the culture of Egyptian cotton in the Southwest, it is obviously important to understand how far the commercial reputation of the Egyptian cotton for uniformity depends on the special methods of sorting and preparing the cotton for market. This will enable us to appreciate the advantage that may be gained by growing a more uniform fiber in the fields and avoiding the necessity of the subsequent labor in sorting and blending the fiber into a uniform product after it comes to the ginhouse.

Some writers have given the impression that the native cultivators rogue out all the Hindi plants during the process of thinning the young cotton early in the spring and thus avoid an admixture of the Hindi fiber. Others have referred to the Hindi cotton as a wild plant in Egypt, or even a common weed, making it seem almost impossible to avoid contamination.

Neither of these impressions seems to correspond with the facts. Though many of the native cultivators will hasten to assure the inquirer that they pull out all of the Hindi plants, a goodly remnant of typical Hindi individuals is to be found in nearly every field. On the other hand, one does not find the Hindi cotton, any more than the Egyptian cotton, outside of regularly planted cotton fields. Seeds scattered near permanent watercourses or about towns may sometimes grow to maturity, but it is not easy to understand how the idea of wild cotton growing at large in Egypt could have gained currency. Other plants that casual observers might mistake for cotton, such as the okra or bamieh (*Hibiscus esculentus*), the Deccan hemp (*Hibiscus cannabinus*), or even the cocklebur (Xanthium), are all strictly dependent upon cultivation and irrigation. It is difficult to believe that a plant of the habits of the cotton could exist as a native or truly wild species in the Nile Valley. And if such a species did exist naturally it would be dependent upon the annual flood for its water, and would be a winter-growing species. The commercial culture of cotton was not developed in Egypt under the historical system of basin irrigation direct from the annual flood

of the Nile. The period of high water comes during the late summer and autumn, the fruiting season of the cotton. Egypt did not gain importance as a cotton-producing country until the modern system of perennial irrigation from stored water was developed, in the nineteenth century.

The Egyptian system of close planting greatly increases the difficulties of finding the Hindi individuals and of counting the Hindi and Egyptian plants to determine the percentages of each. Early in the season, while the plants are still small, each one can readily be seen as a separate individual, but with larger growth they fuse together, as it were, to form a solid mass of foliage. Early inspection has the further advantage of utilizing the differences in the color of the foliage that are readily appreciable in the vegetative phase of development, but tend to disappear after the fruiting stage has been reached, as already explained.

If actual countings are not made, the proportion of Hindi cotton is likely to be seriously underestimated after the plants have reached the adult or flowering stage. It has been said that the Hindi plants can be distinguished from the Egyptian by their taller growth, but this seems to be true of hybrids or of young individuals rather than of mature plants of the true Hindi type. It was noticed at Calioub and at several other points that while many of the hybrid plants ran several inches above their Egyptian neighbors, the true Hindi plants had usually been outgrown by the Egyptian. In fact, some of the Egyptian cultivators consider that the hybrids rather than the true Hindi plants ought to be pulled out. They have noticed that many of the large overgrown hybrids produce very little fruit and are willing to pull them out so they shall not crowd their more productive neighbors. Careful roguing in the early part of the season is more likely to take out all of the true Hindi plants and leave a few of the hybrids, so that careful cultivators are more likely to be familiar with mature hybrids than with mature Hindi individuals.

The true Hindi plants, being less obtrusive when the stage of maturity is reached, are very easily overlooked unless special care is taken to separate and count the plants of each hill. Though two plants are usually left at thinning, regularity in this respect can not be depended upon. It often happens that only one plant survives, or careless cultivators may leave occasional hills with three or four plants.

It may be that the value of countings as the basis of general estimates of the proportion of Hindi cotton would not be seriously impaired by assuming two plants to each hill. The saving of time in this way would enable more extensive counts to be made. This plan was followed in a few of the later countings mentioned below.

at Calioub and Siut, in fields where the plants had grown very large. The hills were each noticed in turn to see whether they contained Hindi plants. Hills with no Hindi were assumed to have two Egyptian plants. The general effect of this plan would be to reduce somewhat the apparent proportion of Hindi plants, since it is probable that in most of the fields there would be more hills with a single plant than with three or four plants. Nevertheless, it might be that the figures obtained in this way would be more reliable, in view of the larger areas that might be inspected in a limited time.

To serve as a general basis of judgment regarding the prevalence of the Hindi cotton in Egypt, countings of individual plants were made in several different localities. In most localities several separate counts were made, usually in fields of different proprietors, or at least of different tenant cultivators. The figures obtained do not represent the full extent of Hindi contamination of the stock, for in most cases a more or less careful roguing out of the Hindi plants had already taken place. The psychological factor of the individual cultivator enters, therefore, as an important element in the calculations. One field might have only a few Hindi plants, while the next would have a considerable percentage. Thus of two adjacent fields at Tanta one showed less than 3 per cent of Hindi, the other 15 per cent.

Questioning of the native cultivators showed wide differences of individual opinion. Some of them were quite alive to the need of pulling out all of the Hindi cotton and showed annoyance or offered excuses if reminded that many Hindi plants were still to be found in their fields. Others took a more languid interest in the matter. One cultivator might claim to have pulled out large numbers of Hindi already, while his neighbor might not think it necessary to admit any responsibility for pulling out the Hindi at all. He would not deny, perhaps, that he had heard of the need of pulling out bad cotton plants, but would insist that very few people did it.

The popular impression in Egypt among people who consider themselves informed about cotton growing is that selection receives proper attention in the Delta region, where the Mit Afifi and Jannovitch, the principal varieties of Egyptian cotton, are grown, but is very much neglected in Upper Egypt, where the Ashmuni and other inferior stocks are produced. It seems, however, that this impression may relate to more careful sorting done in the ginning establishments of the Delta rather than to any really efficient selection in the field. Even about Tanta and Mansurah, the recognized centers of production of high-grade fiber, a conspicuous representation of the Hindi cotton was seen in a large proportion of the fields.

The percentages of Hindi plants counted in fields at Tanta, in Lower Egypt, are about the same as those obtained at Beni-Suef, in

Upper Egypt. (See Table I.) The idea of Hindi cotton seemed to be more common about Tanta, but no indication of a serious effort to eradicate the Hindi type from the fields could be gathered from native cultivators. They are willing to pull out the Hindi plants rather than the Egyptian at the time that the hills are thinned down to the usual two plants, but have no idea of destroying any more plants after the thinning has been done. One very zealous native showed interest to the extent of pulling up some of the Hindi plants that were pointed out to him, where there was an Egyptian plant in the same hill. But when there were two Hindi plants together in a hill he would pull up only one. Nor could he be induced to sacrifice any of the Hindi individuals that stood by themselves, although he believed (as was afterward learned) that a Government inspection was being made. The Egyptian Government sends entomological inspectors through the fields to guard against outbreaks of the Egyptian bollworm.

Beni-Suef is considered the chief center of cultivation of the Ashmuni cotton, this variety being now confined largely to Upper Egypt. Inspection of fields in this locality on June 6, 1910, showed a general prevalence of Hindi and great lack of uniformity in other respects, though not as great nor as obvious as in experiments with this variety in Arizona. There is the same tendency to red spots at the base of the leaves, which is recognized as a mark of this variety to distinguish it from Mit Afifi, Jannovitch, and other more carefully selected varieties. The more general tendency to the red spot may be a result of a more general contamination with the Hindi type of cotton.

A special count was made at Beni-Suef to learn the extent of Hindi contamination as indicated by the presence of the distinct red spot at the base of the leaf. This included true Hindi plants, obvious hybrids, and all other plants that would have been considered as having too red a callus for varieties of Egyptian cotton other than Ashmuni. Of 213 plants examined for the color of the callus 133 had the callus green or only slightly tinged with red, as usual in Egyptian cotton, while 80 plants were noted as having the callus distinctly red, as in the Hindi cotton.

In the oasis of Fayum still less attention seems to be paid to the Hindi cotton than about Beni-Suef. Native cultivators knew that some of the plants produced inferior cotton, but did not claim to be able to distinguish them except by the white flowers. There was evidently no intention of pulling out any of the white-flowered plants. The variety planted at Fayum was not considered to be Ashmuni, but was merely called Beládi, or "native," cotton.

Other countings of Hindi were made in the Beládi cotton at Siut. Cotton is not regularly planted about Siut, but experiments are

being made with seed brought from Fayum. The percentage of Hindi is much larger than appeared at Fayum, though the planter claimed that he had taken out numerous Hindi plants when the field was thinned. In addition to the plants counted as Hindi, much diversity was apparent, almost as much as in a field of Ashmuni cotton grown in 1909 at Somerton, Ariz. Such cases suggest the possibility that transfer to new conditions may have the effect of inducing additional variations in these diverse stocks, but the proportion of Hindi in either parent stock could not be ascertained. Whatever the cause of the phenomenon, it is a significant fact that the proportion of Hindi plants and obvious hybrids may run as high as 20 per cent.

The census of Jannovitch cotton at Tanta was somewhat more rigorous than that at Beni-Suef and included some plants with distinctly red leaf bases; plants with distinctly red leaves and other obviously aberrant tendencies that might have been omitted in the Ashmuni fields, where the red callus is so common a feature. But many other definitely aberrant plants with light-green leaves were not included when they lacked the red callus. These light-colored plants have the more ample and luxuriant foliage of the Hindi hybrids and may represent a second-generation splitting of the Hindi characters. Such a splitting might be expected with a color character like the basal spot that also shows seasonal reversibility.

The smallest proportions of true Hindi plants were found in fields in the vicinity of the barrage (a few miles below Cairo) and at Calioub, in the same district. None of the fields that were inspected in these places showed any large percentages. About two-thirds of the plants counted as Hindi were plants of the type considered as first-generation hybrids. In one field at the barrage and in another at Calioub no true Hindi plants could be found, even after a rather careful search, though several obvious hybrids were present in each field. At Benha, on the contrary, the Hindi percentages not only ran higher but a larger proportion of the plants represented the true Hindi type.

In the neighborhood where the counts were made near Mansurah the native cultivators placed much importance on the elimination of the Hindi plants, though they were known by a different name, "Haga," the word Hindi not being recognized. It was estimated that about 5 to 6 Hindi plants had been removed from each row of 100 to 150 plants at the time of thinning, in addition to those that remained to be counted. This would indicate a total Hindi representation of between 5 to 10 per cent in this stock of seed at the time of planting.

In several instances it was noticed that the Hindi plants seemed to be more numerous on the higher, drier ridges or dikes that bounded

the different sections into which the fields were divided for irrigation purposes. Separate counts were made of plants along some of the dikes, but without securing any definite evidence. It would be interesting to know whether such differences of conditions would have an influence over the expression of the Hindi characters. Other explanations were possible—that the higher ridges had been neglected at the time of thinning the plants or that the Hindi plants had an advantage in germinating in the drier soil of the higher ridges, because of the smooth seeds. The cotton often appears to be more luxuriant on the higher dikes than in other parts of the fields. Indeed, such dikes are usually planted with double rows of cotton, as though to take full advantage of the more favorable conditions.

TABLE I.—*Countings of Hindi cotton plants.*

Location.	Plants counted.	Egyptian type.	Hindi type.	Percentage of Hindi.
Beni-Suef, Upper Egypt (Ashmuni variety)	445	435	10	2.24
	274	242	32	11.67
	512	457	55	10.74
	165	155	10	6.06
	178	161	17	9.55
	446	435	11	2.48
	327	294	33	10.09
	245	224	21	8.56
	130	124	6	4.61
Total	2,722	2,527	195	7.16
Tanta, Lower Egypt (Jannoviteh variety)	595	569	26	4.36
	886	829	57	6.43
	464	441	23	4.96
	464	437	27	5.82
	368	340	28	7.61
	1,028	923	105	10.21
	806	738	68	8.44
	134	118	16	11.92
	566	550	16	2.83
	566	476	90	15.09
Total	5,877	5,421	456	7.77
Barrage, near Cairo	1,149	1,124	25	2.17
	424	410	14	3.31
	551	543	8	1.45
	483	474	9	1.86
	567	559	8	1.41
	334	328	6	1.79
	511	486	25	4.89
Total	4,019	3,924	95	2.36
Calioub, near Cairo	417	412	5	1.19
	497	493	4	.80
	1,216	1,202	14	1.15
	2,130	2,107	23	1.07
Fayum, Upper Egypt (Beládi variety)	871	819	52	5.99
	676	629	47	6.95
Total	1,547	1,448	99	6.41
Siut, Upper Egypt	609	494	115	18.88
	467	398	69	14.77
	316	260	56	17.72
	444	354	90	20.27
	467	383	84	17.98
Total	2,303	1,889	414	17.97
Mansurah, Lower Egypt (Jannovitch and Mit Afifi varieties)	844	829	15	1.77
	476	472	4	.84
	560	555	5	.89
	531	523	8	1.50
	790	758	32	4.05
	669	662	7	1.04
	528	517	11	2.08
	598	584	14	2.34
	934	924	10	1.07
Total	5,930	5,824	106	1.78
Benha, Lower Egypt	857	810	47	5.49
	202	200	2	.99
	461	429	32	6.93
	655	633	22	3.36
	558	542	16	2.86
Total	2,733	2,614	119	4.36
Beteha, Palestine	1,043	954	89	8.53
	889	816	73	8.21
	1,975	1,947	28	1.41
	982	946	36	3.66
Total	4,889	4,663	226	4.62

Count was made of 32,150 plants in all, of which 1,733 were recorded as belonging to the Hindi type, a percentage of 5.39. If the percentages for the different localities are averaged, a somewhat higher general average, 5.98 per cent, is obtained.

One series of countings of Hindi plants was made in an experiment with Egyptian cotton in Palestine, at a locality called Beteha, near

the north end of the Lake of Tiberias, not far from the ancient Capernaum. The first two counts at Beteha were made in late-planted fields that had not yet been thinned or rogued for Hindi. The percentages obtained in these cases, 8.53 and 8.21, may be taken to represent the amount of Hindi contamination represented in the seed before planting. Early-planted fields at Beteha seemed to be as far advanced as any seen in Egypt, the date of the visit being June 23.

In order to obtain a more general and yet a not altogether indefinite indication of the prevalence of the Hindi cotton, the apparent presence or absence of Hindi cotton was noted for a considerable number of fields that could be seen to advantage from the railroad. Such inspection is greatly facilitated by a fact already considered, namely, that the leaves of the Hindi cotton have greater freedom of motion than those of the Egyptian cotton, and that they make pronounced changes of position in order to face the sun in the morning and afternoon. The Hindi plants are much more readily seen from a distance at these times than in the middle of the day, when the leaves are in a horizontal position to face the sun overhead.

The presence of tall hybrids gives a general impression of uneven surfaces to the fields and thus betrays the presence of Hindi cotton, even when details of individual plants can not be made out. But when the broader, fresh-green leaves of the Hindi plants are formed into rosettes to face the sun, they become conspicuous and unmistakable. Indeed, it is sometimes more difficult to distinguish them from the okra that is often planted in the fields than from the Egyptian cotton. The Egyptian okra (bamieh) has broad leaves of the same color as those of the Hindi cotton and also a red spot at the junction with the stem.

Such observations are greatly assisted by the fact that the Egyptian railroads are usually elevated on embankments. By being able to look down on the fields a more accurate impression can be gained than by viewing the plants from the side, as one is obliged to do when standing on the same level.

It is to be expected of course that Hindi plants would be found by more careful inspection in most of the fields where they were not apparent from a passing train. But at least it may be considered that fields showing no apparent Hindi have been rogued. In a large proportion of cases the Hindi plants and hybrids were very conspicuous. Fields that have had the Hindi plants and hybrids rogued out often appear remarkably even in height and color.

Such an inspection could not be made to any advantage after the Egyptian cotton has entered the fruiting phase, when the color changes from a dark to a lighter green, thus destroying the contrast with the Hindi cotton, so marked during the earlier vegetative phase.

In addition to the lighter color assumed by the foliage of the Egyptian plants as the season advances, the proportion of yellow in the fields is increased by the abundance of bracts and flowers. At the time these changes were taking place, about the middle of July, the dark-green tone of the vegetative phase was still shown with much uniformity in some of the fields, while others had gone over to the yellower shades or were still more completely dominated by the abundance of yellowish bracts and still yellower flowers. These changes seemed to have come rather suddenly, for most of the fields seemed to represent one phase or the other quite definitely, only a few showing pronounced individual diversities of coloring among the Egyptian plants.

TABLE II.—*Fields with Hindi cotton apparent from trains*

Fields were noted between towns—	Number of fields.	Fields with apparent Hindi.	Fields without apparent Hindi.
Calioub and Chebin el Kaneter	48	46	2
Chebin and Machetoul	53	50	3
Machetoul and Bilbeis	81	76	5
Bilbeis and Zagazig	82	81	1
Zagazig and Abou-Kebir	88	82	6
Abou-Kebir and Kafr Sakr	24	22	2
Kafr Sakr and Abou el Chekouk	30	24	6
Abou el Chekouk and Simbellaouein	51	[a](10) 44	7
Simbellaouein and Baklieh	13	(3) 13	0
Baklieh and Mansurah	24	(4) 17	7
Mansurah and Samanoud	90	(16) 73	17
Samanoud and Mehalla Kebir	29	26	3
Mehalla Kebir and Mehallet Roh	34	(4) 27	7
Mehallet Roh and Tanta	49	(3) 42	7
Total	696	623	73
Percentage		89.51	10.48

[a] In some localities fields that showed a strikingly large proportion of Hindi cotton were specially noted, and the numbers of such fields are given in parentheses in the table. It would be safe to estimate that the proportion of Hindi cotton and obvious hybrids in such fields was more than 5 per cent. Many fields between Bilbeis and Zagazig appeared to be quite as thickly sprinkled with Hindi as any in Upper Egypt where percentages of 15 and 20 were counted.

In addition to fields noted in Table II, many other inspections were made in the region between Cairo and Tanta. Several hundred fields were seen in Upper Egypt, in every one of which indications of Hindi contamination were found.

In the district between Abou el Chekouk and Mansurah much of the cotton at the middle of July was still too small and irregular to give favorable conditions for seeing Hindi plants from the train. Many of the fields had not begun to flower. In many the stand was irregular, or the plants of irregular sizes, perhaps as a result of alkali in the soil. Fields of rice interspersed among the cotton showed the same irregularity. The unfavorable conditions may be partly responsible for the larger proportion of fields with no apparent Hindi in this district. Fields with larger plants often showed great

abundance of Hindi. Most of the cotton to the west of Mansurah was in better condition and afforded a more reliable indication of the prevalence of Hindi, or rather the prevalence of roguing. Though the proportion of fields apparently clean of Hindi seemed to be distinctly larger than in other districts, many of the fields showed unmistakable Hindi plants in great abundance.

Unless the conditions are favorable for the detection of the Hindi plants such inspections could have very little value, but if made at the right time the presence of the Hindi contamination and the relative amount in different districts could be judged very easily in all localities accessible by railroad. The time would differ with the growth of the cotton in the different localities, probably extending through the month of July. Before June 20 the Hindi plants could seldom be seen from the trains, but during the second and third weeks of July they were easy to see in all except the more backward districts.

CHARACTERS OF HINDI HYBRIDS.

DISTINCTIVE FEATURES OF HYBRIDS.

Except in cases that are especially noted, the plants enumerated as Hindi in the preceding tables comprise two elements, the typical Hindi plants and the pronounced Hindi hybrids, those that resemble the first generation of the crosses that have been made between the Hindi and the Egyptian cottons.

When the fields are in the earlier vegetative phase, the pronounced hybrids can be distinguished from the Egyptian plants by the light color of the leaves and the red pulvinus at the base of the veins, almost as easily as the true Hindi. The larger size of the hybrids also attracts attention. The leaves of the hybrids become larger than those of the true Hindi plants, and most of the larger leaves have five or seven distinct lobes instead of three. The lobes of the hybrids are somewhat folded or channeled, like those of the Egyptian cotton, instead of spreading out nearly flat, as in the Hindi cotton. The larger size of the involucral bracts of hybrids is another feature usually quite obvious. (Pl. IV, *B*.) The teeth do not always run down toward the base of the bracts, as in the Hindi cotton, though there is a general tendency in this direction. In Arizona the Hindi hybrids have shown a marked tendency to sterility or to very late bearing, but in Egypt, early in June, some of the hybrids seemed to be more advanced toward flowering than their Egyptian neighbors.

The countings of the Hindi plants and obvious hybrids do not by any means indicate the full extent of the Hindi contamination in the Egyptian fields. There is background of diversity too multifarious to be counted or even noted in detail without careful inspection

of the characters of individual plants. Crossing between hybrid plants and Egyptian must produce many very dilute hybrids with little or no expression of the Hindi characters. Indeed, it may well be doubted whether any of the Egyptian stock would be found to be entirely free from the Hindi contamination if all of the ancestry could be traced. As yet we have no knowledge of the effects of slight dilutions of the Hindi blood upon the expression of characters, but experiments are being made to obtain information on this point.

Two principal elements might be recognized in the study of the diversity that exists in the Egyptian fields. One element might be ascribed to the prevalence of the Hindi cotton, the other to variation inside the Egyptian type. But in the present state of our knowledge it is often quite impossible to determine at once whether a variant plant is a dilute Hindi hybrid or an unusual example of the Egyptian stock. Evidence on this question can be secured by planting the seed to see whether the progeny "come true" to the characters of the parent, as in a mutation, or show more pronounced reversions to the Hindi type. But many mutative variations are also to be considered as reversions. The practical fact is that the Hindi contamination is responsible for a large amount of diversity outside of the obvious hybrid forms that resemble first-generation crosses.

Among the plants enumerated as Egyptian are many that are appreciably different from the Egyptian type, even in the early part of the season. Without departing seriously from the Egyptian form and habits of growth, some of the plants have broader or narrower leaves, lighter or darker than their neighbors. Though the form of the leaves may be that of the Egyptian cotton, the bases of the veins may be reddened as in the Hindi. Or plants with Egyptian foliage may have unusual habits of growth, the more frequent tendency being toward taller stalks and more strictly upright branches.

The large cordate bracts that characterize the most obvious Hindi hybrids are not entirely confined to that class of plants, but may be found on other large plants with foliage of Egyptian shape and color. The pulvinus may have the Hindi size, shape, and color, though concealed by more abundant hairs. In addition to the large circular, or very deeply cordate bracts, with the teeth running well down, such plants often have the calyx distinctly toothed, though the teeth do not have the long slender points that occur so frequently in the Hindi cotton. (See Pls. III and IV.)

As the season advances such differences become more apparent. When flowering and fruiting begin the hybrid nature of many individuals becomes unmistakable, even in plants that might not have been suspected of hybridity from the vegetative characters alone. Roguing must not be limited to the time of thinning in the early

spring if any complete elimination of the Hindi characters is expected.[a]

The tendency to revert to small bolls is one of the most frequent and least obvious evidences of Hindi contamination. Small bolls can often be found on large-bolled plants, but many individuals produce only small bolls. The shape of the bolls may not suggest Hindi, though other Hindi characters may be found, such as naked seeds, sparse white lint, or pale spots in the flowers.

To make a complete enumeration of all the plants that show any of the Hindi characters it would be necessary to watch a field of cotton through the whole season, for in some plants only the lint and the seeds may betray the Hindi ancestry. Already, at the beginning of the fruiting season in Egypt, it became evident that many of the aberrant Egyptian plants were really Hindi hybrids, in addition to the type of hybrids that had been included in the countings. Even in the fields that had been quite carefully rogued, as at Mansurah, so that only very small percentages of plants with the Hindi foliage were left, many white-flowered individuals remained. The leaves of the white-flowered plants seemed to be a little broader than those of adjacent yellow-flowered Egyptian plants, but the difference was not enough to be noticed if attention had not been attracted by the flowers.

COHERENCE OF CHARACTERS IN HYBRIDS.

It is not yet certain that all of the more Hindi-like hybrid plants are really first-generation hybrids, the direct result of cross-fertilization between Hindi and Egyptian plants. All that is known at present is that the crossing of Egyptian with Hindi does produce plants of the Hindi-like hybrid type. The experiment has been made in Egypt by Mr. Balls and in Arizona by Messrs. McLachlan and Meade. It is possible, however, that some of the Hindi-like hybrid forms may represent the progeny of hybrid parents. According to the Mendelian theory of heredity a part of each generation of hybrids should resemble the first generation, while the remainder should show other combinations of the parental characters. In typical Mendelian hybrids the contrasted parental characters are supposed to have entire freedom of chance combination in the second and later generations.

In reality there does not seem to be such complete freedom of combination of the two sets of characters that represent the two parental types. Plants that have the Hindi foliage, or that of the Hindi-like hybrid type, invariably have the white petals of the Hindi cotton.

[a] Cotton Selection on the Farm by the Characters of the Stalks, Leaves, and Bolls, Circular No. 66, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1910.

White flowers always have the more open, cuplike form of the Hindi cotton instead of the longer and more tubular form of the Egyptian cotton. It very rarely if ever happens that any single Hindi character is brought into definite expression by itself—that is, without being accompanied by the more or less definite expression of other Hindi characters. It is hardly to be supposed that any of the Hindi plants, any more than the Egyptian, are pure bred in the sense of having had no Egyptian ancestors, and yet the Hindi type is nearly as uniform as the Egyptian, in spite of all the selection that has been directed against it. Neither is it reasonable to assume that all of the pronounced hybrid plants have the same proportions of Hindi and Egyptian blood, though they form nearly as definite a group as the parent types.

Hindi-like lint and seeds sometimes occur on plants that give little or no external evidence of Hindi contamination, but plants that have previously shown Hindi leaves or flowers very seldom, if ever, have typical Egyptian bolls or lint of good Egyptian quality. In a field of Jannovitch cotton raised in Arizona in 1909 from imported Egyptian seed numerous individuals were found that seemed, early in the season, to depart from the normal Egyptian type only in the lighter and more pinkish tinge of the purple spot at the base of the petals. But when these plants were examined again in the fall it was found that the bolls and lint also departed from the type of the variety. All the pale-spotted individuals had small bolls, and some of them showed naked seeds and short Hindi-like lint.

That the depth of color of the petal spot can be, in itself, a matter of any direct significance in the economy of the plant is hardly to be believed, but it seems to have an indirect significance as indicating a tendency for the Hindi or other abnormal characters to come into expression. White petals may be considered in the same way as evidence of a still stronger tendency to express the Hindi characters in the parts to be subsequently formed. Very pale yellow flowers were noticed on a few Egyptian-like plants at Mansurah, but in nearly all cases a departure from the normal Egyptian color involved a complete change to the creamy white of the Hindi flowers.

Although white Hindi-like flowers are rarely to be found on plants that have produced Egyptian foliage, such sudden changes in the expression of the characters do not appear to be normal phenomena of heredity, at least in cotton hybrids, for plants with these incongruous combinations of characters are generally infertile and sometimes completely sterile.[a]

[a] Mutative Reversions in Cotton, Circular No. 53, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1910, p. 6.

Coherence of characters is not confined to Hindi hybrids, but apparently has to be reckoned with in any attempt to combine the characters of different types of cotton. The phenomenon was first recognized and described in the study of Egyptian-Upland hybrids in Texas and Arizona. It differs from correlation in affecting whole groups of characters instead of only two or three. Thus a general correlation may be said to run through many different types of cotton—between the shape of the boll and the length of the lint or between the color of the lint and its strength. Correlation refers primarily to the fact that certain characters tend to vary together, one increasing or diminishing in relation with another. The fact that the weight of ears of corn increases with their length is reckoned as a correlation. Coherence refers to the expression of characters in hybrids. It denotes a condition in which characters derived from the same parent remain together in expression instead of being expressed in chance combinations as in Mendelian hybrids.

Correlations often appear entirely arbitrary, unless they are merely mathematical expressions, as in the case of the corn ears. From the mathematical standpoint it seems impossible to understand why long fibers should not be packed into round bolls as well as into pointed bolls or why brown fibers should not grow as long as white fibers. But after the tendency to coherence of much larger groups of characters has been recognized as a fact correlations appear somewhat less mysterious. The general association of longer lint with more pointed bolls in any particular type of cotton may be connected with the other general fact that the long-linted types of cotton have more gradually tapering bolls than short-linted types of cotton. Coherence implies that the expression or nonexpression of one character may determine whether other characters shall be patent or latent.

A striking example of coherence of characters was observed in Egypt in a block of hybrids made by Mr. F. Fletcher, director of the School of Agriculture at Gizeh, between an American Upland variety called Jackson's Limbless and an Egyptian variety called Voltos, somewhat similar to Nubari, Voltos being the male parent. In addition to many other courtesies of hospitality Mr. Fletcher most generously insisted upon a full use of his interesting series of experimental plantings of cotton at Gizeh, which yielded many interesting facts with special relation to problems of diversity.

Instead of the usual tendency of some of the Egyptian traits to predominate in the first generation, this lot of hybrids showed an unusually definite expression of the Upland characters. Very few of the plants would have been taken for Egyptian cotton, even on casual examination, and none of them showed any close approximation to the Egyptian type. On the other hand, a considerable proportion of the plants adhered very closely to the characters of the

Upland type. Several of these were distinctly clustered and some were quite limbless, like the Upland parent, though the majority did not have the shortened internodes.

Coherence of characters was shown very conspicuously in the fact that all of the definitely clustered or limbless plants had the Upland type of foliage, all were quite hairy, and all had white petals, as in Upland cotton. The only definite mark of hybridization on several of these plants was the purple spot at the base of the petals. When the purple spot was lacking there was no definite evidence of hybridization, but some plants that would have been taken for pure Upland in all other respects had very faint spots, showing that they were hybrids.

There was no complete dominance of the yellow flower color as reported in some Egyptian-Upland hybrids. None of the yellow flowers were as yellow as those of Egyptian cotton. All of the yellow flowers had pale-purple spots at the base of the petals. Some of the white flowers had spots as dark as any of the yellow flowers. In this respect the hybrids may be said to afford an example of the Mendelian law of free combination, but these variations occurred in the first generation, where Mendelian crosses are expected to give more uniform results.

Another lot of hybrids produced by Mr. Fletcher by fertilizing an Upland cotton from Cochin China with pollen of the Voltos variety of Egyptian cotton showed quite a contrast in comparison with the preceding series. Nearly all of these plants looked like ordinary first-generation Upland-Egyptian hybrids, except one that showed only Upland features. But the white petals had small purple spots as an evidence that the plant represented a true hybrid, not merely a result of accident in manipulation. The plant was very hairy and the leaves and bolls showed no departure from Upland characters. All other plants of the cross had pale-yellow flowers, and all the flowers had the spots pale, sometimes entirely wanting. The spot character would have to be reckoned as nearly recessive, but not quite completely so. Two plants were found in the same lot that might have been taken for ordinary Egyptian individuals, unless it were for too much hair, but one plant was more hirsute than the other, especially on the under side of the leaves, where the stellate hairs developed into noticeable tufts. This also must be taken as a sign of hybridity. The other plant was somewhat abnormal, in that it produced several sterile involucres composed of only a single bract.

In a third lot of hybrids between the Voltos variety of Egyptian cotton as the female parent and the Cochin China Upland as the male there were several more plants of a complete Upland type. Three of these plants had been grown from fuzzy seeds that appeared in the Voltos cotton, an indication that the variety was not pure.

The habit of these plants was much like the Cochin China parent and also closely similar to that of the Rabinal and Pachon varieties of Upland cotton from Central America. The plants were very hairy and the bracts were unusually well closed, as well or better than in the Rabinal cotton, and being also larger they remained closed to a more advanced stage. This character of the closed bracts was also shown among the hybrids. It was fully expressed, or even intensified, in some of the plants that had yellow flowers and other unmistakable evidences of hybridity. Well-closed hairy bracts have value as a weevil-resistant character, since they exclude the insects from the young buds.[a]

The phenomenon of coherence of characters is not only of interest from the standpoint of the scientific study of heredity, but is of distinct practical importance in relation to the problem of developing and maintaining uniformity in cultivated varieties. It represents on the one hand a limitation of the power of the breeder to make free combinations of the characters of different species, as in ordinary Mendelian hybrids, but on the other hand it assists in maintaining the uniformity of established strains and guarding them against contamination. If there were no coherence in the expression of the characters any Hindi character could come into expression independent of any other. The work of selection would involve a detailed inspection of each plant by all of its characters and would require an amount of time that would make it entirely impracticable as a farm operation, even though the farmer should acquire the necessary skill. In short, it is the fact of coherence of characters that lends value to selection, that makes it possible by roguing to improve or maintain the quality of the crop.

The success of the Egyptian method of securing commercial uniformity by matching the color of the fiber rests also on the fact that variations in the color of the lint are not independent of other characters. The inferior lint of the Hindi plants and hybrids does not have the same color as the lint of Egyptian plants. If there were no coherence of the Hindi characters the brown color would be found in combination with the naked seeds and short lint of the Hindi type, but this seems never to occur.

Recognition of the principle of coherence calls attention to the practical fact that plants seldom make serious changes in the expression of one character without showing changes of expression on other characters. The plants that produce the inferior lint in the fall are those that have departed from the regular courses of development earlier in the season. Indeed, these departures from normal heredity

[a] Weevil-Resisting Adaptations of the Cotton Plant, Bulletin No. 88, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1906.

can usually be recognized much more readily by inspecting the vegetative characters of the plants in the earlier stages of development than after the crop is ripe and the damage of cross-fertilization has been done. It takes only an instant to see that the foliage or the habits of growth of a plant are different from those of its neighbors, much less time than is required to judge plants by their lint and seed characters at maturity, after the external differences of leaves, flowers, and bolls are no longer to be appreciated.

The breeder in search of new varieties may find it desirable to preserve all the sports or freak plants that he can find to see whether in some rare cases they may not prove superior to normal plants of the variety, but the farmer who follows this course will lead his variety to degeneration. He must rely on the fact that the vast majority of the plants that diverge from the characters of the variety represent degenerations. His policy is to pull all the aberrant plants as soon as they can be detected. If allowed to remain, they will destroy the uniformity of the stock.[a]

INTENSIFICATION OF CHARACTERS IN HYBRIDS.

Another deviation from the Mendelian expression of characters in cotton hybrids is found in cases where characters are suppressed or intensified beyond the range of variation of the parental types. The crossing of the Egyptian cotton with short-staple Upland varieties

[a] A writer in the Liverpool Daily Post and Mercury (Saturday, March 12, 1910) maintains that periods of prosperity for the Egyptian cotton industry have followed the introduction of new varieties and that periods of depression ensued as the varieties degenerated:

"It is to be remarked that each time a new variety of seed was sown for the first time of cultivating an increase was immediately obtained of 1 to 1½ cantars weight per feddan, and as high as 12 to 14 per cent in the ginning yield. This increase diminished with the passing years and by slow degrees the seed degenerated. The excellent results of the beginning did not bear out their early promise, and after a lapse of time of more or less duration the seed cultivated had to be abandoned to give place to a new variety. * * *

"And it is the same story. As in 1862, when the Jumel, old and degenerated, had to be abandoned, as in 1892 the Ashmuni had to be replaced by Mitaffifi, so to-day the Mitaffifi seems coming to the end of its career, and no one can deny the degeneration of quality.

"While in 1891, 1892, and 1893 it yielded 7 to 8 cantars per feddan on the best lands and 5 to 6 on the others, at the present day it never gives either 7 or 8 cantars, and in Lower Egypt its production has certainly diminished by 1 to 1½ cantars per feddan on an average. This cotton, which during the first years of its cultivation yielded 110 to 114 in ginning, no longer gives to-day more than 101 to 103, and that with difficulty. * * *

"Seventeen years, therefore, had sufficed for the degeneration of Jumel, and it is exactly after the same lapse of time that we are forced to notice the degeneration of Mitaffifi."

usually results in a favorable intensification of the lint characters in the first generation. Notwithstanding the inferiority of the lint of the Upland parent, the lint of the hybrid is usually longer and stronger than that of a pure Egyptian progeny grown under the same conditions.[a]

A form of intensification occasionally shown in Egyptian-Upland hybrids is an unusual development of the nectaries. An excellent example of this was found in an aberrant plant at Calioub, July 12, 1910. It was probably a Hindi hybrid, though showing no pronounced Hindi characters. It was much taller than its neighbors and had unusually long basal internodes on the fruiting branches, while the other internodes were short and imperfect. Many buds had aborted and no bolls had been set. Each of the involucres that remained on the plant, 15 in number, had a large nectary on each of the three bracts.

In order to give a more definite indication of the extent of intensification shown by the nectaries of this plant, notes were made of the occurrence of nectaries on the involucral bracts of six adjacent plants, one of which happened to be Hindi. The lower buds of the Egyptian plants were generally without nectaries, unlike the Hindi plant which had nectaries on the early as well as on the later involucres, though with no such regularity as in the aberrant plant, to say nothing of the much larger and more regular size of the nectaries of the aberrant plant. Table III shows the distribution of nectaries on all the involucral bracts of the Egyptian and Hindi plants. Bracts with large nectaries are indicated as "N," those with small nectaries as "n," those with no nectaries as "o." No nectaries as large as those of the aberrant plant were found on any of the neighboring Egyptian and Hindi individuals. Several other plants were examined in addition to those that were definitely counted. One of the Egyptian plants had an involucre with only two bracts, a not uncommon occurrence.

[a] Suppressed and Intensified Characters in Cotton Hybrids, Bulletin 147, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1909.

TABLE III.—*Census of nectaries of Egyptian and Hindi cotton plants.*

Adjacent plants.			Aberrant plant.	Adjacent plants.		
Egyptian.	Egyptian.	Egyptian.		Hindi.	Egyptian.	Egyptian.
0 n 0	0 n n	0 0 0	N N N	0 0 0	0 0 0	0 0 0
0 0 0	0 0 0	0 0 0	N N N	0 0 0	0 0 0	0 0 0
0 0 0	n n n	0 0 0	N N N	0 n n	0 n 0	0 n 0
0 0 0	n n n	0 0 0	N N N	n n 0	0 0 0	0 0 n
		0 0	N N N	n n 0		n 0 0
		0 0 0	N N N	0 0 0		0 n n
		0 0 n	N N N	0 n 0		0 0
		0 n n	N N N	0 0 n		0 0 0
		n n n	N N N	n 0 n		0 n n
		0 0 n	N N N			
		0 n 0	N N N			
			N N N			
			N N N			
			N N N			
			N N N			

Another example of a notable departure from parental characters was shown in a block of hybrids produced by Mr. Fletcher by crossing two Egyptian varieties. The whole block showed a remarkable susceptibility to a disease of the roots similar to the wilt of the United States. The whole block of plants was notably different in behavior from either of the three other blocks of hybrids that inclosed it on three sides; the other side bordered on a roadway. All of the plants were small, with a very open habit of growth, and their foliage was tinged with red. Many of the roots were dead or dying and had changed to a grayish-brown color. The contrast between this block and its neighbors was very distinct out to the square corners, with the larger and more healthy plants on either side.

Microscopical examination by Mr. Fletcher found the fibro-vascular bundles of the roots stuffed with fungous mycelium. There seemed to be no escape from Mr. Fletcher's view that this particular stock of hybrids was unusually susceptible to the disease in comparison with the surrounding stocks. The peculiarity may have come, of course, from one of the individual plants that happened to be used as parent of the cross, but this does not diminish the value of the evidence that some members of the Egyptian type may have marked susceptibility to the disease. Mr. Fletcher has noted other indications of such susceptibility and is inclined to believe that the

disease may be an unrecognized cause of much damage to the crop. It appears that the symptoms are generally more pronounced on land that had cotton the year before, but the observations have not extended far enough to establish this point.

RELATIONSHIPS OF HINDI AND EGYPTIAN COTTONS.

The Egyptian cotton in the United States is exposed to the additional danger of crossing with the American Upland type of cotton. It is quite as important to guard against this danger as to exclude the Hindi contamination that has caused so many difficulties and losses in Egypt.

Experiments indicate that the result of allowing the Egyptian cotton to be crossed with Upland pollen will be much the same as with the Hindi, and this is also to be expected from the fact that the Hindi cotton shares many of the characters of Upland cotton, and especially those of some of the types of Upland cotton that have been discovered recently in southern Mexico and Central America.[a]

Though differing in minor details, there is a general agreement between the American Upland types of cotton and the Hindi in the habits of growth, the form, color, and textures of the leaves, involucres, and flowers. The external characters of the bolls are also much the same. The principal difference lies in the character of the seeds. In the American Upland cottons the seeds are generally covered with a dense coat of short fuzz, though some of our varieties show frequent variations in the direction of naked seeds, like those of the Hindi cotton. Indeed, there are occasional variations where the lint and the fuzz are both lacking, showing that the seed characters of the Hindi cotton lie within the range of variation of the Upland type. Thus if the parentage of a hybrid plant is not known it may be impossible to determine whether it represents the Hindi contamination or an Upland cross. In general it may be assumed that plants with hairy stems and leaves represent Upland hybrids rather than Hindi, for the typical Hindi cotton is not hairy. Yet a few hairy Hindi-like plants have been found in Egypt as well as in plantings of imported seed in Arizona.

From the standpoint of the study of heredity it would be very desirable to determine when the Hindi contamination of the Egyptian cotton took place. The Hindi variations may represent a recent admixture or the crossing may have taken place so far back as to represent a general constitutional tendency to reversion pervading the whole Egyptian type. The idea that the Hindi cotton grew as a wild weed in Egypt would allow us to suppose that the process of

[a] Origin of the Hindi Cotton, Circular No. 42, Bureau of Plant Industry, U. S. Dept. of Agriculture.

contamination had been continuous, with some new crosses every year to replace those that were removed by selection. But the idea of wild cotton in Egypt and also the theory founded upon it seem altogether improbable. The sources of the Hindi contamination must apparently be sought farther back.

Another possibility is that the Hindi cotton was formerly cultivated in Egypt before the present so-called Egyptian type was introduced and that the mixing occurred while the Egyptian cotton was replacing the Hindi. A difficulty with this idea is that the lint of the Hindi cotton is so sparse and short as to make its cultivation seem improbable. But it is possible that Hindi plants now appearing as reversions among the Egyptian cotton do not fully represent the possibilities of the Hindi type in the direction of lint production. While there is a general tendency to sparse lint among naked-seeded types of cotton, this is not universal. A strain of Caravonica cotton grown in Hawaii has very abundant lint, in spite of the fact that the seeds are entirely devoid of fuzz, as shown by samples recently deposited with the Department of Agriculture by Dr. E. V. Wilcox, Director of the Hawaii Agricultural Experiment Station. Mr. Fletcher has recent information indicating that Hindi cotton is still planted as a crop in Mesopotamia under the same name as in Egypt. Plants grown at Gizeh by Mr. Fletcher from seed received from Mesopotamia were carefully examined and seemed to show all the essential characters of the Hindi cotton. (See Pl. III.) It is possible, therefore, that the Hindi admixture may be traced by way of Mesopotamia.

The idea that the Mediterranean countries were limited to Old World types of cotton (*Gossypium herbaceum*, and its relatives *indicum*, *arboreum*, etc.) even in ancient times may prove to be erroneous. In southern Italy an Upland-like cotton is cultivated under an ancient name "*bombage*," evidently cognate with the Greek "*bombax*." The plants are quite small and somewhat hairy, like American Upland cotton, but the bracts are very strongly toothed after the Hindi fashion.

In this connection it may be well to mention the fact that a sample of seed of brown, rough-fibered cotton has recently been received from northern Arabia by the United States Department of Agriculture. While these seeds and lint do not closely resemble those of any recognized variety, they show more of an approach to the Egyptian qualities than any samples previously seen from the Old World. Another small sample of seeds and lint, received about the same time from Honduras, has a much closer resemblance to the Egyptian cotton and is stated to represent a native tree cotton. These seeds have the size and shape of Egyptian seeds with tufts of brownish

fuzz at the ends, and the lint is similar to that of the Egyptian cotton, whereas the seeds from Arabia are covered with a brown fuzz.

While at Gizeh there was also opportunity, through the kindness of Mr. W. Lawrence Balls, botanist of the Khedivial Society, to see living plants of a kidney cotton raised from seed brought from the Niam-Niam country in the upper valley of the White Nile, a type considered by Mr. Balls as representing one of the parents of the Egyptian cotton. It has to be admitted that these plants show a notable agreement with the Egyptian cotton in many respects and are quite unlike any of the varieties of kidney-seeded cotton that have been seen in Mexico and Central America or received from those countries.

The Niam-Niam cotton has three external nectaries present with great regularity, reniform-cordate in shape, and usually distinctly emarginate on the upper side. The nectaries are always of a red color, at least on these well-exposed plants. Inner nectaries are also present with much regularity, are broadly V shaped, and often colored red. The surfaces of the nectaries are rather coarsely granular-papillate and without hairs. Cases of supposed intensification of nectaries in Egyptian hybrids might be considered as reversions to such an ancestor as this.

The leaves vary from entire to 5 lobed, the latter usually on the rank growth of new shoots. Occasionally there are 6 or 7 lobes, but the additional lobes are usually small. The leaves are of the Egyptian form and color, somewhat more hairy than usual in Egyptian cotton, but the hairs are short, as in some variations of the Egyptian type. The pulvinus and veins are green or tinged with dull reddish, as in Egyptian cotton. The pulvinus is very hairy and not enlarged, but the outer pairs of veins show an occasional tendency to unite at the base. There are 1 to 3 leaf nectaries, those of the midribs being sagittate.

The stipules of the main stalk and vegetative branches are long and slender as in rank-growing Egyptian cotton, while those of the fruiting branches are unequal, one narrow and the other broad, the latter often with two teeth.

The bracts are usually connate at their base for one-eighth to one-fourth inch, as often occurs in Egyptian cotton. The calyx has very distinct, broadly rounded lobes (Pl. V, *C*), more prominent than is usual in the Egyptian cotton but nearly equaled under some conditions, as in the Egyptian cotton grown near Los Angeles in the season of 1909.

The plants at Gizeh were quite woody and about 10 feet high, and had no tendency to produce elongated fruiting branches. Only one flower was borne on each fruiting branch. The pedicels of the flowers

were very short and subtended by a small leaf, usually with one stipule very much enlarged and often toothed, somewhat like an involucral bract.

One of the most striking peculiarities in which the Niam-Niam cotton agrees with the Egyptian is the tendency to enlargement of one of the stipules of the leaves of the fruiting branches. It has been noticed in Arizona that abnormally large strong-growing plants of Egyptian cotton often have this tendency very pronounced, a fact suggestive of the possibility that such plants may represent reversions toward an ancestral form similar to the Niam-Niam cotton. The unequal development of the stipules has been considered in relation to Hindi hybrids, but such a tendency does not seem to be as pronounced in the Hindi hybrids as in the Egyptian cotton and in this African relative. Enlarged stipules are especially likely to be found in Egyptian cotton on leaves of short branches produced from the fruiting branches and may be connected with the tendency of such branches to produce organs intermediate between the ordinary leaves and the involucral bracts.

While the Niam-Niam cotton must certainly be considered in the study of the relationships of the Egyptian cotton, it seems more likely to prove a collateral relative than a direct ancestor. It is very difficult to believe that the Egyptian cotton descended from a kidney-seeded ancestor or from one that had the fruiting branches so shortened and specialized as the Niam-Niam cotton.

The most significant thing regarding these cottons from Mesopotamia and central Africa is that they may add something to the evidence of the existence of genuine Old World varieties of the Upland type of cottons. The Upland variety from Cochin China recently brought forward by Mr. Fletcher as an ancestor for our American Upland cottons is also very interesting from this standpoint.[a]

As seen growing at Gizeh the Cochin China cotton shows a remarkable resemblance to some of the Central American varieties and especially to two types from the Central Plateau and the Pacific slope of Guatemala, those that have been described as Pachon and Rabinal. The Guatemalan Upland cottons and other related types from southern Mexico show very close agreements with the Hindi cotton in so many of the characters that a rather close relationship must be supposed to exist. This renders the close resemblance of the Cochin China cotton to the Central American varieties all the more interesting.

The Cochin China cotton shows in Egypt the same bushy habit of growth with many upright vegetative branches as the Central Ameri-

[a] Fletcher, F. The Origin of Egyptian Cotton, Cairo Scientific Journal, vol. 2, no. 26, November, 1908.

can Upland cottons when first brought to the United States, though not carried to quite the same extent under the less extreme Egyptian conditions. The stems, leaves, and involucres are densely hairy as in the Central American cottons. The bracts also have the margins hairy and very firmly appressed in the same way as in the Central American cottons and perhaps to an even greater extent.

The lobes of the calyx have the same tendency to grow into long teeth (Pl. V, A), and the bolls have the same conic-oval, abruptly apiculate form which several of the Central American varieties share with the Hindi cotton. In short, the resemblance seems so complete that if the Cochin China cotton had been found in Central America it would have been considered as only one more of the relatively slight local variations shown by the general type represented by the Rabinal and Pachon varieties. The most notable difference was an apparent absence of bractlets, but this condition could probably be found on second-year wood in the Central American varieties. While the Cochin China cotton, like the Central American varieties, appears to be a relative of our American Upland cottons, there are native Mexican varieties that seem to be still more closely related to some of our United States Upland varieties. Yet it is not impossible that Mr. Fletcher's idea of tracing the Cochin China cotton to the United States through an early introduction of so-called "Siam cotton" may turn out to be true of our long-staple Upland type still grown in Louisiana.

If the Cochin China cotton were more nearly identical with our United States Upland cottons it might be looked upon as an introduction from the United States, but it is much less likely that a local Central American variety has been carried to Cochin China. The information of Mr. Fletcher's correspondent, that this cotton was really indigenous in Cochin China, may therefore be credited.[a]

While the existence of these additional relatives of the Egyptian and Hindi types of cotton in the Old World does not affect the evidences of relationship that have been pointed out between these types of cotton and others that appear to be natives of America, it does have a bearing upon the question of how these members of American types of cotton reached the Old World. If many sorts like the Hindi, Egyptian, Niam-Niam, and Cochin China cottons are found in different parts of the Old World it will not be reasonable to believe that they represent recent importations from America, since the time of Columbus. It will be necessary to consider the possibility that American types of cotton, like the coconut palm, sweet potato, and

[a] Fletcher, F. The Botany and Origin of American Upland Cotton. Cairo Scientific Journal, vol. 3, no. 38, November, 1907, p. 263.

other economic plants of American origin, were carried across the Pacific Ocean in prehistoric times.[a]

If our long-staple varieties of Upland cotton originated in the East Indies it is reasonable to expect that other superior types of Upland cotton may be found in that part of the world. Indeed, Mr. Fletcher's Cochin China cotton seems to be a promising type, worthy of attention from the standpoint of acclimatization. The bolls are larger than in our long-staple Upland varieties and the lint is of good length. The very large and well-closed hairy involucral bracts would have value from the standpoint of weevil resistance, like the similar bracts of the Central American varieties which exclude the boll weevils from the young buds, as already noted in describing the hybrids of the Cochin China cotton.[b]

SUPPOSED INCREASE OF HINDI COTTON.

The popular belief in Egypt is that the proportion of Hindi cotton is increasing, though there seems to be no way to obtain definite information on this point. Intelligent natives declare that they

[a] Food Plants of Ancient America, Smithsonian Report 1903, pp. 481–497.

Agricultural History and Utility of the Cultivated Aroids, Bulletin 164, pt. 2, Bureau of Plant Industry, U. S. Dept. of Agriculture, 1910.

History of the Coconut Palm in America, Contributions from the United States National Herbarium, vol. 14, pt. 2, 1910.

[b] The successful cultivation of a so-called "Cambodia" cotton in British India has been noticed in a recent Consular Report, issued while this bulletin was in preparation. The facts are of special interest in view of the many unsuccessful experiments that have been made in India with Upland varieties from the United States. The statement is as follows:

"In Tinnevelly district, Madras Presidency, at the extreme southern end of the peninsula, there had been planted up to October about 17,000 acres in what is known as Cambodia cotton. This is a variety of acclimatized American cotton, introduced into the country about four years ago, which is being quite successfully grown and which yields far more fiber per acre than any of the old varieties.

"Last year a total of 15,000 bales of Cambodia was produced on 15,000 acres of the black soil of Tinnevelly, and this season, in addition to the larger area already reported as planted in that district, the agricultural department is experimenting with it in several other parts of the Presidency with a view to its general adoption by growers. It is said to thrive on irrigated lands, and should it prove even partially as successful in other districts as in Tinnevelly, there is little doubt that within a very few years it will be grown throughout the whole of south India, if not elsewhere in the country.

"As the fiber of the Cambodia compares favorably with that of American Upland cottons, it is not too much to say that India may within a few years become a serious competitor of the United States in meeting the world's demand for the commodity, instead of furnishing only the inferior grades as at present." (*Report of Nathaniel B. Stewart, consul at Madras, India, in Daily Consular and Trade Reports, December 17, 1910.*)

remember when the Mit Afifi or the Jannovitch varieties produced fields of uniform plants, all of the same height, with none of the irregularities now shown by the tall hybrid plants of the Hindi-infested fields. But in the absence of any actual countings in former years it is not possible to determine what change has taken place.

From the standpoint of the Mendelian theory of heredity an increased representation of the Hindi characters would not be expected to occur unless additional contamination took place from outside sources, which appear to be lacking in Egypt. Mathematicians have shown that characters expressed according to the Mendelian theory would not tend to increase, but would remain at the same general proportion in a mixed population.[a]

Nevertheless, an increasing dominance or stronger tendency of expression of the Hindi characters should not be dismissed as impossible, for it has been noticed in experiments with Egyptian-Upland hybrids that the Upland characters seem to attain a more and more predominant expression in the later generations, even when selections are made with a view to preserve the Egyptian or intermediate characters among the hybrids. Though no direct statistical evidence regarding the supposed increase is likely to be obtained, it may be possible to throw light on the question indirectly by the study of the tendencies of expression shown in artificial hybrids between the Egyptian and Hindi types. Experiments of this kind were begun by the making of such hybrids in Arizona in the season of 1909.

The popular impression of a gradual increase in the proportion of Hindi cotton is supported by the general opinion of the commercial world that the quality of the Egyptian cotton is declining. This may mean that poorer qualities are being sent out under the same marks or that the ginning establishments are finding it more difficult to keep their product up to recognized standards. Either of these results, or both, might naturally be caused if the Hindi cotton continues to multiply in the face of the selection that is now being applied.[b]

Considered on a percentage basis, a considerable amount of selection has undoubtedly been directed against the Hindi cotton. In

[a] Hardy, G. H. Mendelian Proportions in a Mixed Population. Science, n. s., vol. 28, p. 48, July 10, 1908.

[b] The idea of a progressive deterioration of the Egyptian product is confirmed by a recent authoritative statement published while the present report was in preparation: "There is no gainsaying the unanimous evidence that the general character of Brown Egyptian cotton [by which Lancashire means Afifi] has gone down most markedly from the standard of 15 years ago. All the spinners of fine counts, to whom strength is everything, speak with regret of the Afifi of those days. Without exception they say that during recent years they have continually been compelled, in order to maintain their standards of strength,

the Delta region a large proportion, probably 50 per cent or more, of the Hindi plants that germinate in the fields are rogued out. The sorting of the fiber in the ginhouses must take out a still larger percentage of the Hindi cotton that is harvested.

Some of the ginners are also said to sift out the smooth seeds, or even to resort to hand picking to keep the smooth Hindi seeds from being planted. While it is to be expected that the various ginning establishments would be found to differ greatly in the thoroughness with which these precautions are observed, the general effect must be to exclude a large proportion of the Hindi seed every year. Under any Mendelian rule or other customary idea regarding the effects of selection it might be expected that the expression of the Hindi characters would have declined long since to a negligible quantity, but the facts certainly do not correspond to this expectation. The result demonstrates instead that the system of selection now in operation is entirely inadequate to eliminate the Hindi variations.

As already noted in connection with the seed characters of the Hindi cotton, the tendency to an increased representation of this type is not limited to the factor of prepotency, but may prove to be due partly or wholly to more prompt germination of the seeds, owing to the absence of fuzz that allows more effective contact with the soil. Experiments with other types of cotton have shown that varieties having less fuzz germinate more promptly, but comparisons will also be made between Egyptian and Hindi.

ESTIMATE OF DAMAGE FROM THE HINDI CONTAMINATION.

As the percentages of Hindi cotton in the Egyptian fields do not represent the full amount of Hindi contamination, so they do not indicate the full extent of damage to the crop. In addition to the true Hindi plants and the obviously Hindi-like hybrids, supposed to represent the first generation, more careful inspection always shows a considerable number of obscure or dilute hybrids as well as many individual variations that may reasonably be ascribed to the same general fact of Hindi contamination. These aberrant plants include those that show the white flowers, the flowers with pale spots, and other peculiarities that can often be detected only by

to raise the mark or grade of cotton they use, and to add increasing proportions of superior varieties, such as Nubari and Jannovitch, merely to obtain the same results as they formerly secured with Afifi alone. Strength is absolutely essential in the manufacture of 'twist' yarns for warping, and in spite of improved spinning processes, greater loss in waste through taking out a larger proportion of short staple, and more careful and costly methods generally, the spinners have had the greatest difficulty in maintaining the quality of their yarns." (See Todd, John A., "The Market for Egyptian Cotton in 1909–1910," L'Egypte Contemporaine, no. 5, January, 1911, p. 5.)

careful comparison of all the parts, including the seeds and lint. A complete census of the aberrant plants of a field requires too much time to make it generally feasible. Moreover, the cotton in Egypt was not yet far enough advanced in July, 1910, to allow such a study to be completed. The visit was made at that season because the vegetative characters of Hindi plants were known to be more readily visible at that time.

Counts made in a field of Ashmuni cotton raised in Arizona in 1909 from imported seed gave over 40 per cent of the plants showing distinct departures from the normal characteristics of Egyptian cotton, mostly in the direction of the Hindi. A similar diversity would probably be found in some of the Egyptian fields representing the same variety of cotton. With the better varieties such as Mit Afifi and Jannovitch the percentage of dilute hybrids and variants, as of true Hindi and obvious hybrids, is doubtless considerably less though by no means a negligible quantity.

It would probably be well within the truth to estimate that the results obtained by counting would at least be doubled if they were to include the later generations of hybrids and dilute crosses that increase the diversity and diminish the value of the crop. If the average of the percentages shown in the different countings of Hindi plants be accepted as the basis of calculation, a total estimate of about 12 per cent would represent the extent of the Hindi contamination that would become visible under a more careful inspection of the Egyptian fields. Estimated even at 10 per cent, the annual damage of the Hindi cotton must run well above $10,000,000, perhaps even to twice that amount. It is true, of course, that any definite figures must be in the nature of guesswork; they can serve only in a general way to indicate the magnitude of the factor of diversity in the Egyptian cotton crop.

While the cotton of the Hindi and other variant plants is not altogether worthless, there can be no doubt that the crop as a whole would be far more profitable to the farmer if all these plants were destroyed, even though nothing took their places. A general diminution in yield is due to the infertility of many of the hybrids and other aberrant plants; a general depreciation of the value of the crop is due to the residuum of inferior cotton that the sorting does not remove, to the expense of the sorting, and to the relative waste of labor in growing and picking the low-grade cotton. These elements of loss recur with every season and represent a large tax upon the industry. They also represent roughly the advantage that American farmers may hope to gain by paying more effective attention to the factor of selection as a means of maintaining the purity and productive efficiency of varieties.

OTHER CAUSES OF DETERIORATION OF THE EGYPTIAN CROP.

While an increase of the proportion of Hindi cotton would explain a reduction in the yield as well as in the quality of the crop, it is probable that other causes are responsible for a share in the decline. Indeed, some writers on the subject, overlooking the Hindi factor, have used considerable ingenuity in imagining other causes of deterioration and are calling for radical measures of reform to check, if possible, the downward tendencies. Statistics indicate a general decline in production at the rate of about 100 pounds of lint per acre during a period of about 12 years. Such a reduction is a very serious matter from the standpoint of the native cultivator who operates on a very small piece of land at a very high rental. Even when the tenant has to pump his own irrigation water his rent may run at the rate of $40 or $50 per acre. Under favorable conditions a return of $100 may be secured, but the margin is often very narrow, only $5 to $10 for a season's work.

In spite of the decline in yield, the increase of the area of production by new irrigation works may maintain or even increase the total output of the country as a whole, though it is evident both in Lower and Upper Egypt that the extension of cotton into newly reclaimed areas is likely to be a very gradual process attended by considerable difficulties. Other possibilities of extensive cotton production are said to exist in the Egyptian Sudan, where many efforts for agricultural progress, including large projects in irrigation, are now being made.

One of the favorite theories to account for the lessening yields of cotton is that the varieties have run out. This theory may be true in the sense already discussed, that of deterioration due to hybridism and resulting diversity, but it is probably not true in the sense that is commonly supposed, that the varieties have weakened and declined in vigor and fertility. With plants long propagated from cuttings, such as strawberries and potatoes, it is believed that old varieties become weaker and less resistant to disease after a period of a few decades, but with open-fertilized, seed-propagated plants like the cotton, the idea of varieties running out is not considered as having received any adequate demonstration. Some of the native cultivators declare that all the plants used to grow as large on their land as the tall hybrids do now and that they were fertile in proportion to their size, but such a difference might be due to a decline in the fertility of the soil as well as to a deterioration of the variety.

The tradition of perpetual fertility of the Egyptian soil, annually renewed by the sediment deposited by the flood of the Nile, does not apply to the cotton lands, for this crop is raised on an entirely different system having no relation to the agriculture of

ancient Egypt. Ancient Egypt depended on winter and spring crops that could be grown during the intervals between the summer inundations, but cotton requires the whole warm season, spring, summer, and autumn. It has to be irrigated in the spring before the floods come and is harvested during the flood period. Cotton can be grown, therefore, only on land that is protected from the floods and provided with canals for perennial irrigation. The only Nile mud that comes to these lands is a very little in the turbid water of the later irrigations that are given to the cotton after the river rises. There is no deposit of mud from large volumes of water turned into basins and allowed to settle as under the old system of irrigation at flood time. Hence there is every reason to expect a gradual decline in the fertility of the cotton lands, a decline likely to be noticed first in the lighter and poorer soils but also likely to affect the others in time. Whether this decline has already become a serious factor in reducing yield might require a very careful investigation to determine, but it is very likely to be a contributing factor.

The use of fertilizers is already recognized as a serious question in relation to the cotton industry. As in the United States, natural and artificial manures are used with pronounced benefit on the poorer and lighter lands while the heavier soils show little or no response. The domestic supply of fertilizing material is greatly reduced by the natives in their universal use of the dung of domestic animals as fuel. Some writers have seen an evidence of agricultural efficiency in the making of such material up into cakes and hoarding it around the native houses, but the object is to cook the family meals, not to fertilize the land.[a]

A theory receiving much attention at present is that the decline of the cotton crop is due to a rise of the water table or level of the subsoil water in the soil, resulting from infiltration from canals and the use of larger quantities of water for irrigation purposes. While it is evidently true in Egypt, as in the United States, that too much water is bad for cotton, it hardly seems probable that the change of the water table has been sufficiently serious and general to be responsible for any very large part of the decline of the crop. The recent improvements of irrigation facilities are making it easy for the cultivators to injure their crops by using too much water, a tendency that seems to be very general in irrigated regions. Indications of such injury could often be seen in the fields. In some cases continued excess of water had evidently interfered with growth, so that the cotton of the water-logged fields remained very small. In other cases excess of water appeared to be responsible for too vigorous

[a] Foaden, G. P. Notes on Egyptian Agriculture. Bulletin 62, Bureau of Plant Industry, U. S. Dept. of Agriculture, pp. 26–33.

growth and late fruiting, with the probable result of a smaller crop. American cotton planters are familiar with the fact that too much rain often cuts down the crop by inducing additional growth near the beginning of the fruiting period. A whole crop of buds or young bolls may be shed that would have grown to maturity if the weather had continued dry.

Cotton growing on lands along permanent watercourses in the Zagazig district, where the water table must have been kept within a few feet of the surface, did not show any serious impairment except for a few rows along ditches or ponds that supplied water practically on the surface. The small size and pale color of one or two rows along the dikes often indicated serious injury by the close proximity to water, but usually there was a rapid improvement farther back. A recent publication gives the results of many investigations of water level in wells and concludes that the modern system of irrigation has had no serious general effect in raising the level of the subsoil water. On the other hand, it is pointed out that a secondary artificial water table may be formed when superfluous irrigation water collects over an impervious subsoil layer.[a]

Disease also may play a part in the decline of production. As pointed out by Mr. Fletcher, in the vicinity of Gizeh some of the fields of cotton show irregular patches of very inferior plants, with

[a] Ferrar, H. T. On the Creation of an Artificial Water Table in Egypt, Cairo Scientific Journal, vol. 4, p. 153, July, 1910.

The conclusions of this paper are stated as follows:

"It is reasonable to suppose that a small quantity of water has been retained by the alluvium each succeeding year, for it is not likely that a great augmentation of subsoil water would take place in a year or two, and in the absence of substantiated evidence we must assume that by degrees water has been accumulating in the soil since the introduction of perennial irrigation. Observations made in the provinces of Menufia and Gharbia have shown that at the present time (May 1) a layer of saturation may be found which is seldom more than two meters below the soil surface. The upper surface of this artificially saturated layer has been called the *artificial water table*.

"Some misapprehension exists with regard to the water which is found in the Nile alluvium and it will be of interest, therefore, to state tentatively two main conclusions drawn from observations made at more than 150 experimental tube wells which have been under observation during the past year. The observations made at these wells in Lower Egypt all support the view that there are two water tables:

"1. *A natural water table which is independent of the works of man, except locally where extra permeability allows a constant supply of irrigation water to be added.*

"2. *An artificial water table which was created by the act of the introduction of perennial irrigation by Mohammed Aly Pasha.* It is thought that *this artificial water table has gradually become higher, owing mainly to excessive watering of crops*, until at the present day it has a deleterious effect upon the fertility of the soil."

some dead and dying. On examination of the roots Mr. Fletcher found the fibro-vascular bundles stuffed with fungous mycelium as in the wilt disease of cotton in the United States. Samples of roots of cotton plants affected in the same way were also sent by Mr. Fletcher some years ago to Mr. W. A. Orton, of the Bureau of Plant Industry, but no definite identification of the disease could be made.

It has been supposed that the Egyptian cotton is resistant to the wilt disease, but that this resistance is not absolute seemed to be shown very clearly in one of Mr. Fletcher's experiments already noted. In a type of cotton practically resistant to such a disease a large amount of unrecognized damage might be done. Mr. Orton states that in the United States the wilt disease is responsible for much damage outside of the most seriously infested areas where the plants are killed.

PROSPECTS OF EGYPTIAN COTTON IN THE UNITED STATES.

Though it is to be expected that the Hindi contamination and other causes of decline of the cotton crop in Egypt will eventually be recognized and removed, there is no reason to expect any sudden or complete change in the present conditions. The yield and quality may be expected to fluctuate somewhat with the seasons, but such differences are likely to be less serious in Egypt than in almost any other country.

The Hindi cotton might be eliminated eventually if a better system of selection were applied or new and uniform strains could be developed and substituted for the present diverse stocks. More extensive fertilizing might counteract the diminishing fertility of the soil. Drainage works are being extended and improved methods of controlling insect pests are being applied. More hardy varieties may also be developed, analogous to the wilt-resistant varieties of Upland cotton bred by Mr. Orton in the United States.

But all of these measures are likely to require considerable periods of time, quite as long, indeed, as would be needed for the elimination of the Hindi, and this will give our newly established cotton-growing communities of the Southwest a fair opportunity to market their first crops, if they decide to undertake the production of Egyptian cotton on a commercial scale, instead of the short-staple Upland cotton they are now planting. One of the difficulties in establishing such an industry is that it needs to begin on a sufficiently large scale to provide the necessary ginning and baling facilities. Manufacturers are not willing to buy small quantities of cotton from a new region.

No assurance can be given, of course, that the present high prices of Egyptian cotton will be maintained for even a few years. The farmer will have to judge for himself whether the normal relations

of supply and demand are likely to continue and to have their normal influence on the prices. The present status of the Egyptian industry is only one factor of the problem, but the prospects in this quarter seem to favor the proposed establishment of an Egyptian cotton industry in the Southwest.

It need not be supposed that the culture of Egyptian cotton in the United States will involve an injurious competition with the Egyptian industry. The irrigated districts of Arizona and southern California where the experiments with the Egyptian cotton have been carried on are not very extensive, nor thickly populated. Settlement is going on in a very gradual way, as irrigation facilities are provided. Moreover, the opening of an additional source of supply of Egyptian cotton would be likely to improve the commercial prospects of this type of fiber. The danger is already recognized in Egypt that if prices remain too high markets may be lost by the further substitution of inferior kinds of cotton in fabrics for which Egyptian has been used.

Recently published results of an investigation of this question show that an extensive substitution of other types of cotton for the Egyptian has already taken place and that there has been a serious decline in some lines of Egyptian cotton goods as a result of improvements in the weaving machinery and finishing processes that make it possible to use cheaper materials not previously employed for such purposes. The plan of substitution seems to have succeeded beyond all expectations, as the following statements will show:

> It is in these lower grade goods that the substitution of American for Egyptian yarns has shown the most marked development. The substitution has taken place in various ways, but all due to the one cause—the great difference in price between American and Egyptian yarns. The high price of Egyptian cotton has compelled the spinners to devote their attention to producing a finer spun yarn from American staple than was formerly thought possible. Until a few years ago 40's were regarded as practically the limit of American spinning. Now by improved processes and the adoption of finer methods of spinning (e. g., combing, which was formerly confined to Egyptian yarns) 60's, 70's, and 80's of satisfactory quality can be spun from American. Though perhaps not equal in strength to the Egyptian yarns of the same count, these yarns have proved an excellent substitute in many branches of the trade. * * *
>
> The secondary difficulty of overcoming the dealers' prejudices against American cotton was of short duration. Most of the goods in question were well-established stock lines which the dealers had sold for some years at fixed prices, and to raise these prices was impossible. But the rise in price of the Egyptian yarns was too great to be covered by any possible sacrifice of profits on the part of the manufacturers or the dealers, and there was no alternative but to abandon the Egyptian yarns. Had such a suggestion been made a few years ago, it would have been ridiculed; but the shopkeepers, more than half persuaded by the obvious excellence of the goods, were compelled to try them, and their success was immediate and astonishing. Customers showed no

hesitation in choosing between the old goods at enhanced prices and the new cheaper goods, and the success of the latter in use rapidly disposed of any fears of their practicability. The customers either did not know the difference or were quite pleased with the substitute. * * * The result is that the trade in those fabrics, where the substitution of cheaper cotton was impossible, has dwindled to very small proportions. The consumers declined to pay the high prices, preferring goods of cheaper quality at something like the old prices. And the manufacturers have not been slow to meet the requirements of the market. Much of the cotton trade is season's goods, and even the established stock lines may suffer a serious loss of demand in one season through the appearance of new goods in competition. The manufacturers have therefore placed before their customers alongside of the old goods at increased prices entirely new and cheaper goods of different materials and new designs which have proved eminently successful. Thus in the end substitution though impossible directly has won its way indirectly to the same result; the old fabrics made from the expensive Egyptian cotton have been largely replaced by new fabrics of cheaper materials mostly American.[a]

It would be a mistake to suppose that the problem of uniformity can be completely solved by breeding and selection, however carefully and efficiently done. The quality of the fiber depends on favorable conditions of growth that often vary in the same field. Even the same individual plant may produce entirely different grades of fiber as a result of changed conditions during the same season. Any sudden forcing or checking of growth is likely to injure both the yield and the quality of the cotton crop. A large amount of experimenting may still be necessary to determine the best methods of culture and irrigation to secure the largest yields and the best quality of lint.

The cultural problems are not the same as with crop plants where the chief object is to promote vigorous growth and a large bulk of plant tissues. With cotton both the yield and the quality are likely to be cut down if the plants are too large and luxuriant. The tendency to overgrowth is a serious difficulty with the Egyptian cotton on some of the very rich new soils in the Southwestern States. How to hold this undesirable luxuriance in check is one of the chief problems. Earlier crops, larger yields, better fiber, and easier picking can all be obtained if the excessive growth of the plants can be restricted. Nor can the new cotton-growing districts be expected to prosper on the basis of a single crop, however profitable it may appear to be at first. To grow cotton continuously on the same soil in an irrigated region is likely to invite disease. Rotations of crops and other forms of diversified agriculture will be needed to insure permanent prosperity.

[a] See Todd, John A., "The Market for Egyptian Cotton in 1909–1910," L'Egypte Contemporaine, no. 5, January, 1911, pp. 3, 4, and 6.

CONCLUSIONS.

The standards of uniformity are higher with the Egyptian cotton than with American short staples, because the Egyptian cotton is used for superior fabrics and for other industrial purposes where strength is required. The prospects of establishing a successful Egyptian cotton industry in America depend on the possibility of producing a uniform crop and avoiding the need of a subsequent sorting of the fiber.

In the Egyptian industry the requirement of uniformity is met, in part, by a system of careful grading and sorting, made possible by cheap labor not available in the United States. Inspection of the fields in Egypt during the early part of the growing season shows a large and very general contamination with the inferior type of cotton known as Hindi that produces only a short, sparse, white lint, quite unlike that of the true Egyptian cotton.

The claim that the Hindi cotton is all removed from the field at the time of thinning the plants is not warranted by the facts, for the Hindi type and obvious hybrid forms are to be found in nearly all the fields, often in considerable proportions, sometimes more than 10 per cent of the total number of plants. Removal of the Hindi plants is practiced only at the period of thinning and very seldom results in any complete elimination of the Hindi cotton from the fields.

The injury caused by the Hindi contaminations is not limited to the proportion of Hindi plants and obvious hybrids that were counted in the fields. Many plants not readily distinguished as Hindi hybrids at earlier stages of growth, give later indications of hybrid nature in white flowers, pale-green bolls, or sparse, inferior lint, or in relative or complete sterility. The Egyptian system of roguing the plants only at the time of thinning would not effect a complete elimination of the Hindi cotton, even if it were generally applied.

An increase of the Hindi contamination is popularly supposed to have taken place in Egypt, in spite of the selection that has been directed against it. Such an increase would be able to cause a serious decline in the yield as well as in the quality of the Egyptian crop, quite independent of other possible causes of deterioration that are supposed to explain the lessened production of the Egyptian fields, such as diminished fertility of the soil, rise of the water level in the soil, plant diseases, and insect pests.

The supposed increase in the proportion of Hindi cotton may prove to be due to the naked seeds that permit a more rapid absorption of water and a more prompt germination than fuzzy seeds. Prompt germination would allow the Hindi seedling plants to make more rapid growth in the earlier stages and thus gain an advantage over Egyptian seedlings in the same hill. It is also possible that the

Hindi characters are prepotent over the Egyptian, like the Upland characters in the later generations of Egyptian-Upland hybrids.

Breeding experiments have shown that it is possible to secure a much higher degree of uniformity in Arizona than now exists in most of the cotton fields in Egypt. Attention to the external characters enables the Hindi cotton and other undesirable variations to be removed from the fields before the flowers open and hence before cross-fertilization becomes possible. If reasonable care be used in maintaining the uniformity of these types, it does not appear that the American-grown Egyptian cotton is likely to suffer any commercial disadvantage on the ground of lack of uniformity in comparison with the Egyptian crop, even though we do not go to the expense of establishing large ginning establishments where the cotton is laboriously sorted by hand.

The greater popularity of the brown-linted varieties of Egyptian cotton may be explained by the advantage that the color gives in sorting out the inferior white Hindi fiber. The exclusion of the Hindi cotton by a more efficient system of selection will enable white varieties to be grown in Arizona and thus produce longer and stronger fiber than brown varieties are likely to afford. A study of many variations and hybrids of the Egyptian cotton shows a distinct tendency for the brown color to be associated with short fibers.

It is possible that the reversions to the Hindi characters may continue to appear in small numbers, even in carefully selected stocks, as in analogous naked-seeded variations occasionally found in uniform carefully selected varieties of Upland cotton. Nevertheless, experiments indicate that such reversions to the Hindi characters are not likely to interfere with the development and preservation of uniform strains of Egyptian cotton in the United States if the proper methods of selection are applied.

PLATES.

DESCRIPTION OF PLATES.

PLATE I. Fig. 1.—Cotton field at Benha, Egypt, showing size and habits of growth of Egyptian cotton plants at the middle of June. Fig. 2.—Closer view of an Egyptian cotton plant with a Hindi plant on either side.

PLATE II. Fig. 1.—View from the outside of the cotton field shown in Plate I. Fig. 2.—General view of a larger field, showing differences in the conditions of the plants at the middle of July.

PLATE III. Bracts and calyxes of Hindi cotton: *A*, From a plant grown at Gizeh, Egypt, by Mr. F. Fletcher from seed obtained in Mesopotamia; *B*, *C*, from two flowers of Hindi cotton from Fayum, Egypt. (Natural size.)

PLATE IV. Bracts and calyxes of cotton from Calioub, Egypt: *A*, Egyptian; *B*, Hindi hybrid. Note the longer laciniæ on the Hindi hybrid bracts; also, that the calyx teeth are intermediate between the Egyptian (Pl. IV, *A*) and the Hindi (Pl. III, *A*, *B*, *C*). The teeth on one side of the Hindi hybrid calyx are rolled back in the photograph. (Natural size.)

PLATE V. Bracts and calyxes of cotton grown at Gizeh, Egypt: *A*, *B*, Of two flowers of Hindi-like Upland cotton from Cochin China, grown by Mr. F. Fletcher; *C*, of a relative of the Egyptian cotton from the Niam-Niam country of central Africa, grown by Mr. W. Lawrence Balls.

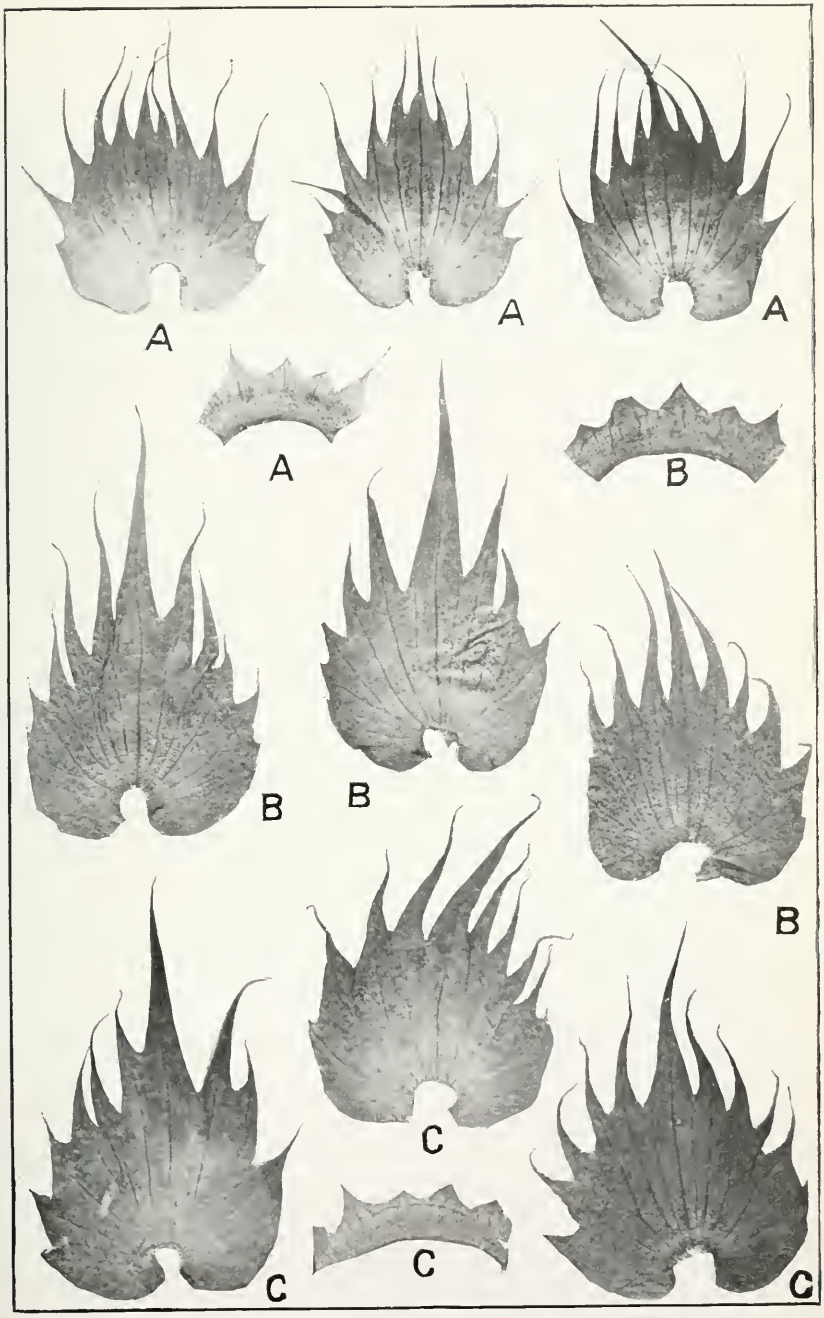
PLATE VI. Bolls of Egyptian and of Hindi cotton grown at Somerton, Ariz., in the season of 1909, showing differences in the shape and the markings of the surfaces: *A*, Egyptian; *B*, Hindi. The tooth calyx of the Hindi cotton can be contrasted with the truncate saucer-like calyx of the Egyptian. (Natural size.)

FIG. 1.—A FIELD OF EGYPTIAN COTTON INTERMIXED WITH HINDI.

FIG. 2.—AN EGYPTIAN COTTON PLANT BETWEEN TWO HINDI PLANTS.

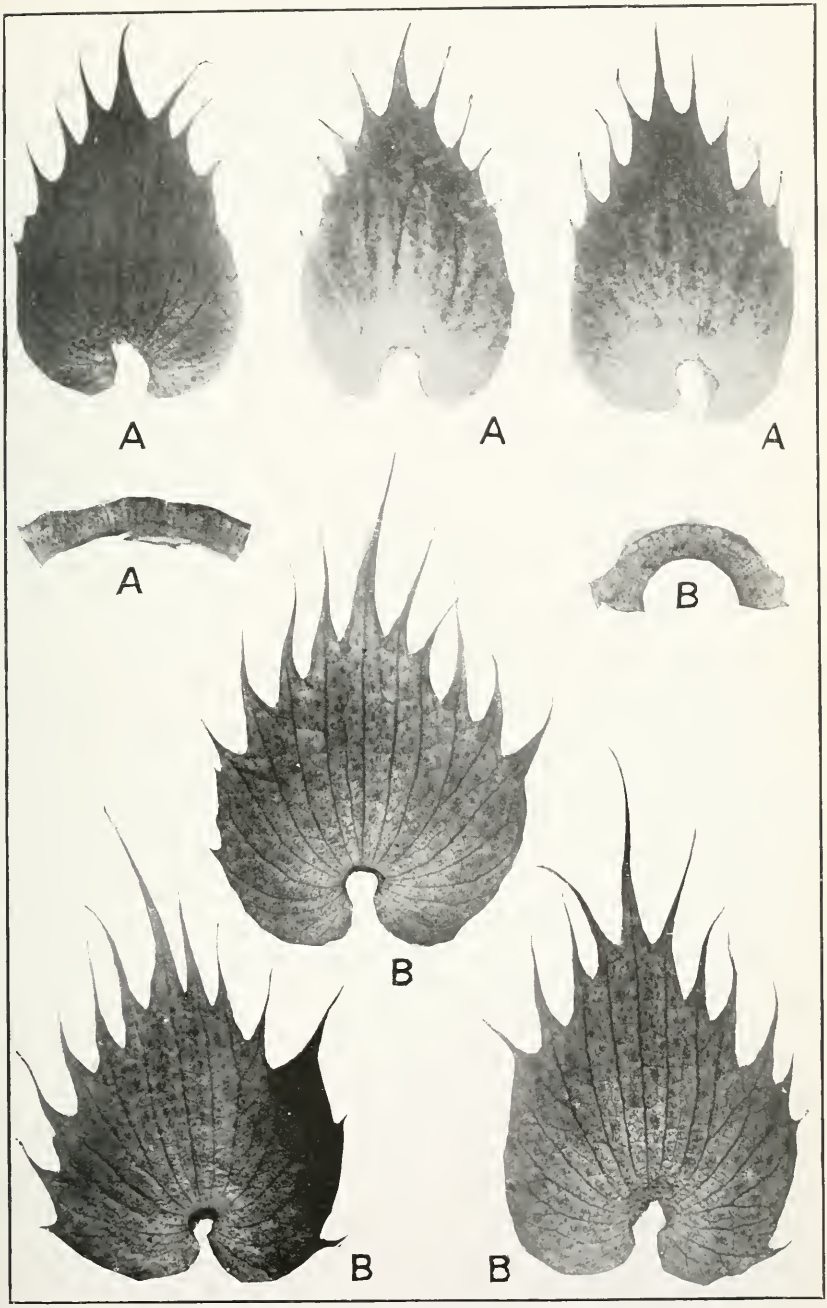
FIG. 1.—SMALL COTTON FIELD AT BENHA, EGYPT.

FIG. 2.—LARGE COTTON FIELD AT BENHA, EGYPT, WITH NATIVES IRRIGATING.



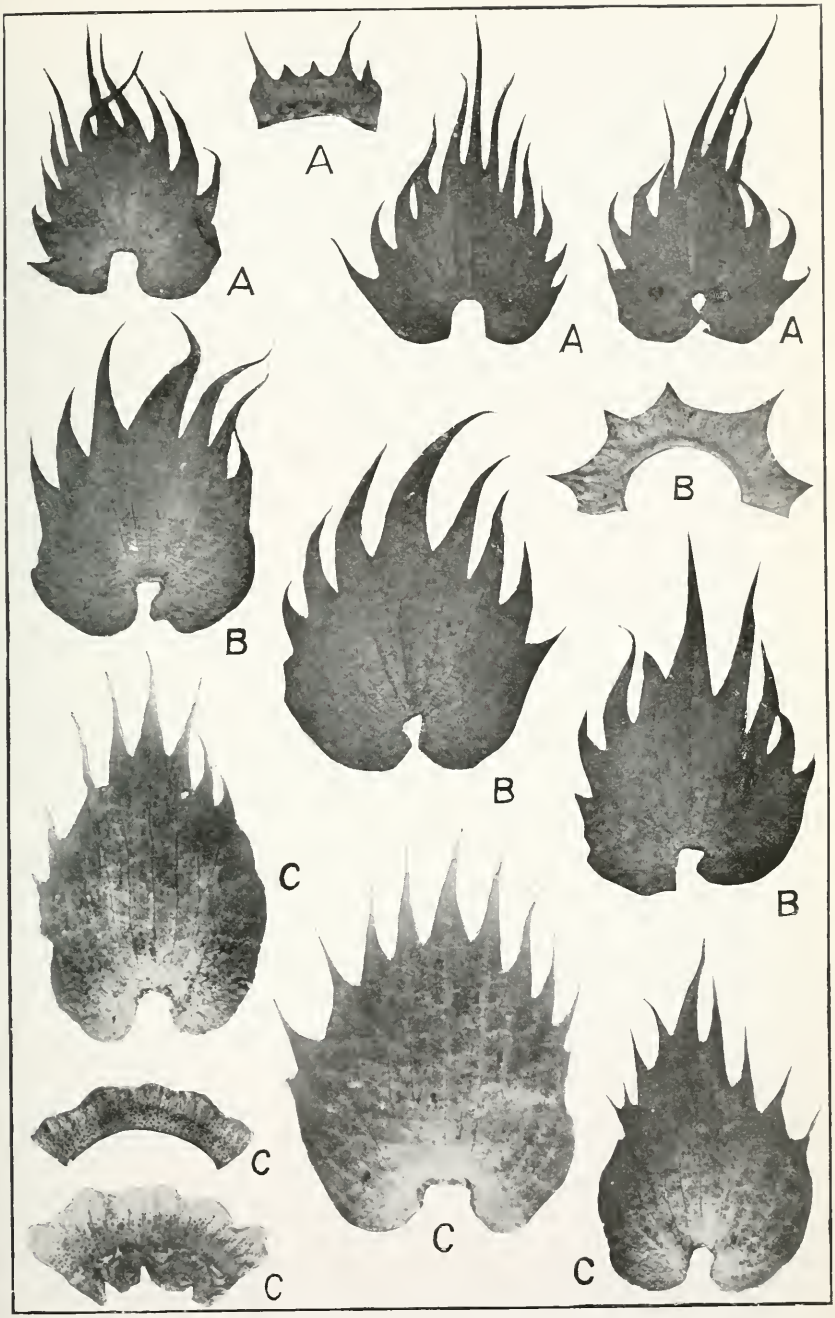
BRACTS AND CALYXES OF HINDI COTTON: A, FROM MESOPOTAMIA; B AND C, FROM FAYUM, EGYPT.

(Natural size.)



BRACTS AND CALYXES OF COTTON: A, EGYPTIAN; B, HINDI HYBRID.

(Natural size.)



BRACTS AND CALYXES OF COTTON: A AND B, HINDI-LIKE UPLAND FROM COCHIN CHINA; C, A RELATIVE OF THE EGYPTIAN FROM CENTRAL AFRICA.

(Natural size.)

BOLLS OF COTTON: A, EGYPTIAN; B, HINDI.

(Natural size.)

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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 211.

B. T. GALLOWAY, *Chief of Bureau.*

BACTERIOLOGICAL STUDIES OF THE SOILS OF THE TRUCKEE-CARSON IRRIGATION PROJECT.

BY

KARL F. KELLERMAN,

Physiologist in Charge of Soil-Bacteriology and Water-Purification Investigations,

AND

E. R. ALLEN,

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ISSUED APRIL 15, 1911.

WASHINGTON:

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Chief of Bureau, BEVERLY T. GALLOWAY.
Assistant Chief of Bureau, WILLIAM A. TAYLOR.
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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., January 17, 1911.

Sir: I have the honor to transmit herewith a paper entitled "Bacteriological Studies of the Soils of the Truckee-Carson Irrigation Project" and to recommend that it be published as Bulletin No. 211 of the series of this Bureau.

These investigations, though in many ways of a preliminary character, indicate some of the possibilities of a bacteriological diagnosis of soils and will be of interest to all who have to deal with problems of soil fertility.

Respectfully,

Wm. A. Taylor,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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BACTERIOLOGICAL STUDIES OF THE SOILS OF THE TRUCKEE-CARSON IRRIGATION PROJECT.

INTRODUCTION.

In making a bacteriological study of any soil or group of soils there are certain fairly well defined groups of micro-organisms whose functions, although as yet imperfectly understood, are recognized as important factors in crop production and are more or less familiar to everyone who has attempted to investigate the problems of soil fertility. These groups of micro-organisms may be roughly separated into four classes, depending upon their physiologic characteristics: (1) Parasites, or organisms important chiefly because they are pathogenic to animals or plants and are frequently found in soils; (2) the cellulose-destroying organisms; (3) the organisms associated with the formation of humus; and (4) the organisms associated with the transformation of soil nitrogen. Only those groups concerned with the transformation of nitrogen, which in the form of ammonia or nitrate is practically the most important of all plant foods, are reported upon at this time.

The data sought in studies of this character may be outlined as follows:

(1) Total numbers of saprophytic bacteria in measured quantities of soil.
(2) Ammonification; the breaking down of nitrogenous organic matter into ammonia.
(3) Nitrification; the oxidation of various compounds of nitrogen to nitrate.
(4) Denitrification; the reverse of nitrification.
(5) Nitrogen fixation, symbiotic and nonsymbiotic; the utilization of atmospheric nitrogen in forming nitrogenous organic compounds.

In the work conducted at Fallon, Nev., during the season of 1909, in cooperation with the Office of Western Agricultural Extension, no quantitative study was made of nitrogen fixation, and the data on the subject of ammonification are very meager. Some preliminary investigations in arid regions had shown that nitrification takes place here at considerable depth. All studies, therefore, were made of a 3-foot zone, keeping separate the samples of soils from different depths.

The comparative nitrifying power of the different samples from the various plats is shown by curves, the parts per million of nitrogen as nitrate and nitrite being plotted as ordinates, and the different depths as abscissæ. These curves show only the gain in nitric and nitrous nitrogen. Chlorids and sulphates are also shown, but seem to be of

little importance. The quantity of nitric nitrogen originally present is shown in the legends under the diagrams (figs. 2–13).

A description of the Truckee-Carson Experiment Farm, at Fallon, Nev., upon which practically all of the work herein reported was conducted, is given in a previous bulletin of this Bureau.[1] The designations of the small plats from which samples were taken for bacteriological study and their location are shown in figure 1.

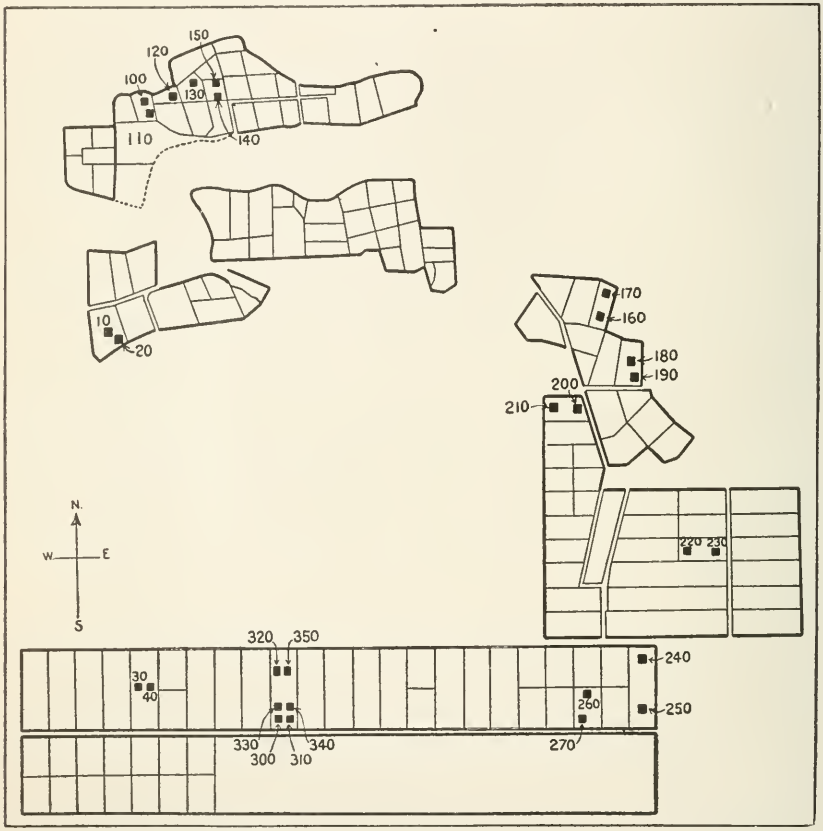

FIG. 1.—Location of sampling plats in the experimental fields of the Truckee-Carson Experiment Farm south of Fallon, Nev.

METHODS EMPLOYED IN BACTERIOLOGICAL INVESTIGATIONS OF THE SOIL AT FALLON, NEV.

REQUIREMENTS TO BE MET.

Investigations in soil bacteriology require first of all the selection and development of satisfactory methods for determining the distribution and activity of the micro-organisms which may occur under

[1] Scofield, C. S., and Rogers, S. J. The Truckee-Carson Experiment Farm. Bulletin 157, Bureau of Plant Industry, 1909.

different soil conditions. Though it is recognized that the methods suggested by different investigators are not adequate for accurate quantitative investigations of bacterial functions and conditions in various soils, the methods which at this time have been found most convenient and suitable for the investigations under discussion are briefly reviewed.[1]

COUNTS OF BACTERIA.

Samples of soil were collected with as strict aseptic precautions as it is possible to observe under field conditions. Sterile salt-mouth bottles were used as containers, and the soil auger used for taking up the soil was carefully cleaned and flamed over an alcohol lamp before sampling each stratum. In the laboratory 1-gram portions were removed from the bottles with a sterile scoop which held the required quantity, transferred to 300 cubic centimeters of sterile water in 500-cubic-centimeter flasks, and the whole shaken thoroughly at short intervals for fifteen minutes. One-cubic-centimeter portions of these infusions were then removed with sterile pipettes and added to 10 cubic centimeters of melted beef agar, and plates poured in the ordinary manner and incubated at 28° C. Counts of bacteria were made at the end of five-day periods.

AMMONIFICATION.

Sterile peptone solutions having the following composition were inoculated with 5 per cent of soil and the ammonia determined at the end of seven and fifteen days by distillation with magnesia:

Peptone	15 grams.
Dipotassium phosphate	3 grams.
Magnesium sulphate	3 grams.
Sodium chlorid	3 grams.
Water	1,000 c. c.

[1] Lipman, J. G. Experiments on the Transformation and Fixation of Nitrogen by Bacteria. Twenty-fourth Annual Report, New Jersey State Agricultural Experiment Stations, 1903, pp. 217–285.

Lipman, J. G., and Brown, Percy E. Methods Concerning Ammonia Formation in Soils and Culture Solutions. Report, Soil Chemist and Bacteriologist, New Jersey Agricultural College Experiment Station, 1908, pp. 95–105.

Lipman, J. G., and Brown, Percy E. Notes on Methods and Culture Media. Report, Soil Chemist and Bacteriologist, New Jersey Agricultural College Experiment Station, 1908, pp. 129–136.

Lipman, J. G. Azotobacter Studies. Report, Soil Chemist and Bacteriologist, New Jersey Agricultural College Experiment Station, 1908, pp. 137–143.

Löhnis, F. Ein Beitrag zur Methodik der bakteriologischen Bodenuntersuchung. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, pt. 2, vol. 12, no. 6–8, pp. 262–267, June 24, 1904; no. 11–16, pp. 448–463, July 14, 1904; vol. 17, no. 14–16, pp. 518–528, December 7, 1906; vol. 20, no. 24–25, pp. 781–799, April 15, 1908; vol. 24, no. 5–7, pp. 183–192, August, 1909.

Remy, Theodor. Bodenchemische und Bakteriologische Studien. Landwirtschaftliche Jahrbücher, vol. 35, Supplement 4, pp. 1–62. Berlin, 1906.

NITRIFICATION.

Samples of soil were collected with the precautions previously described. In some cases 1-gram portions for counts of total numbers of bacteria were removed from the bottle of soil and the remainder of the sample used for nitrification studies.

Because of the great variation in the fertilty of different fields it was considered necessary to determine at what depths the nitrifying bacteria existed; therefore, instead of emptying the soil from the container and allowing it to dry, thus exposing it to some contamination, one-half of the soil, approximately 50 grams, was removed with a sterile spatula and used for "original" determinations. Five cubic centimeters of 0.4 per cent ammonium sulphate was then added to the portion remaining in the bottle and the sample placed in the incubator at 28°C. With the original moisture of the soil this additional 5 cubic centimeters frequently made the water content of the soil somewhat above optimum, but owing to the rapid evaporation in an arid climate this rapidly decreased and was adjusted as nearly as possible in subsequent waterings. All samples were weighed at 3-day intervals, and as any appeared to fall below optimum the required quantity of sterile distilled water was added to restore them. The incubation period was two weeks, the temperature being maintained at 28°C.

The chemical work presented no little difficulty. The analytical determinations may be considered in two phases: (1) The preparation of the aqueous extract of the soil both before and after incubation with ammonium sulphate and (2) the determination of nitrites and nitrates in original and incubated samples.

In the preparation of the aqueous extract considerable difficulty was experienced. All of the soils used contained variable and frequently quite large proportions of very fine clay, which would not settle out and leave a clear supernatant liquid, even on prolonged standing. It was thought advisable to determine the chlorids and sulphates in the original samples; therefore the common salts containing these radicals could not be used to flocculate the clay, although this method was sometimes used in the examination of the samples after incubation where only nitrites and nitrates were determined. Pressure-pump facilities were inadequate for the large number of samples used, the more so as the fine clay particles clogged the porcelain filter and caused filtration to be extremely slow with the low pressure available.[1] Heating the sample in the oven at different temperatures previous to adding the water seemed to have no effect, so the supernatant liquid was first drawn off turbid, evaporated to dryness, baked at 90° to 100° C., and then filtered. In all of the

[1] Approximately 25 pounds to the square inch.

baking experiments it was noticed that the nearer a set of samples was baked at 100° C. the better the subsequent filtering, probably indicating that the clay is siliceous.

The Griess method is the standard for determining nitrites, but owing to the delay in getting chemicals at Fallon the potassium-iodid-starch method was used for a large part of the work. This method, while primarily a qualitative one, was found to be fairly reliable for quantitative determinations if a large quantity of reagent was used when the nitrites were high, as indicated by a rapid development of the blue-black color. The Grandval-Lajoux phenol-sulphonic acid method as modified by Syme [1] was used for estimating nitrates; before determining nitrates the nitrites were removed by urea in acid solution in accordance with Piccini's method.

Chlorids were frequently high in soil solutions in which nitrates were to be determined, and it was necessary to remove them when present in concentrations greater than 50 or 70 parts per million. This was accomplished by the use of silver sulphate.

Chlorids [2] were determined by the Mohr method, titrating the neutral solution with N/10 silver nitrate and using potassium chromate as an indicator. Sulphates [2] were determined by the turbidity method described by the Bureau of Soils.[3]

DENITRIFICATION.

Studies of denitrification were made by inoculating Dunham's peptone solution containing 0.2 per cent potassium nitrate with soil and with a Frost scale measuring roughly the quantity of free nitrogen evolved. Either ordinary fermentation tubes or test tubes inverted in salt-mouth bottles were used. The latter method is preferred, as it permits the use of larger quantities of soil for inoculations.

NITROGEN FIXATION.

Leguminous plants were examined for the presence of nodules, and Azotobacter cultures were isolated from soil samples.

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[1] Syme, W. A. The Colorimetric Determination of Nitrates in Soil Solutions Containing Organic Matter. Thirty-first Annual Report of the North Carolina Agricultural Experiment Station, for the Year Ending June 30, 1908, pp. 64–65.

[2] Both of these salts were determined by Mr. C. A. Jensen, of the Office of Western Agricultural Extension of the Bureau of Plant Industry.

[3] Schreiner, Oswald, and Failyer, George H. Colorimetric, Turbidity, and Titration Methods Used in Soil Investigations. Bulletin 31, Bureau of Soils, U. S. Dept of Agriculture, 1906.

NITRIFYING POWER OF SOILS AT DIFFERENT DEPTHS.

In investigations in soil bacteriology in the eastern United States only the surface soil shows great variations. The soil of the arid sections is much deeper, however; that is, the subsoil is less "raw" than in regions of heavier rainfall, a fact that has come to be more or less familiar to everyone studying soil conditions over extensive areas.

Figure 2 shows the nitrification of samples from plats 100 and 110. These plats, which are practically duplicates, are in a productive

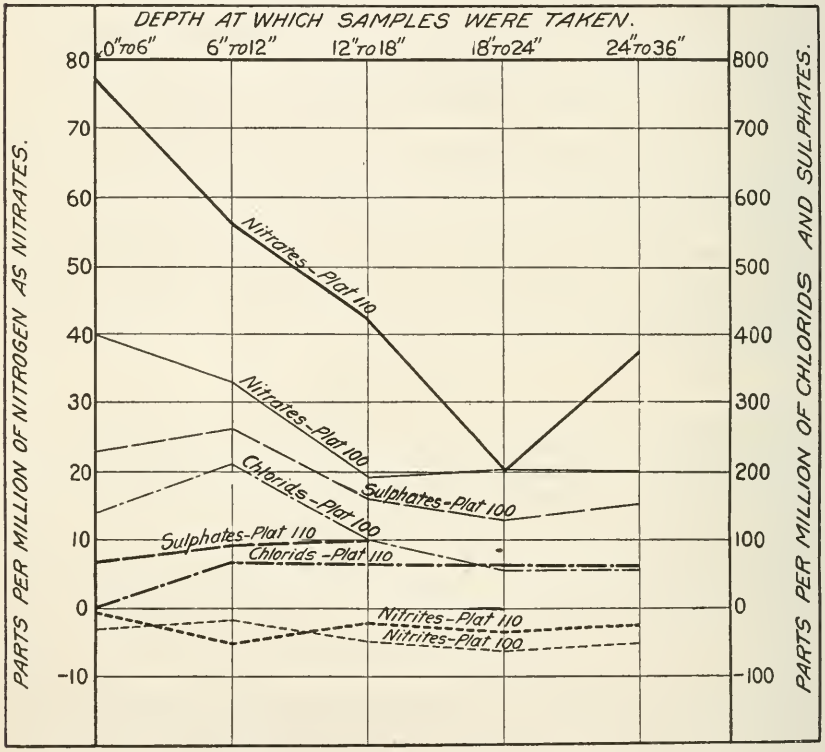

FIG. 2.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plats 100 and 110, Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 100: Depth, 0 to 6 inches, 8 parts per million; 6 to 12 inches, 15; 12 to 18 inches, 9; 18 to 24 inches, 4.8; 24 to 36 inches, 6.56. From plat 110: Depth, 0 to 6 inches, 9 parts per million; 6 to 12 inches, 7.4; 12 to 18 inches, 5.2; 18 to 24 inches, 4.8; 24 to 36 inches, 3.12.

alfalfa field which has been under cultivation for several years. The soil is loose and sandy throughout the 3-foot depth. The nitrate curves show that there is a gradual decrease in nitrifying power with depth.

Figures 3 and 4 show the nitrification in samples from plats 120 and 130. These are in a fertile alfalfa field similar to the one mentioned

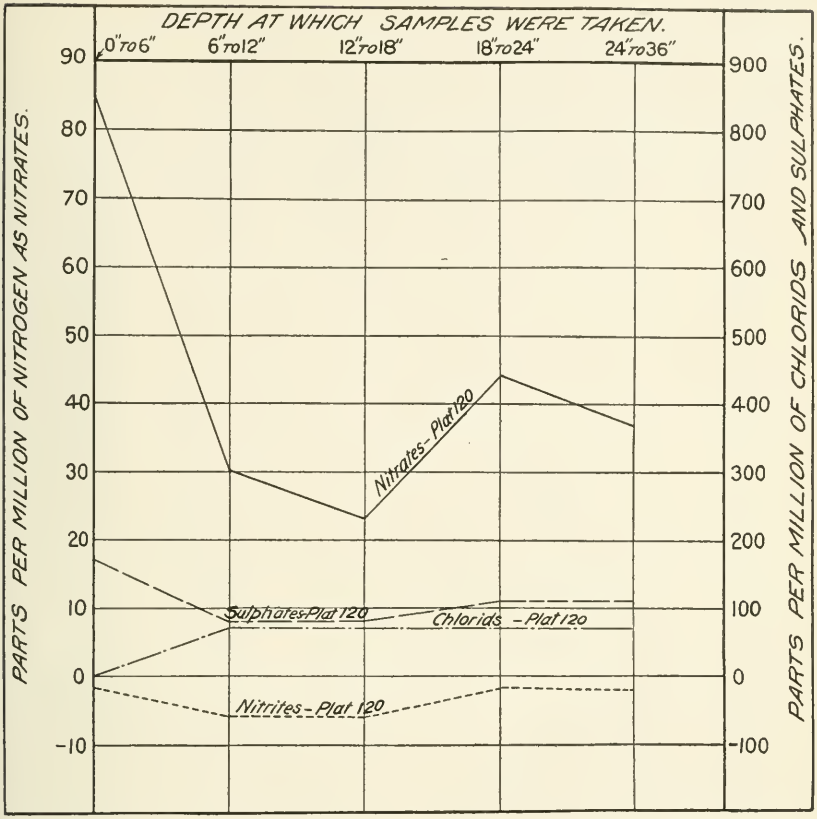

FIG. 3.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 120, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 15.36 parts per million; 6 to 12 inches, 8.64; 12 to 18 inches, 6.72; 18 to 24 inches, 3.84; 24 to 36 inches, 2.88.

in the previous paragraph. The samples from plat 120 show nitrification varying rather irregularly with depth. Samples from plat 130

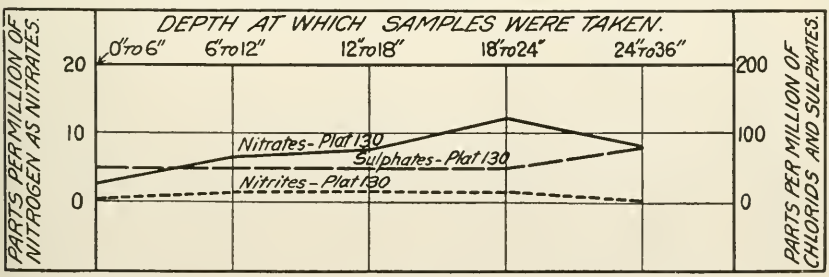

FIG. 4.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 130, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 13.3 parts per million; 6 to 12 inches, 6.72; 12 to 18 inches, 9.6; 18 to 24 inches, 7.23; 24 to 36 inches, 14.4.

practically failed to nitrify,[1] although the two plats appear to be very similar.

Figure 5 shows the relative nitrifying power of good and poor soils collected from adjoining plats. Plat 160 has a loose sandy soil to a

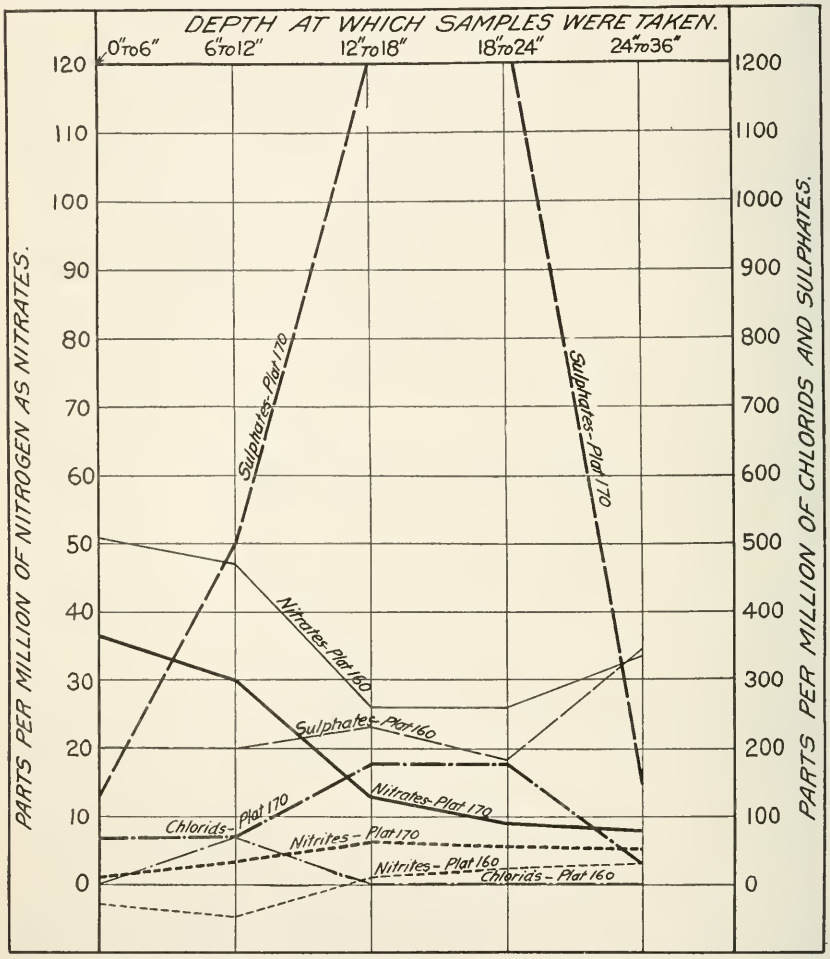

FIG. 5.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plats 160 and 170, Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 160: Depth, 0 to 6 inches, 8.64 parts per million; 6 to 12 inches, 2.88; 12 to 18 inches, 4.8; 18 to 24 inches, 6; 24 to 36 inches, 4.8. From plat 170: Depth, 0 to 6 inches, 4.32 parts per million; 6 to 12 inches, 6; 12 to 18 inches, 3.84; 18 to 24 inches, 3.6; 24 to 36 inches, 3.

depth of 18 inches; below this it is very heavy, but below 26 and 30 inches it is again lighter in texture. At the time of sampling, this plat was supporting a fine growth of alfalfa. Plat 170 is in the northeast corner of the same field, and was very similar except that the

[1] This field had been irrigated a short time before the samples were collected.

surface was a little more compact and the alfalfa was practically a failure. The nitrification curves show the same general variations, but the one of the poor soil is consistently below that of the productive soil.

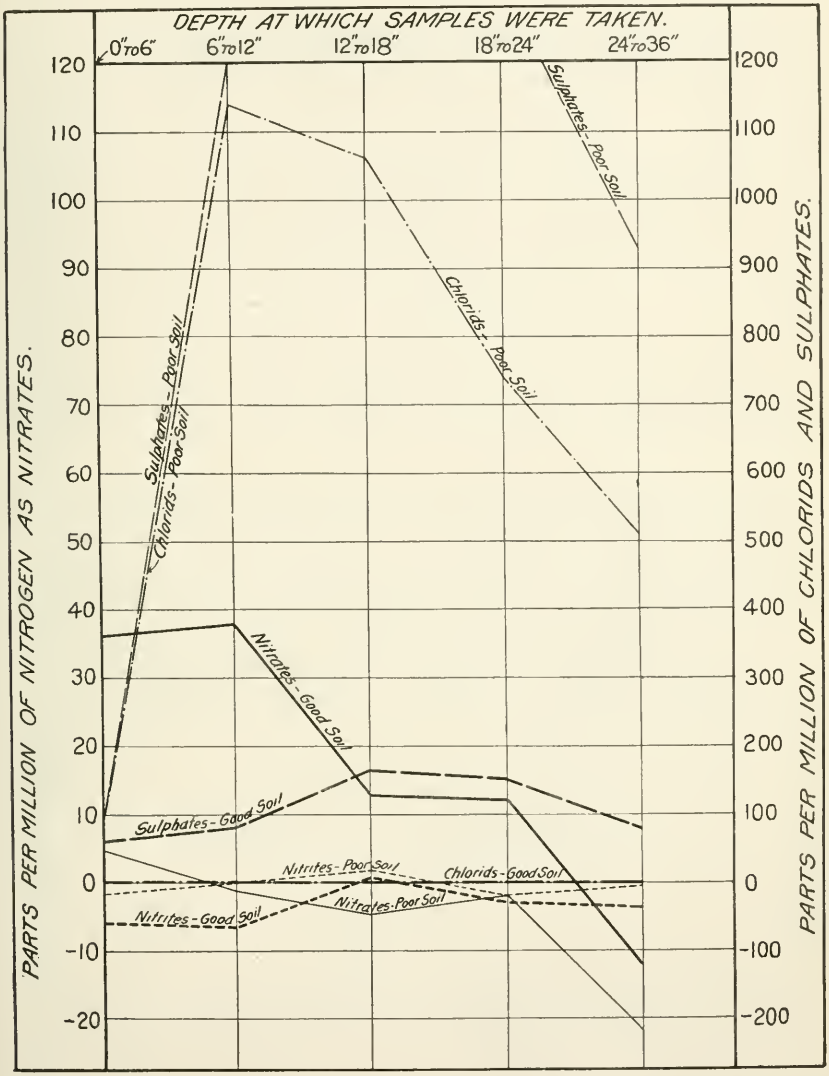

FIG. 6.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 180 (poor soil) and plat 190 (good soil), Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 180: Depth, 0 to 6 inches, 2 parts per million; 6 to 12 inches, 3.5; 12 to 18 inches, 8.25; 18 to 24 inches, 4.5; 24 to 36 inches, 25.75. From plat 190: Depth, 0 to 6 inches, 4.5 parts per million; 6 to 12 inches, 15.75; 12 to 18 inches, 11.25; 18 to 24 inches, 20.75; 24 to 36 inches, 21.75.

Plats 180 and 190 are located upon poor and good spots. The texture of the samples is very similar, both being sandy, but the surface of plat 180, the unproductive soil, is hard and compact as if

held together by some cementing material. As shown in figure 6, the nitrifying power of samples from plat 180 is almost nothing. In this figure the chlorid and sulphate curves are of interest, as those of plat 180, the poor soil, are far above those of plat 190, the good soil.[1]

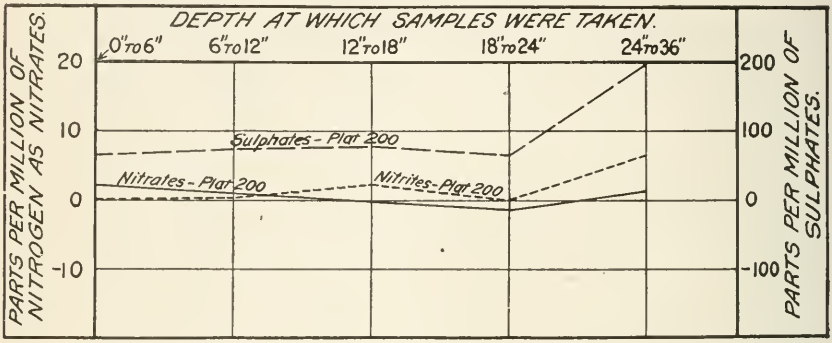

FIG. 7.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 200, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 7.68 parts per million; 6 to 12 inches, 5.8; 12 to 18 inches, 3.93; 18 to 24 inches, 4.32; 24 to 36 inches, 1.82.

Figures 7 to 10, inclusive, show the nitrifying power of samples of soil from plats 200, 210, 220, and 230. They are in fields which have only recently been leveled and irrigated; in fact, 1909 was the first year they had been cropped. They produced a fair crop of barley, but the

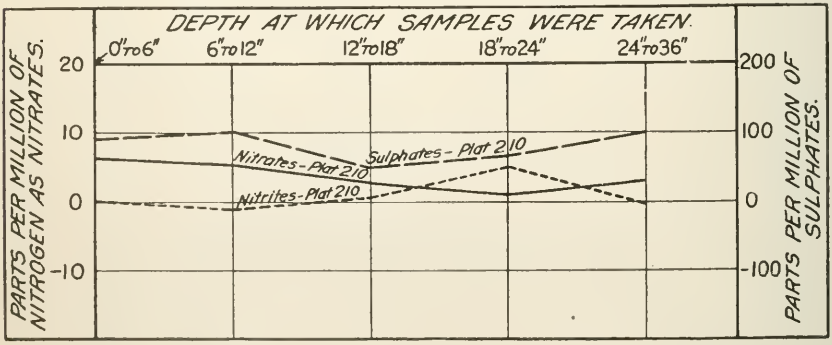

FIG. 8.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 210, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 2.66 parts per million; 6 to 12 inches, 4.8; 12 to 18 inches, 4.16; 18 to 24 inches, 3; 24 to 36 inches, 2.

young alfalfa sown in the barley was doing only fairly well. The curves from all of these plats show a very low nitrifying power, yet a glance at the figures shows that nitrates were present in moderate quantities in the original samples.

[1] Bridge readings on these samples were made by Mr. Jensen.

Figures 11 and 12 present the results obtained from samples of soil from plats 240, 250, 260, and 270. The fields in which these plats are

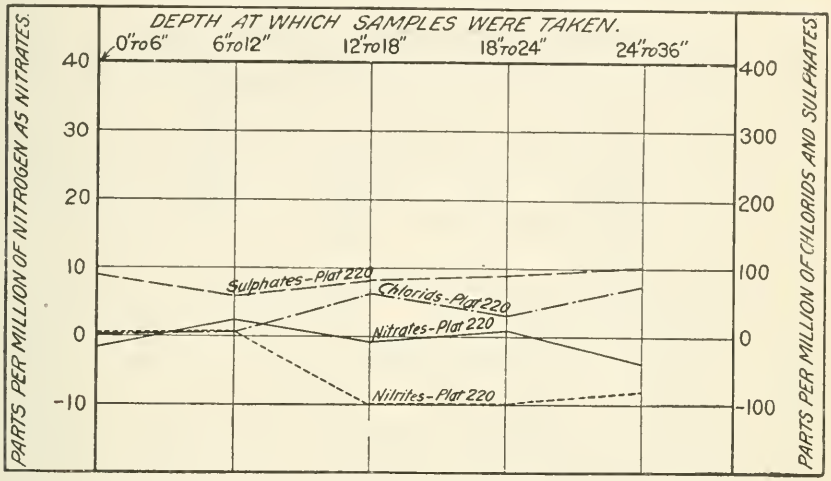

FIG. 9.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 220, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 7.68 parts per million; 6 to 12 inches, 6.91; 12 to 18 inches, 10; 18 to 24 inches, 5.64; 24 to 36 inches, 6.

located have been merely leveled and left fallow, receiving regular applications of irrigation water. The field containing plats 240 and 250 is never cultivated, while that containing plats 260 and 270 is

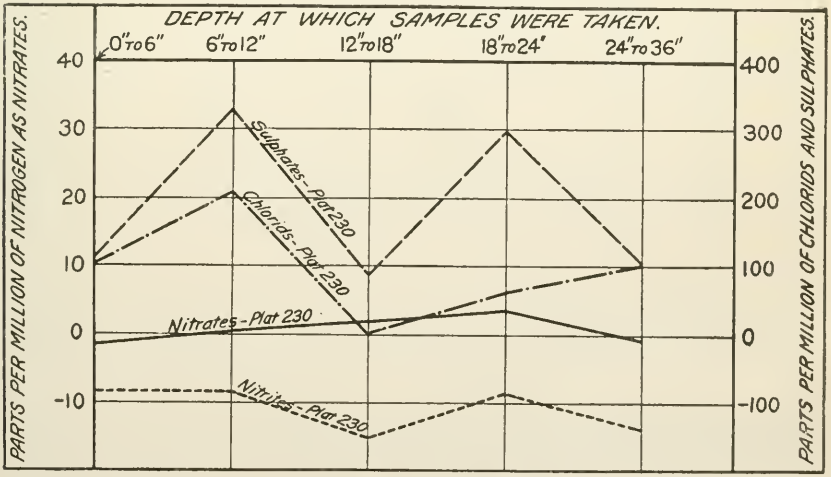

FIG. 10.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plat 230, Truckee-Carson Experiment Farm. Original nitrate present in samples: Depth, 0 to 6 inches, 10 parts per million; 6 to 12 inches, 8.16; 12 to 18 inches, 5; 18 to 24 inches, 4.56; 24 to 36 inches, 9.6.

cultivated according to thorough summer-fallow methods. As the conditions are abnormal it is not surprising that the curves of chlorids

and sulphates, as well as the curve showing nitrification, should be so erratic and variable.

Figure 13 shows the nitrifying power of samples from plats 280 and

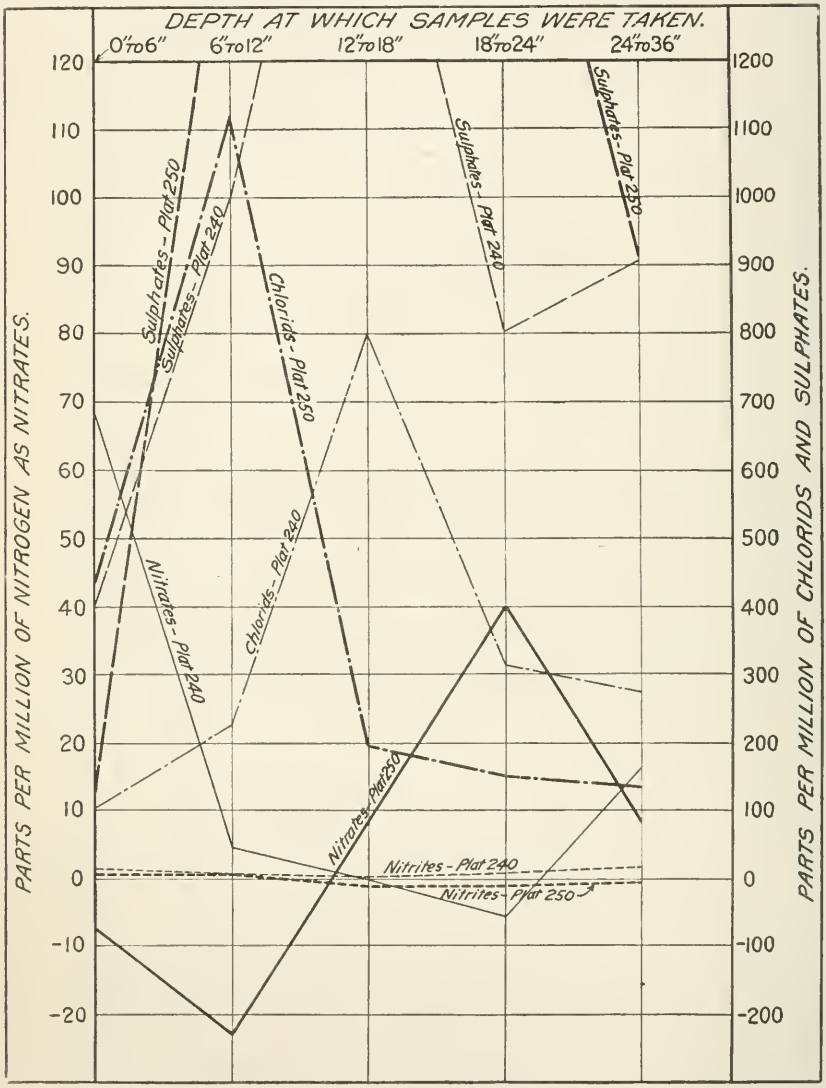

FIG. 11.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plats 240 and 250, Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 240: Depth, 0 to 6 inches, 6.8 parts per million; 6 to 12 inches, 8; 12 to 18 inches, 10.4; 18 to 24 inches, 6; 24 to 36 inches, 5. From plat 250: Depth, 0 to 6 inches, 28 parts per million; 6 to 12 inches, 48; 12 to 18 inches, 6; 18 to 24 inches, 5.2; 24 to 36 inches, 7.

290, located in an old alfalfa field just north of Fallon. These soils are very productive, and it was expected that they would show a greater nitrifying power than they did. This may possibly be

explained, however, by the original high nitrate content of the soil, as there is often a tendency for the nitrifying power of a soil to decrease as nitrates accumulate.

NITRIFICATION OF SAMPLES IN SOLUTION.

In order to further test for the presence of nitrifying bacteria and also to study some of their characteristics, inoculations were made

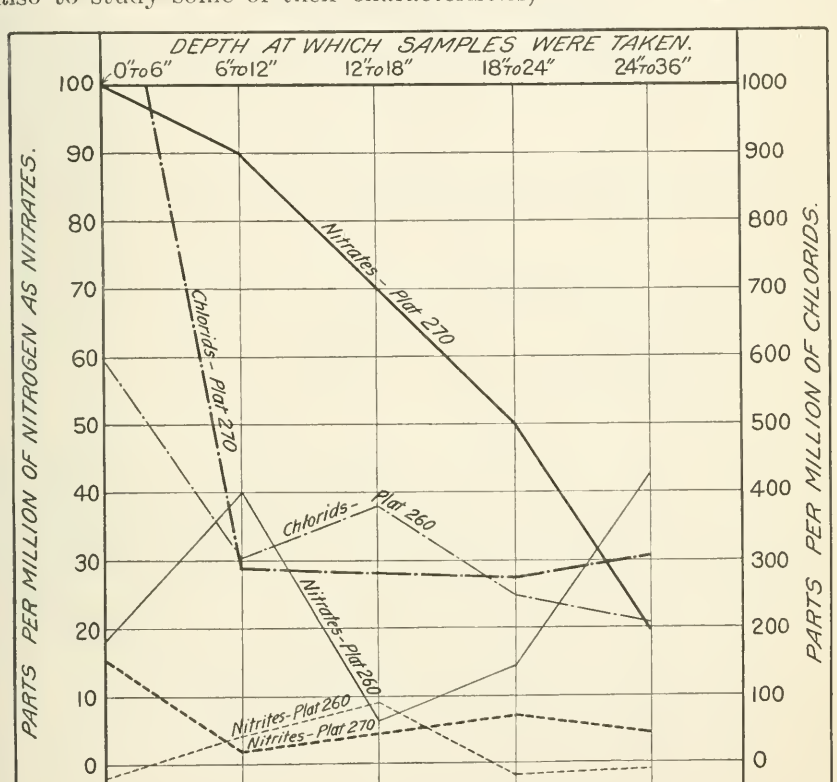

FIG. 12.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plats 260 and 270, Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 260: Depth, 0 to 6 inches, 62 parts per million; 6 to 12 inches, 30; 12 to 18 inches, 18.75; 18 to 24 inches, 35.7; 24 to 36 inches, 30. From plat 270: Depth, 0 to 6 inches, 100 parts per million; 6 to 12 inches, 27.7; 12 to 18 inches, 50; 18 to 24 inches, 40; 24 to 36 inches, 50.

into media consisting entirely of inorganic material which is not suitable for the growth of saprophytic bacteria.[1] Curves have not been plotted from the data thus obtained, as the conditions were too abnormal to warrant considering the differences from a quantitative

[1] Winogradsky and Omelianski's Fluid Culture-Medium for Isolating the Nitrate Bacteria from Soils, and Winogradsky and Omelianski's Fluid Culture-Medium for Isolating the Nitrite Bacteria from Soils. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, vol. 5, pt. 2, 1899, pp. 537–549.

standpoint. The results are all expressed in Table I as parts of nitrogen per million of the solution.

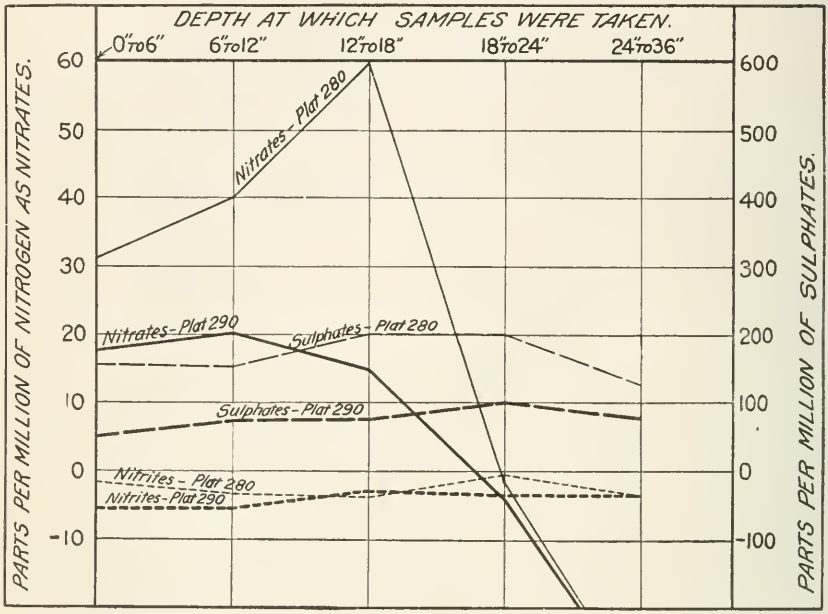

FIG. 13.—Diagram showing the nitrification of ammonium sulphate in samples of soil from different depths from plats 280 and 290, Truckee-Carson Experiment Farm. Original nitrate present in samples from plat 280: Depth, 0 to 6 inches, 12 parts per million; 6 to 12 inches, 10; 12 to 18 inches, 6; 18 to 24 inches, 15; 24 to 36 inches, 62.5. From plat 290: Depth, 0 to 6 inches, 60 parts per million; 6 to 12 inches, 60; 12 to 18 inches, 55.4; 18 to 24 inches, 60; 24 to 36 inches, 60.

TABLE I.—*Nitrification in solution of samples of soil from plats 100, 110, 180, 190, 220, 260, 270, and 280.*[1] *Truckee-Carson Experiment Farm. Incubated at 28° C.*

No. of plat.	Depth of soil.	Ammonia to nitrite (parts per million).[2]		Nitrite to nitrate (parts per million)[3]	
		6 days.	12 days.	10 days.	20 days.
	Inches.				
100	0–6	6.50	25	91.60	96.00
	6–12	5.00	25	64.80	60.00
	12–18	6.50	18	86.40	81.60
110	0–6	.50	15	.....	.....
	6–12	3.25	20	.....	.....
	12–18	8.00	25	.....	.....
180	0–6	0.00	15	24.00	86.40
	6–12	0.00	15	3.60	2.40
	12–18	0.00	00	2.40	3.00
190	0–6	1.00	16	72.00	74.00
220	0–6	0.00	00	13.20	80.00
	6–12	0.00	00	3.60	5.32
	12–18	0.00	00	2.40	5.60
260	0–6	5.00	17	76.80	74.00
	6–12	3.00	10	57.60	54.40
	12–18	3.00	10	2.88	17.55
270	0–6	6.50	20	9.60	43.20
	6–12	0.00	00	8.64	60.00
280[1]	0–6	5.75	20	21.60	81.60
	6–12	1.00	20	40.80	81.60
	12–18	1.00	20	38.40	81.60

[1] Plat 280 is located in an old alfalfa field one-fourth mile north of Fallon.
[2] Used medium for isolating nitrite bacteria. [3] Used medium for isolating nitrate bacteria.

It will be seen that the nitrifiers and especially the nitrate bacteria develop quite well in solutions. It should be noted that the only samples that failed to produce nitrites were those taken at 6-inch, 12-inch, and 18-inch depths from plat 220, which failed to nitrify in soil. (See fig. 9.) This soil, however, produced nitrates quite readily. This suggests the possibility that the lack of nitrification in this soil may be due to lack of nitrite bacteria.

CHLORIDS AND SULPHATES.

In alkali studies it is recognized that as a rule the chlorid type is more injurious to ordinary farm crops than the sulphate type. Further, in some investigations in the soils of the arid regions it has been found

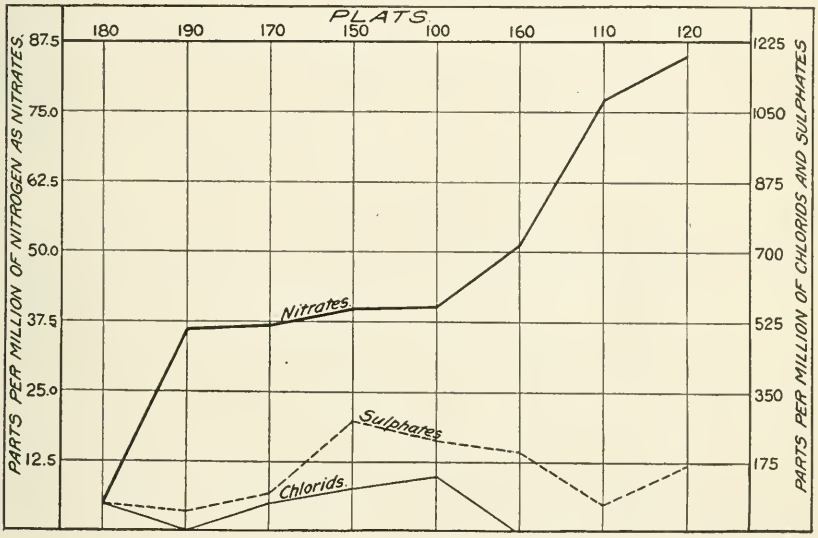

FIG. 14.—Diagram showing the relation between the quantity of alkali and the nitrification in samples of soil from plats 180, 190, 170, 150, 100, 160, 110, and 120, Truckee-Carson Experiment Farm. Samples taken from depths of 0 to 6 inches.

that high nitrates correlate with the sulphate type, while low nitrates are usually associated with the chlorid type. It was thought, therefore, that it would be of interest in connection with this work to study the relation of chlorids and sulphates to the nitrifying power.

In plotting these curves the different plats are arranged in such an order that the nitrification of ammonium sulphate by the different samples, which is the index of the difference of their powers of nitrification, forms an ascending series. Four diagrams are presented (figs. 14 to 17), one for each depth from which samples of soil were taken. Figure 14, representing the surface samples, shows no relation between the concentration of soluble salts and nitrifying power. Figures 16 and 17, representing the deeper samples, are

not in close agreement, although high alkali consisting of both chlorids and sulphates is apparently correlated with low nitrification. Little if any difference is to be noted between the effect of the chlorid and the sulphate types of alkali.

DENITRIFICATION.

In order to test for the presence of denitrifying bacteria several inoculations were made into Dunham's solution containing 0.2 per

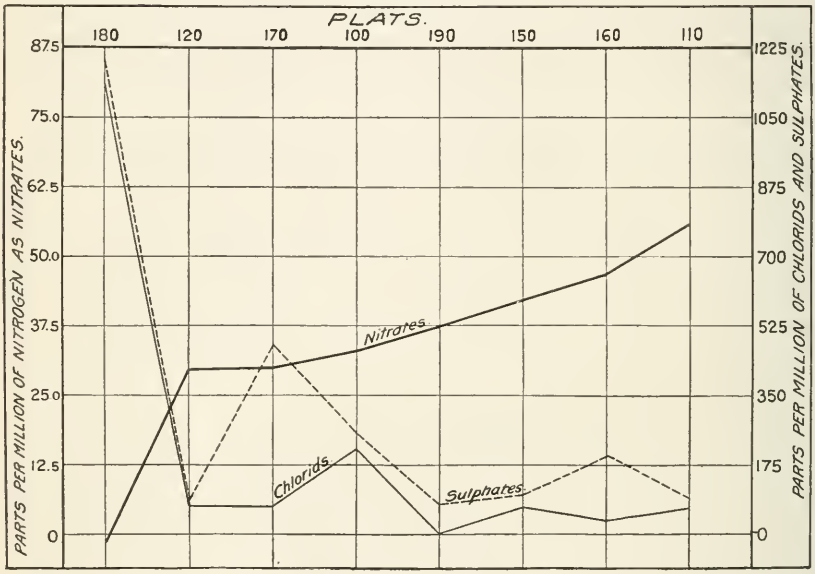

FIG. 15.—Diagram showing the relation between the quantity of alkali and the nitrification in samples of soil from plats 180, 120, 170, 100, 190, 150, 160, and 110, Truckee-Carson Experiment Farm. Samples taken from depths of 6 to 12 inches.

cent of potassium nitrate, and the free nitrogen gas evolved was measured. This medium favors the growth of this class of bacteria. The conditions thus produced are abnormal and the quantitative differences shown in Table II should not be taken too seriously. It will be seen from the table that denitrifying bacteria are present and active in almost all of the soils tested.

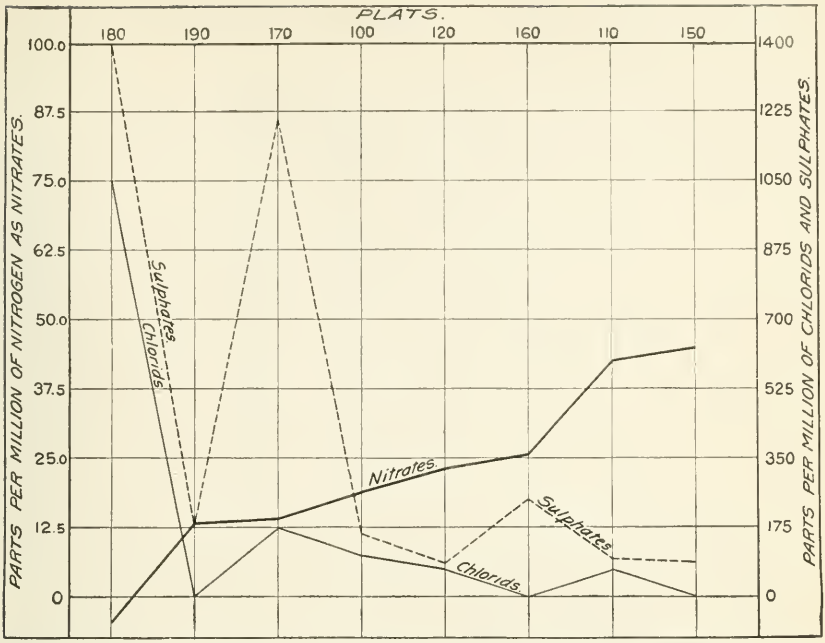

FIG. 16.—Diagram showing the relation between the quantity of alkali and the nitrification in samples of soil from plats 180, 190, 170, 100, 120, 160, 110, and 150, Truckee-Carson Experiment Farm. Samples taken from depths of 12 to 18 inches.

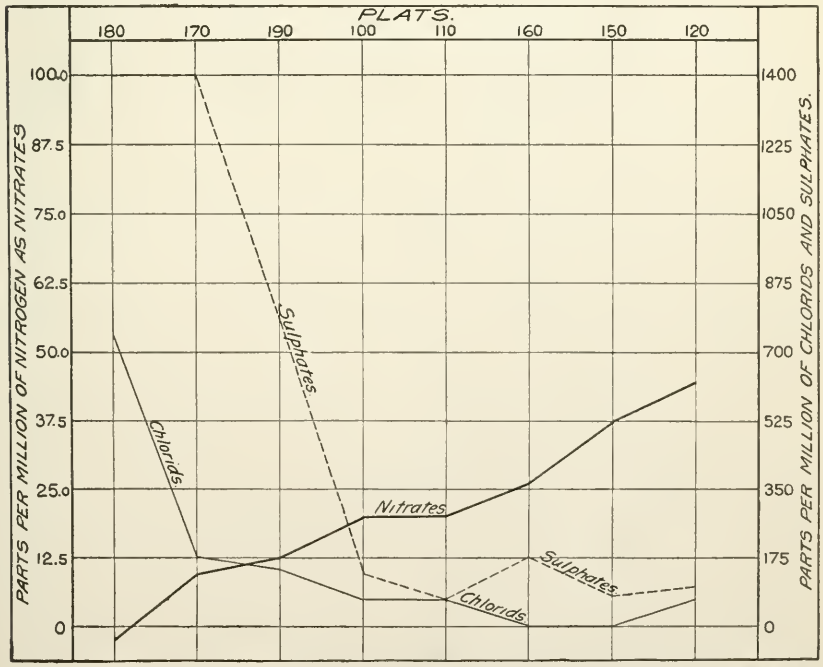

FIG. 17.—Diagram showing the relation between the quantity of alkali and the nitrification in samples of soil from plats 180, 170, 190, 100, 110, 160, 150, and 120, Truckee-Carson Experiment Farm. Samples taken from depths of 18 to 24 inches.

TABLE II.—*Denitrification in solution of samples of soil from plats 180, 190, 230, 260, 270, 280,[1] and 290,[1] Truckee-Carson Experiment Farm.*

No. of plat.	Depth of soil.	Gas formed in 7 days.	Gas formed in 15 days.
	Inches.	*Per cent.*	*Per cent.*
180	0–6	25	25
	6–12	20	30
	12–18	20	25
190	0–6	22	32
	6–12	30	40
	12–18	32	42
230	0–6	20	30
	6–12	21	30
	12–18	20	35
260	0–6	40	53
	6–12	15	23
	12–18	20	30
270	0–6	20	30
	6–12	20	30
	12–18	20	30
280[1]	0–6	00	00
	6–12	Trace.	Trace.
	12–18	Trace.	Trace.
290[1]	0–6	22	40
	6–12	10	18
	12–18	45	62

[1] Plats 280 and 290 are located in an old alfalfa field one-fourth mile north of Fallon.

RELATIVE NUMBERS OF BACTERIA IN DIFFERENT SOILS.

An estimation of the number of bacteria in a gram of soil that would develop aerobically upon beef agar was made for many of the sampling plats in accordance with the method previously described, the results of which are shown in Table III. In accord with the reports of other investigators,[1] the data presented in Table III clearly show that the numbers of bacteria found in the different samples bear no consistent relation to the fertility or crop-producing power of the respective fields.

No attempt was made to determine the relative numbers of protozoa in samples of soil from the good and poor areas. If the development of protozoa is determined by their food supply,[2] in other words, by the numbers of bacteria existing in the soil, it is obvious that in this region the crop-producing power can not be limited[3] by the abundance of protozoa.

[1] Löhnis, F. Ein Beitrag zur Methodik der bakteriologischen Bodenuntersuchung. Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, pt. 2, vol. 12, no. 6–8, June 24, 1904, pp. 262–267.

Chester, Frederick D. The Bacteriological Analysis of Soils. Bulletin 65, Delaware College Agricultural Experiment Station, March 1, 1904.

Voorhees, Edward B., and Lipman, Jacob G. A Review of Investigations in Soil Bacteriology. Bulletin 194, Office of Experiment Stations, U. S. Dept. of Agriculture, October 26, 1907.

[2] Russell, E. J., and Hutchinson, H. B. The Effect of Partial Sterilization of Soil on the Production of Plant Food. Contributions from the Laboratory of the Rothamsted Experimental Station, October, 1909, pp. 111–144.

[3] Hall, A. D. The Fertility of the Soil. Science, n. s., vol. 32, no. 820, September 16, 1910, pp. 363–371.

TABLE III.—*Number of bacteria per gram of soil and nitrifying power of samples from plats 10, 20, 30, 40, 180, 190, 290,[1] 240, 260, and 270, Truckee-Carson Experiment Farm.*

No. of plat.	Depth of soil.	Number of bacteria per gram.	Nitrifying power of soils (parts per million).	Character of soil.
	Inches.			
10	0–6	435,000	4.4	Very poor.
	6–12	251,000	2.0	
	12–18	26,650	.0	
	18–24	146,250	3.0	
	24–36	1,000	.0	
20	0–6	19,500	54.2	Very productive.
	6–12	11,250	6.8	Good growth of alfalfa.
	12–18	30,000	1.0	
	18–24	4,500	.0	
	24–36	3,000	.0	
30	0–6	160,000	3.0	Poor and compact.
	6–12	65,000	.0	
	12–18	262,000	.0	
	18–24	19,855	.0	
	24–36	10,000	.0	
40	0–6	210,000	20.4	Productive.
	6–12	20,000	4.0	Good growth of alfalfa.
	12–18	135,000	1.0	
	18–24	45,000	.0	
	24–36	1,000	.0	
180	0–6	60,000	4.72	Very poor.
	6–12	175,000	1.54	Alkali high. (See fig. 6.)
	12–18	180,000	4.32	
	18–24	4,000	2.54	
190	0–6	3,600	36.30	Productive.
	6–12	168,000	37.45	
	12–18	1,554,000	12.75	
	18–24	704,000	12.25	
290[1]	0–6	273,000	30.00	Productive.
	6–12	396,000	20.00	Old alfalfa field. (See fig. 13.)
	12–18	262,500	14.60	
	18–24	327,000	4.00	
240	0–6	52,000	69.00	Fallow. (See fig. 11.)
	6–12	78,700	4.50	
	12–18	56,000	.40	
260	0–6	81,000	18.00	Fallow. (See fig. 12.)
	6–12	153,300	40.00	
270	0–6	72,000	100.00	
	6–12	2,790,000	92.30	

[1] Plat 290 is located in an old alfalfa field one-fourth mile north of Fallon.

DETAILED STUDY OF SOIL TYPICAL OF EXTENSIVE AREAS.

Plats 300 to 350 are representative of a somewhat extensive type of soil of the Truckee-Carson project. This soil is very unproductive as a rule, almost barren in many cases, yet all through it, wherever properly leveled and irrigated, are spots of a few square rods in area that are normal and productive. The difference between these two conditions seemingly can not be explained by any of the now known causes of infertility. There is a certain difference in texture, or rather in the physical properties; the productive soil is loose and sandy, while the unproductive type, although sandy, contains a small quantity of clay which when shaken up with water remains suspended indefinitely and the soil cements on drying. These physical differences, while no doubt factors, do not seem adequate causes of the extremely low fertility. The total alkali content is not high enough

to produce toxic effects, and a lack of mineral plant food in the virgin soils is almost out of the question.

Both soils are low in organic matter, as are all arid soils. Good soil management in other somewhat similar regions would indicate that the addition of organic matter to these soils in the form of barnyard manure or green manure should produce beneficial physicochemical effects, and such treatments have been applied somewhat extensively as a matter of experiment during the last two or three years. The poor soil apparently has not been benefited to a noticeable degree. The good soil has been somewhat improved, although even here the improvement has not been striking. A minute field examination of these good and poor spots a year or more after they had received applications of organic matter revealed a remarkable difference; all traces of the organic material had disappeared from the fertile spots, while the larger part of the manure added to the infertile spots was in an almost perfect state of preservation. Another peculiar difference was that in the poor spots, at depths of 6 to 28 inches, an irregularly distributed, dark-colored, foul-smelling layer was found, undoubtedly due to the presence of a peculiar organic decomposition product, while such a layer was never found associated with good soil. It should not be inferred from this description that this black layer was found only where organic matter has been added as a treatment; it was quite generally distributed through these infertile soils and is presumably due to the decay of such material as was turned into the soil when it was first reclaimed, such as sagebrush, greasewood, rabbit brush, and other desert plants, together with the roots of these plants which have been accumulating for long periods of time. Laboratory samples showed that this black substance was easily oxidized, for when a sample was taken to the laboratory, dried, and subsequently moistened for physiological experiments, all traces of the black color and peculiar odor disappeared.

These unusual conditions of the decay of organic matter are necessarily somewhat closely associated with improper bacteriological conditions; that is, the improper utilization of organic fertilizers is due either to an improperly balanced or incomplete bacterial flora or to physical or chemical conditions preventing the performance of the normal activities of the bacteria present.

Titrations of some of the aqueous extracts indicated that sodium carbonate (black alkali) was present in the poor soils but not in the good soils. It was also apparent that calcium sulphate and gypsum, when applied in large quantities, produced a decided effect in flocculating the finely divided or colloidal clays. Samples were collected with a sterile spatula from the sides of freshly dug holes and placed in sterile containers. Portions of these samples were inoculated into

Winogradsky's solutions and also into flasks of sterile water, from which counts were made. The remaining portions of the samples were then emptied on clean sheets of paper in the culture room and left to dry under conditions as free as possible from chance contaminations. Fifty-gram portions from each sample were removed for original nitrate determinations, and another equal portion was replaced in the original containers, brought up to optimum moisture content with 5 cubic centimeters of 0.4 per cent sulphate of ammonia and distilled water, incubated for two weeks at 28° C., and the nitrification determined. A duplicate series to which was added a 2 per cent solution of calcium sulphate was prepared. At the beginning of the incubation period the total weight of the container and soil at optimum moisture was taken, and the loss from evaporation was restored with sterile distilled water at 3-day intervals during the incubation period. The results of the experiment appear in Tables IV and V.

TABLE IV.—*Effect of calcium sulphate upon nitrification in samples of soil from plats 300 and 310, Truckee-Carson Experiment Farm, representing poor soil conditions. Incubated 15 days at 28° C.*

NO CALCIUM SULPHATE ADDED TO SAMPLES.

No. of plat.	Depth of soil.	Nitrogen as nitrite (parts per million).			Nitrogen as nitrate (parts per million).		
		Original.	Final.	Gain.	Original.	Final.	Gain.
	Inches.						
300	0–6	1.2	5.60	4.40	9.12	56.40	46.28
	6–12	2.0	3.12	1.12	7.68	00.00	− 7.68
	12–18	1.2	1.68	.48	6.14	00.00	− 6.14
	18–24	.0	1.20	1.20	4.56	00.00	− 4.56
310	0–6	1.0	7.28	6.28	4.80	96.00	91.20
	6–12	1.0	2.10	1.10	1.60	00.00	− 1.60
	12–18	1.0	.80	− .20	4.32	00.00	− 4.32
	18–24	1.0	1.20	.20	3.07	00.00	− 3.07

WITH 2 PER CENT CALCIUM SULPHATE ADDED TO ALL SAMPLES.

300	0–6	1.2	2.80	1.60	9.12	57.00	47.88
	6–12	2.0	2.80	.80	7.68	00.00	− 7.68
	12–18	1.2	2.70	1.50	6.14	00.00	− 6.14
	18–24	.0	.88	.88	4.56	00.00	− 4.56
310	0–6	1.0	4.00	3.00	4.80	96.00	91.20
	6–12	1.0	2.40	1.40	1.60	1.20	− .40
	12–18	1.0	1.68	.68	4.32	0.00	4.32
	18–24	1.0	1.56	.56	3.07	0.00	− 3.07

TABLE V.—*Effect of calcium sulphate upon nitrification in samples of soil from plat 320, Truckee-Carson Experiment Farm, representing good soil conditions. Incubated 15 days at 28° C.*

NO CALCIUM SULPHATE ADDED TO SAMPLES.

No. of plat.	Depth of soil.	Nitrogen as nitrite (parts per million).			Nitrogen as nitrate (parts per million).		
		Original.	Final.	Gain.	Original.	Final.	Gain.
	Inches.						
320	0–6	1.4	4.00	2.60	3.84	80.64	76.80
	6–12	1.5	1.20	−.30	18.24	76.80	58.56
	12–18	.0	1.60	1.60	15.90	5.00	−10.90
	18–24	.8	1.40	.60	15.36	0.00	−15.36

WITH 2 PER CENT CALCIUM SULPHATE ADDED TO ALL SAMPLES.

320	0–6	1.4	5.00	3.60	3.84	81.60	77.76
	6–12	1.5	1.68	.13	18.24	76.80	58.56
	12–18	.0	1.82	1.82	15.90	4.56	−11.36
	18–24	.8	.60	−.20	15.36	.00	−15.36

The gain in nitrates, or the nitrifying power of these samples of soil, is shown in figure 18.

These experiments on nitrification indicate that the difference in productiveness is not due to a suspension of nitrification, and also that it is not due to the presence of sodium carbonate, as the addition of calcium sulphate to the samples had absolutely no effect; the treated and untreated samples could really be considered duplicates. It would seem also that the infertility or the lack of decay of organic substances is not due to lack of air. It might be argued that laboratory conditions were not such as would favor the compacting or cementing of the samples, yet it must be remembered that the cornfield containing plats 300, 310, and 320 was kept well cultivated and no crust was allowed to form during the growing season. Tables VI and VII show the nitrification of the different samples in Winogradsky and Omelianski's media.

TABLE VI.—*Nitrite formed from ammonia by samples of soil from plats 330, 340, and 350, Truckee-Carson Experiment Farm, in medium for nitrite bacteria. Incubated at 28° C.*

No. of plat.	Depth of of soil.	Nitrite formed in 5 days (parts per million).	Nitrite formed in 10 days (parts per million).	Character of soil.
	Inches.			
330	0–6	0.0	4.8	Poor.
	6–12	.0	.0	
	12–18	.0	.0	
	18–24	.0	.0	
340	0–6	9.6	10.4	Do.
	6–12	12.8	12.8	
	12–18	12.8	12.8	
	18–24	.0	12.2	
350	0–6	.0	Trace.	Good.
	6–12	.0	4.8	
	12–18	.0	.0	
	18–24	.0	.0	

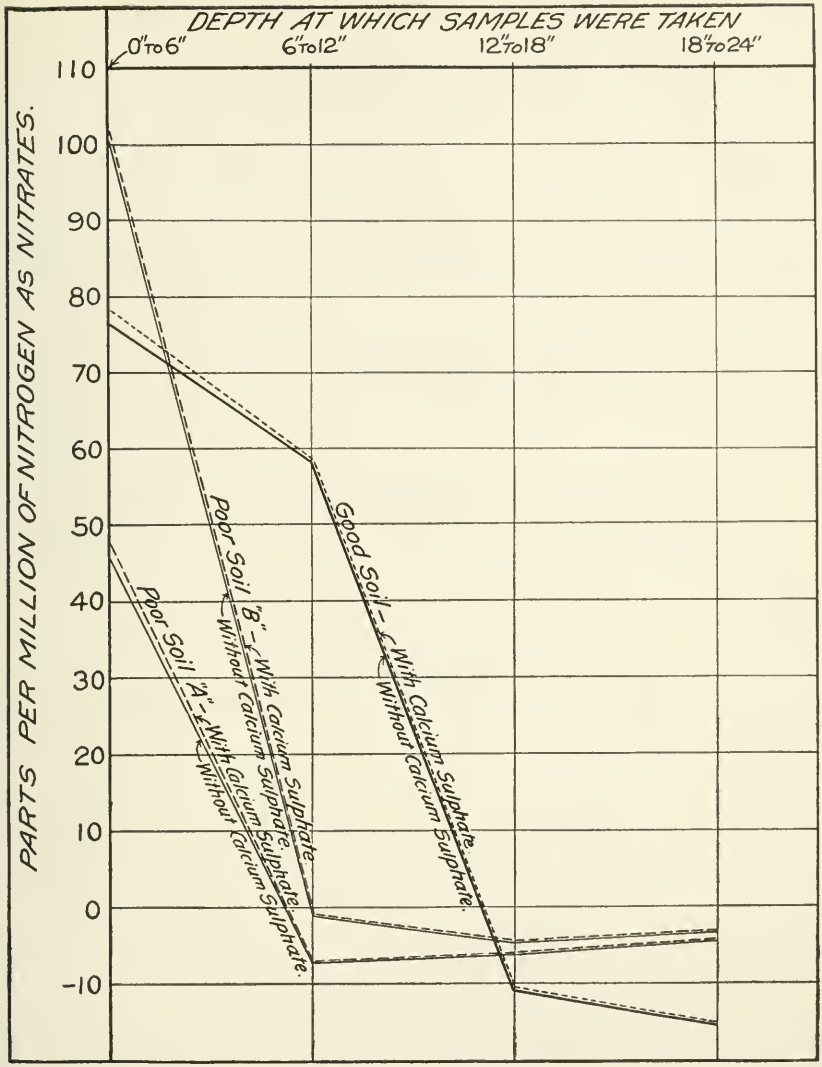

FIG. 18.—Diagram showing the effect of calcium sulphate upon nitrification of ammonium sulphate in samples of soil from plat 300, Truckee-Carson Experiment Farm, representing poor soil "*A*"; plat 310, representing poor soil "*B*"; and plat 320, representing good soil.

TABLE VII.—*Nitrate formed from nitrite by samples of soil from plats 330 and 350, Truckee-Carson Irrigation Project, in medium for nitrate bacteria. Incubated at 28° C.*

No. of plat.	Depth of soil.	Nitrate formed in 10 days (parts per million).	Nitrate formed in 20 days (parts per million).	Character of soil.
	Inches.			
330	0–6	24.00	90.32	Poor.
	6–12	19.68	90.32	
	12–18	12.60	81.28	
	18–24	19.80	90.32	
350	0–6	12.50	54.19	Good.
	6–12	24.00	72.25	
	12–18	.....	.....	
	18–24	4.12	90.32	

The fact that nitrification was feeble in the good soil and also in one of the poor soils should not be overemphasized, for soils that will nitrify under normal conditions frequently fail to do so in solutions. On the other hand, the rapidity with which the nitrate bacteria worked in solutions, even when they failed to do so in the soil, is interesting and almost without parallel. It is not surprising that a soil should fail to nitrify in solution, but it is remarkable that samples which failed to nitrify when kept warm and moist—ideal conditions for nitrification—should produce nitrates rapidly when inoculated into solutions.

The production of ammonia from organic material by soil bacteria furnishes a means of measuring the power of the soil flora to break down nitrogenous organic substances. Thus it would seem that the soils of the plats in which organic matter remained indefinitely in a

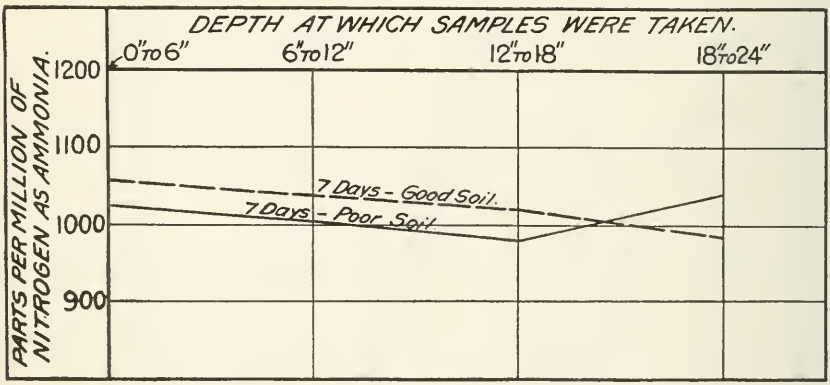

FIG. 19.—Diagram showing the ammonification of peptone in 7 days in samples of soil from plat 350 (good soil) and from plats 330 and 340 (poor soil), Truckee-Carson Experiment Farm.

state of preservation must have a very low ammonifying power. The medium described previously, consisting of 1.5 per cent peptone and inorganic salts, was inoculated with samples from plats 330, 340, and 350, and the ammonia produced determined at 10-day and 20-day incubation periods by distillation with magnesia.[1] The results of this experiment are shown in figures 19 and 20.

As the ammonification of the samples of poor soil, plats 330 and 340, was very similar, the results are averaged and shown as a single curve.

The fact that there is no increase between the 7-day and 15-day periods indicates that the maximum had been reached before any

[1] Dr. J. G. Lipman has recently suggested the use of dried blood as a source of nitrogen for work of this character. See Lipman, Jacob G., and Brown, Percy E., "Experiments on Ammonia and Nitrate Formation in Soils," in Centralblatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten, pt. 2, vol. 26, no. 20–24, April 9, 1910, pp. 590–632.

determinations were made. Yet these results show conclusively that in both good and poor soils there are large numbers of ammonifiers which are physiologically active if proper conditions are provided for them to develop. The relative differences in their ammonifying power and whether or not there are conditions in the soil to prevent their normal activities remain to be shown by further experiment.

Denitrification is of two kinds: The reduction of nitrates to lower forms or transformation into organic form, and the complete breaking down of the nitrogenous substance with the evolution of free nitrogen as a gas. Either of these processes could be a source of infertility.

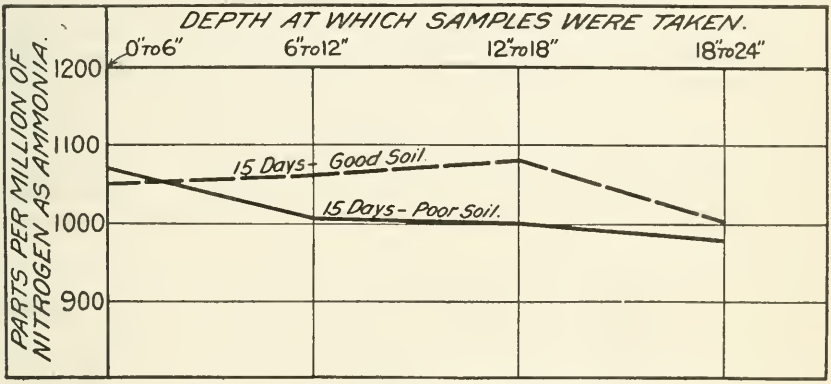

FIG. 20.—Diagram showing the ammonification of peptone in 15 days in samples of soil from plat 350 (good soil) and from plats 330 and 340 (poor soil), Truckee-Carson Experiment Farm.

The evolution of free nitrogen was determined by measuring the nitrogen gas produced from peptone-nitrate solutions at intervals of 7 and 15 days. The results are rather erratic, as is shown in Table VIII.

TABLE VIII.—*Denitrification by samples of soil from plats 330, 340, and 350, Truckee-Carson Experiment Farm.*

No. of plat.	Denitrification.			
	Depth of soil.	Gas formed in—		Character of soil.
		7 days.	15 days.	
	Inches.	*Per cent.*	*Per cent.*	
330	0–6	30	35	Poor.
	6–12	1	10	
	12–18	2	5	
	18–24	2	5	
340	0–6	35	40	Do.
	6–12	40	40	
	12–18	20	25	
	18–24	30	40	
350	0–6	2	7	Good.
	6–12	1	3	
	12–18	1	5	
	18–24	20	20	

Table IX shows the difference between the good and poor soils in regard to total numbers and distribution of bacteria. The difference in the floras is more strikingly brought out when we consider the difference in the colonies from the different soils. The plates from the 6-inch and 12-inch layers of plats 300 and 310, which show low numbers, chiefly contained peculiar colonies surrounded by a wine-colored diffusible pigment. The colony itself was but slightly colored and, surrounded as it was by this pigment, produced a very striking appearance on the plates. One plate from plat 310 was apparently a pure culture of this organism. Such a plate obtained from soil where the growth or flora is almost always rich and varied is very rare, and is the only unusual condition thus far encountered that seems to correlate consistently with the unusual conditions of infertility. This peculiar colony was never seen on soils from the fertile spots, and the fact that it was so predominately present in the infertile soils and in those strata in which the peculiar black layer occurred certainly indicates that further study should be made of this point. Microscopic examination of the colony showed that it was a micrococcus associated with a mold.

TABLE IX.—*Total number of bacteria present in 1-gram samples of soil from plats 300, 310, 320, 330, 340, and 350, Truckee-Carson Experiment Farm.*

No. of plat.	Depth of soil.	Number of bacteria per gram.	Character of soil.
	Inches.		
300	0–6	458,400	Poor.
	6–12	45,000	
	12–18	48,900	
	18–24	178,500	
310	0–6	1,930,500	Do.
	6–12	729,000	
	12–18	15,900	
	18–24	409,500	
320	0–6	507,000	Good.
	6–12	351,000	
	12–18	419,000	
	18–24	429,000	
330	0–6	1,835,000	Poor.
	6–12	915,000	
	12–18	840,000	
	18–24	1,197,000	
340	0–6	4,200,000	Do.
	6–12	525,000	
	12–18	4,620,000	
	18–24	3,780,000	
350	0–6	672,000	Good.
	6–12	.....	
	12–18	636,000	
	18–24	210,000	

CONCLUSIONS.

(1) Nitrifying, denitrifying, and ammonifying bacteria are well distributed and universally present in the soils of the Truckee-Carson Irrigation Project and become physiologically active if favorable conditions are provided for their development.

(2) The lack of proper decay and humification of organic matter in many of the unproductive soils is due either to unfavorable bacterial conditions brought about by certain physical and chemical conditions or to an unusual bacterial flora.

(3) The nitrifying bacteria in the soils of Fallon, Nev., are active at greater depths than in eastern soils and also seem to be unusually virile in solutions, although the data on these points are not conclusive.

(4) In general, the conditions at Fallon, as in any arid region, favor nitrification, which frequently becomes intense; the conditions rarely favor denitrification. Lack of nitrification, therefore, will not be a limiting factor in crop production, nor is there evidence of overnitrification or injury from excessive quantities of nitrate. Humification studies are probably of paramount importance.

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U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 212.
B. T. GALLOWAY, *Chief of Bureau.*

A STUDY OF FARM EQUIPMENT IN OHIO.

BY

L. W. ELLIS,

Assistant in Farm Management, in Cooperation with the Department of Cooperation of the Ohio Agricultural Experiment Station.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., February 1, 1911.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 212 of the series of this Bureau the accompanying manuscript, entitled "A Study of Farm Equipment in Ohio," prepared by Mr. L. W. Ellis, Assistant in the Office of Farm Management.

This paper is based on a detailed study, made in cooperation with the Department of Cooperation of the Ohio Agricultural Experiment Station, of the equipment and the distribution of investment on a large number of Ohio farms. Few people realize the relationship of land, buildings, equipment, stock, machinery, cropping systems, and working capital to successful farming. This paper points out these relationships as they were found to exist on farms of various types in Ohio, and is believed to be a valuable contribution to the science of farm management.

Respectfully,

Wm. A. Taylor,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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A STUDY OF FARM EQUIPMENT IN OHIO.

INTRODUCTION.

Successful farm management presupposes a proper relation between the various factors of production. The process of adjusting land, labor, and capital into harmonious relationship is consciously or unconsciously followed by all farmers. In the course of time the successful farmer reaches the point where productive area, live stock, cropping system, labor, equipment, and working capital are properly balanced and a profitable routine may be followed. Before that point is reached, however, many expensive mistakes are usually made, and perhaps none are more keenly felt than those arising from improper distribution of capital.

The study of farm equipment was undertaken for the purpose of determining from the study of successful farms the proper relationships that should exist between investments in land, improvements, live stock, machinery, and tools.

This report presents the results of a study of equipment on a number of Ohio farms where conditions were unusually favorable for obtaining the desired information. The data and observations would undoubtedly have been more complete and satisfactory had a thorough analysis of the situation been possible in the light of later knowledge. They are here presented in order to illustrate by concrete example numerous problems that arise in this field of investigation. A portion of the data obtained in these investigations has already been published.[1]

METHODS OF PROCEDURE.

The work was done under the joint auspices of the Office of Farm Management and the Department of Cooperation of the Ohio Agricultural Experiment Station. During February and March, 1909, in connection with the annual inventories on the farms of about 35 statistical cooperators, a detailed study of the equipment was made in so far as it was possible to obtain information from the proprietor

[1] Circular 44, Bureau of Plant Industry, "Minor Articles of Farm Equipment." This circular will be sent free on application to the Secretary of Agriculture.

or manager. Specially prepared forms were used in order to embody full details. Previous surveys of the various farms by Mr. H. C. George, of the Ohio Agricultural Experiment Station, gave accurate data as to the size of each, the areas devoted to different purposes, the length and character of fences, and certain other details. Measurements and sketches were made of the buildings, and numerous details as to their character and condition were noted. The extent, character, and cost of water supply and drainage systems were studied. The usual inventory of live stock, machinery, tools, and supplies was made to include many details in addition to mere values. Messrs. Abbott, Bugby, Elser, and Lloyd, of the staff of the Ohio Agricultural Experiment Station, assisted at various times in the field work, and Mr. C. A. Massaro, of the station staff, assisted in the compilation of the data.

Difficulty was encountered on every farm studied in obtaining all the details desired. Especially was this true in the matter of cost of permanent improvements, the installation of which usually antedated the tenure of the incumbent proprietor. The determination of the present value of real and personal property was also especially difficult, as a uniform basis could not be maintained for the reconciliation of exchange value with the value in use.

Previous to the work just mentioned about 20 successful Ohio farms were visited by Mr. H. C. Thompson, of the Office of Farm Management, and less complete equipment studies made. Some data from this source are included in this report. A third source of data consists of circular letters dealing with corn and tillage machinery which were sent out in 1908 to a selected list of Ohio farmers. Over 100 carefully prepared reports of this character have been drawn upon for material.

CHARACTER OF FARMS STUDIED.

The farms from which data are embodied in this report are probably above the average type in the character of the proprietors, methods and equipment, yet they are not necessarily examples of exceptionally successful management. They are well scattered over the State, as shown in figure 1. Only those visited in 1909 were analyzed as to the chief enterprises conducted. For convenience, these farms have been numbered as in the various tables presented later. On 23 of these farms it was found possible to make a complete distribution of investment by enterprises, and this report has chiefly to deal with the farms so distinguished, but data from two of them are excluded from the averages here given because one was a small truck farm and the other a general farm on which special conditions had operated to

reduce the equipment investment to an abnormally low figure. Figures from both these farms, as well as from a number of farms on which the analysis could not be completed, are nevertheless made available for comparison.

The 21 farms represented in the tables showing average distribution of investment range in area from about 50 to 400 acres, the average being about 166 acres. In this and other particulars they differ materially from the State averages as reported in the Twelfth

FIG. 1.—Map of Ohio, showing the location and the numbers of the farms referred to in the tables of this bulletin.

Census (1900). According to the census report 32.4 per cent of the farms of the State were between 50 and 100 acres in area, and 24.3 per cent were between 100 and 175 acres. Table I presents a comparison of the average values for all farms in the State, as shown by the census, with the average values for the 21 farms. It will be remembered, however, that the census valuations are made on the basis of sale values. In taking the inventories of the farms included in this investigation, consideration was given to both the sale value and the

original cost of the property, less a reasonable depreciation charge based on its condition, the length of time already in use, and its expected total life. Contemplation of this difference of method will lessen the apparent difference between these farms and the average for the State.

TABLE I.—*Comparison of average values for all Ohio farms (census of 1900) with average values for a group of 21 farms of this investigation.*

Items of valuation.	For the State (average area 88.5 acres; 78.5 per cent improved).[1]			For 21 farms of this investigation (average area 165.88 acres; 80.9 per cent improved).[1]		
	Per farm.	Per acre.	Per cent.	Per farm.	Per acre.	Per cent.
Total of land, improvements, live stock, and machinery	$4,333	$48.96	100.00	$14,461.10	$87.17	100.00
Of land, fences, drainage, water supply, etc.	2,953	33.37	68.16	8,748.56	52.72	60.48
Of buildings	793	8.96	18.30	3,049.47	18.38	21.08
Of implements and machinery	132	1.49	3.04	773.92	4.67	5.36
Of live stock	455	5.14	10.50	1,889.15	11.40	13.08

[1] In the average for the entire State the item of improved land includes all land regularly tilled or mowed, land pastured and cropped in rotation, land lying fallow, land in gardens, orchards, vineyards, or nurseries, and land occupied by buildings. No instructions were given to census enumerators as to the disposition of public and private roads, all or part of which may be included in the farm areas covered by deeds. In the average for the 21 farms, waste land, roads, and barn lots are classed together as nonproductive. Pastures, tilled fields, and orchards constitute 80.9 per cent of the total area. (See Table II for details of acreage.)

Of the 21 farms 6 include dairying as the principal enterprise, 1 is devoted largely to feeding sheep, and 2 others place greater emphasis on the feeding of cattle than the average farm, but in no instance are the equipment and management those of a highly specialized type of farm. They represent, on the whole, the most common type of farm to be found in the State.

Concerning the farms visited by Mr. Thompson and those covered by circular letter it may be said that they represent the general rather than any special type, and are probably better organized, equipped, and managed than the average of all farms in the State. It is the equipment of this class rather than that of highly specialized farms or that of groups including both the best and poorest examples of farming that has been studied in the endeavor to establish logical relationships between the land, improvements, stock, and machinery required for successful operation.

The data here presented are conclusive only in so far as the farms studied are typical. It is held, however, that similar analyses of a large number of farms in any section would afford reliable averages from which the proper distribution of capital in equipment for a given farm could be predetermined with scientific accuracy.

DISTRIBUTION OF INVESTMENTS.

Three distinct objects are sought in this study of farm equipment: (1) The amount of equipment necessary and its first cost; (2) the inventory valuation at a given time; and (3) the equipment charge on farm operations, a portion of which is represented in the difference between the first cost and a succeeding inventory valuation. The second phase will be discussed first; that is, the present distribution of investment as shown by the inventory. Land, buildings, fences, drainage, water supply, live stock, machinery and tools, and produce and supplies are regarded as the principal classes of equipment. These classes are also divided among the enterprises. The enterprise rather than the farm is regarded as the unit.

LAND.

Table II shows the distribution of acreage for 1909 by enterprises for the various farms. The term "General" includes areas in lots, lanes, waste spots, public roads, and all other lands belonging to the farm which can not properly be charged to one enterprise or to a group of enterprises. "Household" includes the dooryard, the family garden, and also the orchard where the growing of orchard fruits is not at all a commercial proposition. Tenant yard, garden, etc., are charged to "Labor." "All stock" refers to all lots and fields devoted exclusively to live stock. Where pastured fields contain any considerable growth of trees, the judgment of the surveyor was relied upon for a division of the field into pasture and woodland. Temporary pastures are included under this head; hence, the areas devoted to "All stock" and "All crops" would vary from year to year. The term "All crops" includes all tilled and mowed fields. On several farms certain groves, considered as permanent, were maintained largely for the production of maple sugar or sirup, hence the occurrence of a "Sugar" enterprise. The term "Orchard" includes only fruit orchards largely commercial in their nature. "Woodland" comprises not only natural tracts but areas planted for the production of wood, posts, etc. The value given for the bare land represents as accurately as possible the value exclusive of all improvements.

TABLE II.—*Acreages devoted to various enterprises on 23 farms and the value of the land minus all improvements, with the average and the percentage of the total for a group of 21 of these farms.*[1]

Designation of farm.	General.	Household.	Labor.	All stock.	All crops.	Sugar.	Orchard.	Woodland.	Total.	Per cent in crops.	Value of bare land per acre.
1	0.93	0.88	.....	56.43	35.86	.....	.....	22.10	116.20	30.8	$61.62
2	3.86	2.33	.....	54.42	68.14	.....	.....	35.36	164.11	41.5	19.53
3	3.68	1.66	.....	16.97	53.71	.....	14.43	13.80	104.25	51.5	41.44
4	4.38	1.36	.....	25.96	56.22	16.35	4.07	.....	108.34	51.8	31.15
6	4.28	3.57	.....	37.50	73.65	23.27	.....	1.05	143.32	51.4	24.18
7	4.07	1.69	.....	18.98	20.80	.....	.....	4.07	49.61	42.0	33.00
8	2.94	2.03	.....	14.52	58.15	.....	.....	1.00	78.64	74.0	87.74
9	5.41	1.02	0.35	31.93	82.96	.....	.....	26.00	147.67	56.1	65.99
10	5.44	1.34	.....	5.00	84.19	.....	.....	4.03	100.00	84.2	71.00
12	1.83	2.20	.....	28.34	104.60	.....	.....	20.00	156.97	66.7	50.14
13	4.93	3.63	.....	33.02	140.76	.....	.....	15.91	198.25	71.0	45.55
14	8.98	3.67	.....	122.60	197.00	.....	.....	56.67	388.92	50.7	60.00
15	4.83	3.26	.....	67.75	128.85	.....	.....	15.13	219.82	58.6	43.90
16	3.35	1.62	.....	11.84	116.42	.....	.....	39.29	172.52	67.5	45.97
17	10.38	2.65	.49	84.93	124.47	23.05	.....	30.02	275.99	45.1	64.89
18	8.40	1.45	.....	26.32	123.20	.....	3.71	44.75	207.83	59.4	56.49
19	3.00	1.65	.....	21.81	69.42	.....	.....	7.93	103.81	66.8	40.17
20	7.18	.50	.....	84.31	84.56	.....	.99	7.71	185.25	45.7	43.97
21	14.11	1.47	.73	62.44	68.58	.....	4.79	76.50	228.62	30.0	22.26
22	3.33	1.46	.....	103.84	31.17	.....	2.44	13.76	156.00	19.9	25.55
23	10.31	3.36	.....	67.66	77.30	.....	10.49	8.15	177.27	43.5	29.59
24	3.85	1.56	.....	47.00	79.23	.....	.....	23.04	148.38	48.2	19.61
25	.65	.23	.....	.....	9.97	.....	.....	.....	10.85	91.9	40.10
For the group of 21 farms:[1]										(mean)	(mean)
Average	5.51	2.04	.08	46.50	85.71	2.98	1.95	21.11	165.88	52.8	45.96
Per cent of total acreage	3.32	1.23	.05	28.01	51.68	1.80	1.18	12.73	100.00	.....	.....

[1] Nos. 5 and 11 omitted; Nos. 24 and 25 not included in average.

An examination of the table shows that the mean average acre valuation of bare land for 21 farms is $45.96. For farm 1 the acre valuation of bare land is $61.62. For farm 2 it is $19.53. These are both dairy farms in the northeastern part of the State. Farm 1 is 1½ miles from town, on a stone pike, while farm 2 is 5 miles out, on a dirt road. Part of the woodland of farm 1, but no distinct area, produces maple sirup in commercial quantities. Farm 4, with an acre valuation of $31.15, and farms 8, 9, and 10, with acre valuations of $87.74, $65.99, and $71, respectively, are all level farms. No. 4 needs considerable drainage. Nos. 8, 9, and 10 are well equipped with tile drains. Nos. 8 and 10 show high percentages (74 and 84.2, respectively) of land in crops, as compared with the mean average of 52.8 per cent for the 21 farms. Farm 25, with 91.9 per cent of land in tilled crops, and situated within a stone's throw of an interurban railway, shows a bare-land valuation of $40.10 per acre. This farm, however, lacks tile drainage and is overequipped with buildings as compared with other farms. (See Table III for data on building equipment.) Farm 3, with an acre valuation of $41.44, has a very expensive building equipment, and even when the latter is placed at a very low figure compared with its cost it leaves a low

figure for bare land. Farm 14, although the largest of all, with a total of 388.92 acres, has but 50.7 per cent of the land in crops. It contains, however, a large acreage of productive bottom land, has a low building investment per acre, and has good roads to a shipping point, so that the bare land has an acre valuation of $60 as compared with the average of $45.96 for the 21 farms. Farms 20, 21, 22, and 23, with bare-land valuations of $43.97, $22.26, $25.55, and $29.59, respectively, are all located in the hill section (southeastern part) of the State. No. 20 (valuation $43.97) shows an unusually low area in waste and timber land for a hill farm and is connected with town by 6 miles of pike road. No. 23 (valuation $29.59), with nearly the same area, distribution of acreage, and distance from railway station, is separated by 3 miles of hilly dirt road from the pike leading to town. No. 21 (valuation $22.26) has considerable waste and timber land; and No. 22 (valuation $25.55) has been wisely kept in pasture for the greater part, though a greater area in crops would have made it more attractive to a buyer. Farms 12 to 17, inclusive, range in bare-land value from $43.90 for No. 15 to $64.89 for No. 17 and are located in the large-farm area of central and southwestern Ohio. Only one of this group falls below the average bare-land valuation of $45.96. These farms are well equipped with buildings and are easily reached by pike roads from good towns. Most of them show a higher percentage of crop land than the mean of the whole number and are in a high state of productivity. Farm 24, with a bare-land valuation of $19.61, is located in a rougher section in southern Ohio, is underequipped in buildings, and is conservatively valued rather than otherwise.

From these examples the land values due to good roads, good drainage, high percentage of crop areas, good topography, and adequate improvements can be plainly seen.

PERMANENT IMPROVEMENTS.

The appraisement of the true value of permanent improvements in this study was extremely difficult and the values given must be accepted with due allowances. Wherever practicable the basis for fixing values should be that expressed in the following question: "What is the value of this item as a part of the equipment of this farm, remembering that the sum of these values must equal the value set upon the farm as a whole?" Land values have increased in nearly every section, unfortunately not through improvement of the land by farming, but through an advance in the value of land as a raw material. We have no means of determining the present producing power of a given farm as compared with that at the outset, nor what its rate of appreciation or depreciation has been in this

respect. It seems well established that where no systematic steps have been taken to prevent it or to repair damage there has been a steady depreciation in the productiveness of these farms. The buildings and other improvements on any farm may clearly have undergone a process of deterioration, yet the sale value of the farm may have been enhanced, not only by the rise in land values, but also by increase in value of the raw materials from which improvements are constructed. Well-planned improvements may add value to the farm above their cost of installation, while others may immediately represent the loss of a large part of their cost, if measured by their effect on the farm value. Each farm, therefore, was studied as an individual problem and is most interesting when considered in that light.

DRAINAGE.

Tile drains are so intimately associated with the land that it may be impracticable to consider them separately. With the possible exception of the cost of water supply, the outlay in tile drainage is only one which can be depended on to add its face value or more to the value of the bare land and continue to do so indefinitely. The drains occasionally become clogged and require cleaning, but in this study they have been appraised at the full cost of installation. To attempt to appraise them accurately on the basis of their effect on the farm value would be impossible from the information at hand. No valuation has been placed on natural drainage channels considered aside from the land. The investment in artificial drainage systems has been attributed directly to the portions of the farm drained.

WATER SUPPLY.

On many Ohio farms there are natural sources of water supply, which, like natural drainage, can scarcely be valued apart from the land. Their value may not equal their cost, as in the case of streams which permanently render a considerable area unavailable for cropping or which subject fields and fences to damage from high water. On the other hand, the value of a continuous supply of pure water in a convenient place, without expense or labor, can not be estimated by comparing it with the cost of installing artificial water systems, which may represent several failures before a satisfactory supply is obtained and will surely represent a continual expense for labor and maintenance. In studying the distribution of the investment, only the cost of installing the water system has been considered, less a fair amount for depreciation of pumps, tanks, windmills, etc. This total investment in water system has been divided as accurately as possible among the various enterprises on the basis of use. This naturally

places the heaviest charges on the household and those classes of live stock which do not have access to natural supplies in the fields.

FENCES.

Fences well planned and constructed undoubtedly add at first more than their cost to the value of farms, yet, if not well located, they may prove a handicap to the most profitable cropping systems. They are subject to rapid deterioration, involving considerable attention and expense; hence, overequipment in fences may tend to reduce land values.

Certain phases of the fence question were studied in detail and will be discussed later, but in ascertaining the investment in fences the first cost and the condition at the date of inventory were the only points considered. The cost of construction was difficult to obtain, owing to the fact that practically all fences are built by farm labor, and standard costs per rod have not been established, as has been done, for instance, for the digging of ditches for tile drains, which is often paid for on a unit basis. The price of posts varies widely in different localities and has generally advanced since the building of the older fences.

The value of fences, therefore, was based largely on the cost of replacing them, less a fair percentage for depreciation. Worm rail fences constitute a large proportion of the total on many Ohio farms. When built, the value of the material was practically disregarded and labor costs were very low as compared with the present rates. It would be impossible to replace these fences except at a prohibitive cost, yet their real value to the farm is no more than that of modern fences. Many are in an excellent state of preservation, yet occupy enough additional ground to offset any advantages they may have over wire fences. As an expedient they have been valued at a figure approximating the labor cost of building. All fences were charged to "General enterprises," only the farm's share of division fences being included.

BUILDINGS.

Many buildings found on the farms studied are from 40 to 75 years old and of a type of construction not commonly used at present, the frames being composed of large, hewn timbers. Much of the other material has been cut and sawed on the farm, the value of the timber at that time being very low as compared with present prices. These buildings, as a rule, are still in such condition as to be capable of long service without excessive repairs. The first cost of material and labor was low, yet on the present basis it would be almost out of the question to duplicate the buildings.

It follows, then, that neither the cost of building nor the cost of replacing these structures can be relied upon absolutely in appraising

their value. As previously stated, the cost of the more modern buildings is not a true indication of their value to the farm, but insurance figures are quite largely based on their condition and the cost of replacing them. A comparison of the sale values of land without buildings and land with buildings, all in the same neighborhood and of equal productiveness, shows that the difference in favor of the buildings is almost without exception greatly insufficient to equip the unimproved land with those structures which are absolutely necessary to the conduct of an independent farming enterprise. The real value of farm buildings as a part of the total investment is therefore very difficult to ascertain, and depends largely on the point of view.

In this study the building values are a compromise between the cost of equipping the farm with similar structures, less a proper amount for depreciation, and the sale value of the buildings as suggested by comparing the values of land with and without buildings. The value shown for the bare land, therefore, is reduced somewhat by this method, possibly as much as it was increased by the method of appraising the fence, drainage, and water-supply systems.

It can safely be said that buildings represent not only the most expensive class of farm equipment, but the least negotiable. Leaving out household buildings, the remainder on the farms studied shows a much greater variation in investment per acre than any other class of equipment, and a greater variation in percentage of the total investment than land, water supply, live stock, or machinery. Fences, artificial drainage, and water systems may often be dispensed with wholly or to a great extent; hence they are scarcely comparable with land, buildings, live stock, and machinery as regards the relative investment.

One of the most important phases of a study of farm equipment is the determining of the relation that should exist between buildings and the farm enterprises, in order to reduce the wide variation in investment per acre in buildings designed for the same purposes. Prior to a study of the cost and construction of buildings there should be established standard space units to be used in determining the actual building requirements of the farm for the storage of products and machinery, the housing of live stock, and the transaction of the farm affairs. In this study buildings were investigated from that standpoint, but insufficient data were gathered to allow of generalizations.

For purposes outside of this study it became desirable to make a division of building investment by enterprises. As the floor and cubic space devoted to each enterprise had been calculated for the various buildings, a division on the basis of cubic space was worked out and is presented later in tables and discussions.

It will be at once apparent that a division of space on the basis of cubic feet devoted to various enterprises in barns, for instance, is open to serious criticism. This subjects such products as hay, straw, etc., stored in mows, to greater building charges than horses and cattle, for which greater expense is incurred in constructing stalls, mangers, floors, etc. In order to correct this error additional study of the cost of construction of the various portions of the buildings would be necessary, and the need for this did not occur in time to include it in the scope of this study.

Factors for the relative cost of various portions of farm buildings of ordinary construction could no doubt be worked out, by means of which the cubic space devoted to any enterprise could be made the basis for an equitable division of the total value. Some method is desirable, as it is incorrect to charge live-stock enterprises with the investment in portions of the buildings devoted to other enterprises. Animals may be fed grain in a barn for a short time each day and pastured outside, while both hay and grain may be stored in the barn continuously for market. A storage charge, in the latter case, should unquestionably be added to the cost of production. It is only logical to base the unit charge on the amount of the commodities stored, taken in connection with the total annual cost of that part of the building designed exclusively for storing products. A unit-storage charge based on cubic space would place on the proper classes of live stock the burden of the large amount of storage space required for roughage. A division of the entire building charge on the basis of the number of 1,000 pounds head of stock sheltered, or on the floor space occupied, might be unjust to the hog enterprise, for which a comparatively small space is required for storage of feed. A tool room, workshop, driveway, or other space may be used for storing tools, wagons, and machinery, for storing and preparing seed, and for other purposes which are obviously not associated with live-stock enterprises. Conceding the partial inaccuracy of a division of building values on the basis of cubic space occupied, it is contended that even this method results in a distribution of building charges more nearly correct than one based on the number, size, or value of live stock alone. Considering the importance of the building equipment, it is unfortunate that so little investigation has been conducted with a view to discovering the fundamental principles involved in the economical planning of farm buildings.

PERSONAL PROPERTY.

All personal property has been valued with due consideration for both exchange value and value in use. Marketable live stock and products were invoiced at the prevailing prices. Work animals and machinery, however, have a value to the farmer not necessarily the

same as that which would prevail at either public or private sale. This fact has been taken into consideration; hence, the values presented for the work horses, mules, and machinery are usually higher than sale values. The sale values if estimated would have been only approximate at best. All products of the farm, all feed, seed, building material, fuel, and supplies of any kind held in storage for sale or for the use of the farm (not household) business, were inventoried at actual values so far as they could be determined.

Table III shows the total investment in different classes of equipment for the various farms, distributed as here explained.

TABLE III.—*Total investment in the different classes of farm equipment for 25 Ohio farms.*

Designation of farm.	Area (acres).	Land.	Buildings.		Fences.	Drainage.	Water supply.	Live stock.	Machinery, etc.	Produce, supplies, etc.	Total.	Acre investment.
			Farm.	Household.								
1...	116.20	$7,160	$1,025	$1,500	$245	$45	$50	$1,265.00	$667.50	$220.85	$12,178.35	$104.81
2...	164.11	3,205	1,000	800	250	85	60	1,651.20	623.95	520.22	8,195.37	49.94
3...	104.25	4,320	2,800	2,500	455	250	100	1,363.75	664.25	671.95	13,124.95	125.90
4...	108.34	3,375	1,405	700	320	30	170	1,336.25	682.34	329.02	8,347.61	77.05
5...	342.00	9,570	6,250	6,110	1,255	2,795	700	3,549.00	1,065.80	1,942.15	33,236.95	97.18
6...	143.32	3,465	900	900	565	.....	170	1,767.68	1,086.68	323.50	9,177.86	64.04
7...	49.61	1,637	440	310	223	60	80	959.75	630.38	131.65	4,471.78	90.14
8...	78.64	6,900	1,000	1,879	400	1,100	250	654.40	547.05	351.45	13,081.90	166.30
9...	147.67	9,945	1,490	2,060	95	500	110	1,804.00	1,267.10	1,298.23	18,369.33	124.32
10...	100.00	7,100	1,215	1,525	590	1,770	300	1,496.50	645.10	274.75	14,916.35	149.17
11...	186.71	15,001	1,525	1,225	630	.....	290	2,942.00	1,313.34	1,203.52	24,129.86	129.24
12...	156.97	7,870	3,830	1,800	395	1,830	275	2,516.75	788.75	653.00	19,958.50	127.15
13...	198.25	9,228	2,250	2,850	1,130	680	350	2,438.55	980.25	1,390.60	21,297.40	107.43
14...	388.92	23,335	1,720	1,585	890	345	157	3,936.70	1,115.45	1,478.40	34,562.55	88.87
15...	219.82	9,650	930	900	800	135	125	1,975.65	679.90	609.55	15,805.10	71.90
16...	172.52	7,930	825	720	625	.....	100	1,286.50	679.40	527.70	12,693.60	73.58
17...	275.99	17,910	1,225	2,900	1,070	220	135	3,450.00	1,024.00	275.25	28,209.25	102.21
18...	207.83	11,740	3,277	1,843	315	345	150	1,917.78	1,044.67	1,009.75	21,642.20	104.14
19...	103.81	4,170	2,370	1,020	400	300	70	2,550.00	731.55	926.90	12,538.45	80.22
20...	185.25	8,145	2,235	1,150	660	.....	50	2,883.75	556.10	817.80	16,497.65	89.05
21...	228.62	5,090	724	1,726	700	.....	550	1,362.50	807.90	369.95	11,330.35	49.56
22...	156.00	3,985	1,060	1,500	365	.....	70	1,281.50	346.60	285.25	8,893.35	57.01
23...	177.27	5,245	580	1,570	720	.....	285	1,774.00	683.10	804.70	11,661.80	65.78
24...	148.38	2,910	100	800	250	.....	20	860.00	173.20	115.45	5,228.65	35.21
25...	10.85	435	350	500	45	.....	20	238.15	156.20	10.10	1,754.45	124.43

The area given for farm 5 (342 acres) was not verified by the surveyor from the Ohio experiment station. The proprietor of farm 11 could not give the extent nor the value of the tile drains, hence this value is included in that of the land. A very slight quantity of tile is included in the land value for farm 16. The total investment is shown for the different farms to vary from $35.21 to $166.30 per acre, and this variation is brought out even more clearly by Table IV, which reduces each class of investment to the acre basis. The variation in total value of household buildings ($310 to $6,110) is interesting from the fact that the investment in this direction is usually not based on the absolute needs of the farm. The variation in the amount of produce and supplies on hand ($10.10 to $1,942.15) is due partly to the fact that the work of taking the inventories lasted over a period of six weeks, during which time, of course, the consumption of feed continued. For comparable data all inventories,

particularly of supplies, should be taken on the same date. In this study, except as affecting the percentages of the total investment shown later, the quantity of supplies on hand is unimportant.

Table IV shows the acre investment in the various classes of equipment for 30 farms. With the exception of Nos. 5 and 11 all farms, to and including No. 23, have also been included in tables showing the division of investment by enterprises; hence, they are separated in this table from those for which the data are less complete. Farms 5, 27, 28, and 30 had not been surveyed by the station surveyor up to the time these data were compiled; hence, the acreages are only approximate. For several farms the value of improvements was not separated from that of the land for want of sufficient information. The land value of such farms includes all permanent improvements not otherwise shown. These incomplete data are presented for comparison with the mean and average for the 21 farms. While the data for farms 24 and 25 were complete, they are excluded from the summary as not representative, the former because of the extremely low investment and the latter because of the low acreage.

TABLE IV.—*Average investment per acre in land, improvements, and personal farm property on each of 30 Ohio farms, with the mean and the average for a group of 21 of these farms.*

Designation of farm.	Area (acres).	Land.	Buildings.		Fences.	Drainage.	Water supply.	Live stock.	Machinery, etc.	Produce, supplies, etc.
			Farm.	Household.						
1	116.20	$61.62	$8.82	$12.91	$2.11	$0.39	$0.43	$10.89	$5.74	$1.90
2	164.11	19.53	6.09	4.88	1.52	.52	.37	10.06	3.80	3.17
3	104.25	41.44	26.85	23.98	4.37	2.40	.96	13.08	6.37	6.45
4	108.34	31.15	12.96	6.46	2.96	.28	1.57	12.33	6.30	3.04
6	143.32	24.18	6.29	6.29	3.94	.....	1.19	12.32	7.58	2.26
7	49.61	33.00	8.87	6.25	4.50	1.21	1.61	19.35	12.70	2.65
8	78.64	87.74	12.70	23.90	5.08	13.98	3.17	8.31	6.94	4.48
9	147.67	65.99	10.08	13.93	.64	3.38	.74	12.20	8.56	8.80
10	100.00	71.00	12.15	15.25	5.90	17.70	3.00	14.97	6.45	2.75
12	156.97	50.14	24.40	11.46	2.52	11.66	1.75	16.02	5.03	4.17
13	198.25	46.55	11.35	14.38	5.69	3.43	1.77	12.30	4.95	7.01
14	388.92	60.00	4.43	4.07	2.29	.89	.40	10.11	2.87	3.80
15	219.82	43.90	4.23	4.09	3.65	.61	.57	8.99	3.09	2.77
16	172.52	45.97	4.78	4.17	3.62	.....	.58	7.46	3.94	3.06
17	275.99	64.89	4.44	10.51	3.88	.79	.49	12.50	3.71	1.00
18	207.83	56.49	15.78	8.86	1.52	1.66	.72	9.24	5.02	4.85
19	103.81	40.17	22.81	9.81	3.86	2.89	.68	24.58	7.05	8.93
20	185.25	43.97	12.07	6.20	3.56	.....	.27	15.57	3.00	4.41
21	228.62	22.26	3.16	7.55	3.06	.....	2.40	5.95	3.54	1.64
22	156.00	25.55	6.79	9.62	2.35	.....	.44	8.21	2.22	1.83
23	177.27	29.59	3.27	8.86	4.06	.....	1.60	10.01	3.85	4.54
For the group of 21 farms:[1]										
Mean (farm unit)	165.88	45.96	10.59	10.16	3.39	2.94	1.18	12.12	5.36	3.97
Average (acreage unit)	165.88	46.25	9.27	9.11	3.22	2.21	1.04	11.40	4.67	3.81
For the entire State:										
Average (census of 1900)	88.50	33.37	8.96	.....	.....	.....	.....	5.14	1.49	.....
5	342.00	27.98	18.29	17.87	3.66	8.17	2.04	10.38	3.14	5.68
11	186.71	80.34	8.17	6.56	3.38	.....	1.55	15.76	7.04	6.44
24	148.38	19.61	.67	5.39	1.68	.....	.14	5.78	1.17	.78
25	10.85	40.10	32.25	46.09	4.15	.....	1.84	21.95	14.39	.93
26	156.86	65.00	.....	.....	.....	.....	.....	7.53	4.51	1.38
27	180.00	69.98	9.30	10.16	.....	.....	.....	11.95	3.60	2.45
28	504.00	70.00	17.11	6.55	.....	.....	.....	24.12	7.56	4.73
29	156.00	76.92	7.20	15.88	.....	.....	.....	9.30	7.19	4.02
30	79.00	48.04	6.33	31.64	1.46	.....	1.14	13.20	6.13	2.84

[1] Nos. 5 and 11 omitted.

A close study of Table IV will reveal striking differences in the investment per acre for different purposes. As a basis for comparing the individual farms the mean and the average of the data from 21 farms are both included. The mean is obtained by adding together the figures per acre for the 21 farms and dividing by 21, while the average is computed by taking the total investment for the 21 farms and dividing by the sum of their acreages. The mean, then, is an average having the farm as a unit, while the average regards the acre as the unit. These two might vary widely, and the fact that they do not adds to the value of the table. In this study of farms the mean is regarded as the more suggestive, since it takes into account the effect of the size of the farm upon the acre investment.

The range of investment per acre in farm buildings is seen to be from 67 cents on farm 24, where a very old barn and several equally old sheds, etc., constituted the building equipment, to $32.25 for farm 25, where the value of a small barn and poultry house is divided by a small acreage. The investment varies with the condition and number of buildings, but the number and cost do not vary with the acreage.

Farms 13 to 17 are similar in character and location, yet the building equipment on farm 13 is $11.35 per acre, while on Nos. 14 to 17, inclusive, the valuation does not reach $5 per acre on any farm. This is due to the fact that farm 13 is really composed of three farms formerly separate. On the other hand, farms 3, 5, 12, 18, 19, and 28, ranging in size from 104 to 504 acres, show an investment in farm buildings of $15.78 to $26.85 per acre, while farms 7, 8, 10, and 30, varying in size from 49.61 to 100 acres, have an investment in farm buildings of but $6.33 to $12.70 per acre.

In household buildings (dwellings) there is a variation from $4.07 to $46.09 per acre. The 21 farms as a whole have practically the same investment in farm buildings and in household buildings ($10.59 and $10.16, respectively), but among the 30 farms wide extremes are represented. Farms 4, 12, 18, 19, 20, and 28 show two to three times as great an acre investment ($12.07 to $24.40) in farm buildings as in household buildings ($6.20 to $11.46), while on farms 8, 21, 23, 24, 29, and 30 the investment in household buildings ($5.39 to $31.64) is two to five times as great as in farm buildings ($3.16 to $12.70 per acre).

No particular need is apparent for such a wide variation in practice, and on a number of the most successful farms the investment in household and farm buildings is about equal. On farm 24, with a farm-building investment of $0.67 per acre and a household-building investment of $5.39 per acre, a new barn was to be erected within a year or two which would bring about nearly the same relative

condition as exists on farm 18, on which a $3,000 barn had just been completed and on which the farm and household building investments were $15.78 and $8.86 per acre, respectively. The owner of farm 30 moved from the city only a few years ago and invested the greater part of his ready capital in remodeling the dwelling. His percentage of total investment represented by the household building is much higher than that of any other farm except No. 25, the small-truck and poultry farm, and slightly exceeds even that. This owner noted the lack of certain essential machinery, which lack was directly due to the excessive outlay in household buildings and conveniences.

New buildings for either household or farm use tend, of course, to vary the relation, as does also the presence of tenant houses, which are classed with household buildings, yet the few farms studied would indicate that the investment in buildings for the two purposes should be approximately equal for farms of the general class.

A large part of farm 9, with an investment for fencing of only 64 cents per acre, is unfenced, and on several others a large extent of rail fence accounts for a low investment per acre. Attention is called to farms 7 and 8, with fencing investments of $4.50 and $5.08 per acre, respectively, on which the proportion of road fence is particularly large. Farm 13 has considerable road fence, but the high investment ($5.69 per acre) is largely due to the recent construction of woven-wire fences and the generally good condition of those previously installed.

The acre investment in tile drainage and water supply depends largely on the natural advantages of the farm. The extremes are, for drainage, 28 cents on farm 4 and $17.70 on farm 10, the average being $2.21. The extremes for water supply are 37 cents on farm 2 and $3.17 on farm 8, with an average of $1.04 for the 21 farms. Farms 8 and 10 have a high investment in all improvements and are the two highest in the valuation of tile drainage, $13.98 and $17.70 per acre, respectively, yet they show the highest bare-land values, $87.74 and $71 per acre, respectively. Both are connected with town by good stone roads, but the thorough drainage undoubtedly is a large factor in maintaining the value of the land.

The small acreage of farms 7 and 25 (49.61 and 10.85, respectively) makes the acre investment in water systems large, even though the systems are not extensive. Farms 8, 21, and 23, with an acre valuation for water supply of $3.17, $2.40, and $1.60, respectively, have more or less extensive water conveniences in the dwellings. Farms 21 and 23, with investments of $2.40 and $1.60 per acre, respectively, for water, are to be contrasted with farms 18, 19, 20, and 22, with the respective valuations of 72, 68, 27, and 44 cents. These four farms are also in what is known as the hill section; hence, water might easily be obtained from springs, but the water conveniences have

not been extended to the dwellings. Gasoline engines used only for pumping add to the investments on farms 10, 12, and 13, with the acre valuation for water supply of $3, $1.75, and $1.77 per acre, respectively.

The live-stock inventory, like that of produce, supplies, etc., should be taken on the same date for all farms in order to be comparable. This fact is brought out strikingly by farm 12. The inventory in 1908 showed $1,700 worth of steers on hand, or nearly $11 per acre for this class of stock alone. Several days previous to the 1909 inventory 39 head were sold, hence this farm, which is usually heavily stocked with cattle, shows a lower acre investment ($16.02) than its average for the year. The inventory of live stock, even if taken on the same date each year for all farms, would not show the average investment accurately, as on some farms feeding stock are purchased, fed, and marketed between succeeding dates of inventory. This would entail the investment of a considerable amount of capital for the greater part of the year which would not be apparent in a study of inventories. The study of investment in live stock can best be made in connection with Table VIII (p. 27) which shows the relative importance of the various live-stock enterprises.

With the exception of 4 farms the acre investment in machinery, wagons, harness, tools, etc., ranges within comparatively narrow limits (from $2.87 for farm 13 to $7.56 for farm 28.) The four exceptions are farm 22 (acre valuation $2.22), for which much of the machinery was borrowed; farm 24 (acre valuation $1.17), for which machinery was generally bought second hand; and farms 7 and 25 (valuations $12.70 and $14.39), which are low in acreage. With the exception of farms 22, 24, 25, and 28, the total machinery investment per farm is seen by reference to Table III to vary only about 136 per cent, as compared, for instance, to 1,275 per cent for the total value of farm buildings and 835 per cent for household buildings. Two large farms (5 and 14) containing 342 and 388.92 acres, respectively, show low acre investments in machinery ($3.14 and $2.87, respectively), while farm 28, the largest, containing 504 acres, ranks among the highest, showing an acre investment of $7.56 and indicating overequipment.

The total and percentage of investment per acre in real and personal property is given in Table V, together with the mean and average for the group of 21 farms. The odd cents shown in the values of the real estate are due to the fractional parts of an acre in the farm areas, these usually being disregarded by the farm owners. The land with improvements is seen to range from $27.48 to $146.57 per acre, though nearly all farms are valued considerably higher than the State average as shown by the Twelfth Census, viz, $42.33 per acre. The amount of personal property per acre, $7.73

to $40.65, is higher than the State average, $6.63, in every case. It is to be remembered, however, that for comparison the value of produce, etc., is to be deducted from that of the personal property shown, the census values including only live stock and machinery. Excluding produce, etc., the average of the 21 farms shows 81.4 per cent of the total farm value in real estate and 18.6 per cent in personal property, as compared with 86.5 per cent and 13.5 per cent, respectively, for the State. The greater value of personal property on these farms argues the correctness of the statement previously made that the farms under consideration are more successful than the average.

Including produce, etc., a mean of the 30 farms shows 77.34 per cent of the total inventory value to be due to land and improvements. The mean of the 21 shows 77.6 per cent in real estate and the average 78.14 per cent. Seventeen out of 30 farms range between 77 per cent and 83 per cent in real estate, these having a mean of 79.8 per cent. These figures should serve as an indication of approximately the proper division of equipment capital on farms of this class, the cash and other assets of course not being considered in this study.

TABLE V.—*Total investment and percentage of investment per acre in real estate and personal property for each of 30 Ohio farms, with the mean and average for a group of 21 of these farms.*

Designation of farms.	Area (acres).	Real estate.		Personal property.		Total investment per acre.
		Total per acre.	Per cent.	Total per acre.	Per cent.	
1	116.20	$86.28	82.30	$18.50	17.70	$104.81
2	164.11	32.91	65.90	17.03	34.10	49.94
3	104.25	100.00	79.40	25.90	20.60	125.90
4	108.34	55.38	71.80	21.67	28.20	77.05
6	143.32	41.88	65.70	22.16	34.60	64.04
7	49.61	55.44	61.50	34.70	38.50	90.14
8	78.64	146.57	88.10	19.73	11.90	166.30
9	147.67	94.76	76.20	29.56	23.80	124.32
10	100.00	125.00	83.70	24.17	16.30	149.17
12	156.97	101.93	80.20	25.22	19.80	127.15
13	198.25	83.17	77.40	24.26	22.60	107.43
14	388.92	72.09	81.10	16.78	18.90	88.87
15	219.82	57.05	79.40	14.85	20.60	71.90
16	172.52	59.12	80.30	14.46	19.70	73.58
17	275.99	85.00	83.30	17.21	16.70	102.21
18	207.83	85.03	81.60	19.11	18.40	104.14
19	103.81	80.22	66.40	40.65	33.60	120.78
20	185.25	66.07	74.20	22.98	25.80	89.05
21	228.62	38.43	77.50	11.13	22.50	49.56
22	156.00	44.75	78.50	12.26	21.50	57.01
23	177.27	47.38	72.00	18.40	28.00	65.78
For the group of 21 farms:[1]						
Mean (farm unit)	165.88	74.22	77.60	21.45	22.40	95.67
Average (acreage unit)	165.88	72.10	78.14	18.88	21.86	90.98
For the entire State:						
Average (census of 1900)	88.50	42.33	86.50	6.63	13.50	48.96
5	342.00	78.01	80.30	19.17	19.70	97.19
11	186.71	100.00	77.40	29.24	22.60	129.24
24	148.38	27.48	78.00	7.73	22.00	35.21
25	10.85	124.43	77.00	37.27	23.00	161.70
26	156.86	65.00	82.90	13.42	17.10	78.42
27	180.00	90.00	83.30	18.00	16.70	108.00
28	504.00	93.66	72.00	36.41	28.00	130.07
29	156.00	100.00	83.00	20.51	17.00	120.51
30	79.00	88.61	80.00	22.17	20.00	110.78

[1] Nos. 5 and 11 omitted.

The percentage of the total investment represented by each class of equipment is given in Table VI. The uniformity in the percentage of value in land on farms 14 to 17 ($67.52, $61.10, $62.46, and $63.49, respectively) and farms 20 to 23 ($49.36, $44.90, $44.80, and $45, respectively) is interesting. The former are large level farms in the southwestern quarter of the State and the latter are large hill farms in the southeastern quarter. The influence of size of farm is to be seen in farms 7 and 25, and of large building equipment on several others already noted.

TABLE VI.—*Percentage of the total investment represented by each class of equipment on 30 Ohio farms, with the mean and the average for a group of 21 of these farms.*

Designation of farms.	Area (acres).	Land.	Buildings.		Fences.	Drainage.	Water supply.	Live stock.	Machinery, etc.	Produce, supplies, etc.
			Farm.	Household.						
1	116.20	58.79	8.42	12.31	2.01	0.37	0.41	10.39	5.48	1.82
2	164.11	39.12	12.20	9.74	3.05	1.04	.73	20.16	7.62	6.35
3	104.25	32.90	21.35	19.05	3.46	1.91	.76	10.39	5.06	5.12
4	108.34	40.42	16.64	8.29	3.84	.36	2.04	16.01	8.17	3.94
6	143.32	37.74	9.78	9.78	6.25	.....	1.85	19.25	11.82	3.51
7	49.61	36.60	9.85	6.93	4.99	1.34	1.79	21.46	14.10	2.94
8	78.64	52.72	7.64	14.36	3.05	8.39	1.90	5.00	4.17	2.77
9	147.67	53.06	8.11	11.21	.52	2.72	.60	9.82	6.89	7.07
10	100.00	47.60	8.15	10.23	3.95	11.87	2.01	10.03	4.32	1.84
12	156.97	39.42	19.18	9.02	1.98	9.18	1.38	12.60	3.96	3.28
13	198.25	43.35	10.55	13.39	5.30	3.20	1.64	11.44	4.60	6.53
14	388.92	67.52	4.97	4.58	2.58	1.00	.45	11.40	3.22	4.28
15	219.82	61.10	5.89	5.70	5.06	.80	.79	12.50	4.30	3.86
16	172.52	62.46	6.50	5.67	4.95	.....	.79	10.12	5.35	4.16
17	275.99	63.49	4.34	10.29	3.80	.78	.48	12.23	3.62	.97
18	207.83	54.24	15.14	8.51	1.46	1.59	.69	8.86	4.84	4.67
19	103.81	33.20	18.90	8.10	3.20	2.40	.60	20.30	5.90	7.40
20	185.25	49.36	13.55	6.97	4.00	.....	.30	17.48	3.37	4.97
21	228.62	44.90	6.38	15.22	6.20	.....	4.90	12.00	7.10	3.30
22	156.00	44.80	11.91	16.89	4.10	.....	.80	14.40	3.90	3.20
23	177.27	45.00	4.97	13.46	6.18	.....	2.42	15.21	5.86	6.90
For group of 21 farms: [1].										
Mean (farm unit)	165.88	48.04	11.08	10.61	3.54	3.07	1.23	12.68	5.60	4.15
Average (acreage unit)	165.88	50.82	10.20	10.01	3.54	2.43	1.14	12.54	5.13	4.19
For the entire State:										
Average (census 1900)	88.50	68.14	18.36	.....	.....	.....	.....	10.48	3.02	.....
5	342.00	28.80	18.80	8.39	3.78	8.41	2.10	10.68	3.20	5.84
11	186.71	62.15	6.32	5.08	2.61	.....	1.20	12.20	5.45	4.99
24	148.38	55.65	1.91	17.22	4.78	.....	.38	16.45	3.31	2.21
25	10.85	24.79	19.95	28.50	2.56	.....	1.14	13.58	8.90	.58
26	156.86	82.90	.....	.....	.....	.....	.....	9.60	5.74	1.76
27	180.00	65.35	8.62	9.38	.....	.....	.....	11.04	3.34	2.27
28	504.00	53.80	13.16	5.04	.....	.....	.....	18.60	5.80	3.60
29	156.00	63.80	6.00	13.20	.....	.....	.....	7.72	5.96	3.32
30	79.00	43.38	5.71	28.56	1.31	.....	1.03	11.92	5.53	2.56

[1] Nos. 5 and 11 omitted.

The average land value for the State should be compared with the total for land and all improvements except buildings on the 21 farms. The mean of the 21 farms shows 55.9 per cent and the average 57.9 per cent in land, fences, drainage, and water supply as compared to 68.1 per cent for the State. The mean shows 21.7 per cent and the

average 20.2 per cent in all buildings as against 18.4 per cent for the State. Both percentages for the State would be lowered if "Produce, supplies, etc.," had been included in the census. The percentage invested in fences varies even more widely than the acre investment, while the percentages in drainage and water supply usually vary with the natural features of the farm. Farms 5, 8, 10, and 12 (percentages of 8.41, 8.39, 11.87, and 9.18, respectively) have been tile drained over the greater part of their areas. A large part of the investment in water supply on farm 21 is chargeable to household.

The percentage invested in live stock is within the limits of 10 and 20 per cent except for a very few farms. Farm 8 (live-stock investment, 5 per cent) as shown by Table IV, has a low acre investment in live stock ($8.31 as against an average of $11.40) and a high land value ($87.74 as against an average of $46.25). The low percentage is explained by the fact that the owner has limited his farming operations with advancing age. The percentages invested in live stock and machinery as shown by the inventories are lower than they would be on a basis strictly comparable with the State average, as the 4 or more per cent in "Produce, supplies, etc.," is included in this study and not in the census data. If the last item were omitted the average percentages for the 21 farms would be as follows: Land and all improvements except buildings, 60.4; buildings, 21.1; live stock, 13.1; machinery, 5.4. The values placed on live stock and machinery were probably on a higher basis in these inventories than census valuations, and all prices are undoubtedly higher than in 1900, hence, the comparison with the State averages is of less value than would at first appear. Farm 6 (with a machinery percentage of 11.82) has equipment for manufacturing butter and maple sugar in addition to the ordinary machinery; and No. 7, a small farm with a machinery percentage of 14.10, has a portable gasoline engine and wood-sawing outfit, only a part of which should have been charged to the farm. Aside from these two farms the variation of the percentage invested in machinery is small as compared with other classes of equipment.

DISTRIBUTION OF INVESTMENTS BY ENTERPRISES.

Reference has already been made to the division of investment by enterprises. Table VII shows the average distribution of capital for the 21 farms, on the basis previously set forth.

It will be noted that the land value is divided on the basis of acreage, no differences in quality of land on the same farm being recognized. This suggests that a farm inventory be made to show the relative value of the various kinds of land, as, for instance, waste,

dooryard, pasture, barn lots, crop land, orchard, and woodland. The crop land is included in one item under "All crops," owing to the annual variation in acreage for the different crops.

TABLE VII.—*Average inventory for a group of 21 Ohio farms, showing the distribution of investment by classes of equipment and by enterprises.*

Enterprise.	Land.		Buildings.	Fences.	Drainage.	Water supply.	Live stock.	Machinery, etc.	Produce, supplies, etc.	Total.	Per cent.
	Area (acres).	Value.									
General	5.51	$246.44	$325.42	$533.95	$1.09			$237.29		$1,344.19	8.90
Household	2.04	91.01	1,437.05		.71	$72.48		11.07		1,612.33	10.70
Labor	.08	3.91	74.29			1.19				79.39	0.53
Produce, supplies, etc			766.57						$631.93	1,398.50	9.26
Horses			77.85			28.52	$891.66	77.46		1,075.51	7.13
Cattle			153.74			37.86	582.26	32.48		806.35	5.34
Sheep			65.50			10.81	201.05	3.06		280.42	1.86
Hogs			34.70			16.38	158.34	12.17		221.59	1.46
Poultry			40.83			4.53	52.60	4.89		102.85	.68
Bees							3.23	1.59		4.82	.03
All stock	46.50	2,037.10	63.89		2.14			10.59		2,113.72	14.00
All crops	85.71	4,157.92			362.48			102.71		4,623.11	30.63
Corn								83.38		83.38	.56
Small grain								70.98		70.98	.47
Hay								65.83		65.83	.44
Potatoes			3.57					20.44		24.01	.16
Sugar	2.98	122.27	6.05					35.36		163.68	1.08
Orchard	1.95	69.39						4.00		73.39	.49
Woodland	21.11	948.39								948.39	6.28
Beets								.59		.59	.004
Total	165.88	7,676.42	3,049.47	533.95	366.43	171.76	1,889.15	773.92	631.93	15,093.03	
Per cent		50.82	20.21	3.54	2.43	1.14	12.54	4.67	4.19		100.00

The division of building values, based on the cubic space occupied by different enterprises, seems out of proportion, emphasizing as it does the much larger amount of space occupied in proportion to the value of "Produce, supplies, etc.," ($766.57) than of "Live stock" ($436.51). The "Produce, supplies, etc.," item under "Buildings" might be divided between "All stock" and "All crops" but for the annual variation in the proportion of products fed and sold. The "All stock" building charge is based on space devoted to sheds, alleys, etc., or used in caring for several or all classes of stock. Buildings wholly or partly devoted to workshops or to the storage of machinery, wagons, and tools give rise to the amount charged to "General" ($325.42). A potato storage house and several sap houses were found. The term "Buildings" includes both household and farm buildings.

The machinery and utensils charged to household ($11.07) were those which on some farms might be used for either domestic or farm purposes. Each class of live stock is charged with the articles pertaining directly to it; also each crop enterprise. Vehicles for transportation and a large proportion of the smaller tools are charged to "General" ($237.29), and plows, harrows, and other general crop machinery are charged to "All crops" ($102.71).

TABLE VIII.—*Percentage of total farm capital invested in each enterprise on 25 Ohio farms, with means and averages for a group of 21 of these farms.*

Designation of farm.	General.	Household.	Labor.	Storage	Horses.	Cattle and dairy.	Sheep.	Hogs.	Poultry.	Bees.	All stock.	All crops.	Corn.	Small grain.	Hay.	Potatoes.	Miscellaneous.	Woodland.
1	4.47	13.00	.....	5.88	1.76	11.86	.....	1.07	0.52	.....	28.65	18.76	0.60	0.37	0.23	.....	1.64	11.19
2	10.01	10.7	.....	9.50	7.86	16.40	.....	.87	.88	.....	13.54	19.15	.54	.49	.88	0.38	.40	8.40
3	9.02	20.2	.....	16.90	10.27	1.25	4.36	.08	.83	.....	7.25	19.38	.29	.63	.37	.02	4.78	4.36
4	11.73	9.78	.....	11.01	7.85	13.20	.....	1.25	.61	.....	9.91	22.25	.42	.47	.75	.07	10.33	.....
6	13.60	12.11	.....	7.87	13.08	8.10	.....	.99	1.73	.....	9.90	21.30	.61	1.65	1.01	.97	7.70	.28
7	19.15	8.89	.....	7.72	17.65	3.20	2.96	.97	1.72	0.39	14.32	17.55	.45	.01	.59	1.43	.....	3.00
8	8.83	16.45	.....	6.75	3.34	1.53	.....	3.61	.63	.....	10.10	47.19	.13	.10	.66	.01	.....	.67
9	6.03	9.50	2.74	10.36	3.95	7.65	.....	1.36	.33	.....	11.60	33.19	1.49	.74	.31	1.42	.....	9.33
10	9.36	12.00	.....	7.16	7.19	3.06	1.02	.48	1.33	.....	2.50	52.50	.10	.38	.61	.31	.08	1.92
12	7.20	9.81	.....	15.21	11.29	3.48	.....	2.18	.47	.04	7.64	35.92	.93	.53	.28	.....	.....	5.02
13	9.40	15.00	.....	12.85	8.53	3.04	1.17	1.51	.93	.....	7.89	34.82	.36	.52	.41	.07	.....	3.50
14	6.64	5.31	.....	6.85	6.22	4.37	.24	2.19	.14	.02	21.31	35.76	.52	.39	.20	.....	.....	9.84
15	8.18	7.00	.....	7.08	7.64	3.96	.....	2.44	1.02	.....	19.23	37.35	1.19	.59	.12	.....	.....	4.20
16	9.55	6.63	.....	6.63	9.04	1.42	.88	1.81	.39	.....	4.35	42.96	1.61	.63	.21	.....	.....	14.22
17	7.73	9.75	1.55	2.96	3.57	7.24	2.83	.....	.42	.01	19.95	30.07	.37	.38	.20	.....	6.07	6.90
18	8.50	9.22	.....	12.02	3.46	1.21	4.79	3.70	.52	.....	7.60	34.40	.50	.44	.70	.....	1.25	11.69
19	8.80	8.80	.....	17.40	11.10	11.60	.....	1.80	.90	.....	9.20	25.80	.50	.40	.90	.30	.....	2.50
20	11.17	4.38	3.10	10.62	7.92	1.15	11.75	.....	.42	.26	23.37	22.62	.15	.20	.56	.26	.....	2.06
21	13.92	16.29	1.91	6.33	6.42	7.18	3.08	1.12	.59	.02	12.50	13.74	.10	.39	.38	.06	.94	15.01
22	8.28	17.90	.....	7.32	8.73	4.51	5.27	.....	1.98	.12	31.07	9.31	.07	.08	.44	.....	.97	3.95
23	12.20	15.36	.....	8.57	8.15	9.44	.....	1.03	.72	.09	17.52	20.52	.21	.53	.55	.26	2.78	2.07
For group of 21 farms: [1]																		
Average (acreage unit)	8.90	10.70	.53	9.26	7.13	5.34	1.86	1.46	.68	.03	14.00	30.63	.56	.47	.44	.16	1.57	6.28
Mean (farm unit)	9.70	11.337	.443	9.365	7.85	5.945	1.827	1.354	.813	.045	13.78	28.325	.53	.472	.493	.264	1.759	5.719
5	8.74	18.56	1.12	16.02	6.04	4.49	5.59	.57	.47	.....	11.65	19.04	.45	.27	.39	.02	.35	6.23
11	7.49	5.95	.....	6.81	6.59	4.52	3.53	1.19	.75	.....	4.70	46.00	.73	.63	.35	.....	.77	9.99
24	7.70	16.05	.....	3.12	11.00	3.40	1.72	1.05	.66	.13	17.60	27.60	.38	.43	.12	.....	.47	8.60
25	12.40	29.57	.....	.58	9.50	3.36	.....	.....	19.43	.....	.96	23.13	.14	.29	.....	.....	.64	.....

[1] Nos. 5 and 11 omitted.

Table VIII gives by enterprises the percentages of total investment for 25 farms, together with the mean of the percentages for the individual farms and the average percentages for the 21 farms considered as a unit. Miscellaneous enterprises are grouped under the column so headed. These include maple sugar, sirup, etc., on farms 1, 2, 5, 6, and 17; orchards on farms 3, 21, 22, and 23; sugar beets on farm 10; tobacco on farm 24; and market garden on farm 25. On farm 4, 8.65 per cent is invested in the maple-sugar enterprise and 1.68 per cent in orchard; on farm 18, 0.28 per cent is in sugar and 0.97 per cent in orchard. Bees, also included with miscellaneous enterprises, average 0.03 per cent of the total, amounting to less than 0.4 per cent on any farm represented in Table VIII. On farm 29, however, this enterprise represents 2.51 per cent of the total investment.

The relative importance of the various live-stock enterprises can readily be ascertained from Tables VII and VIII. On high-priced land the "All crop" enterprise naturally bears a higher proportion of the total investment. The investment in special crop machinery is relatively small. The low figures (0.15, 0.10, 0.07, and 0.21) for corn machinery among the hill farms (20 to 23, inclusive) are to be noted.

The distribution of capital for each farm is worthy of consideration by itself. It is not easy to generalize in this connection, all the factors discussed up to this point governing the selection of equipment. The various tables, and especially Table VIII, will show the difficulty of studying the farm instead of the enterprise as a unit. Farms 1, 2, 6, 9, 21, and 23 might be classed as dairy farms, yet in the distribution of investment among the various enterprises they are far from uniform. With the exception of these and farms 20 and 25, the farms studied can best be classed as "General," and among these occur variations in the distribution investment to the understanding of which an analysis of the farm as a combination of enterprises is essential.

EQUIPMENT OF THE AVERAGE FARM.

In the foregoing pages the distribution of capital at the time of inventory has been discussed. The next phase of the study, and really the first in logical order, is the enumeration of the items that make up the equipment of an average farm. The average equipment of the 21 farms which have been studied will, of course, serve only for farms having approximately the same conditions as this average farm. The various classes of equipment will be dealt with

separately in the following pages and in sufficient detail to permit the application of the data to farms diverging from the type under consideration. It is impossible to make a general recommendation as to equipment, owing to the complex and varying combinations of enterprises on different farms; the summary presented later is therefore valuable in a suggestive way only.

REAL ESTATE.

The average value previously shown for the bare land is taken as a basis instead of the mean value, as all other data relating to the first cost of equipment are based on averages. The cost and present value of drainage systems were regarded as equal, as before stated, but the first cost of buildings, fences, and water supply will be higher than the values shown in the preceding pages. The various improvements will be discussed separately.

HOUSEHOLD BUILDINGS.

The great variation in the tastes and circumstances of farm owners is largely responsible for the variation in the cost of household buildings, and it is almost impossible to arrive at a satisfactory basis for determining the proper outlay in this respect. Table VII shows that on the 21 farms studied the inventory value of household and tenant buildings was approximately equal to that of farm buildings, each being about $1,500. This, however, does not represent the present cost of construction. Household buildings were not studied closely as to size and cost, but from the values shown in Table III (p. 18) and such data as are at hand it is estimated that to replace those found on the 21 farms would involve an expenditure of $600 to $4,000 per farm, averaging close to $2,500. This would include dwellings for owners, tenants, and laborers; woodhouses; smokehouses; milk cellars; ice houses, etc., some of which might also be used to some extent for the farm.

SPACE NEEDED IN FARM BUILDINGS.

The farm buildings must usually provide for the shelter of horses, cattle, sheep, hogs, and poultry, and for a certain allotment of space to be used by or devoted to the care of several classes of live stock. They must usually accommodate all or a large part of the products of the farm fields, including roughage, grain, and seed. They should provide space for the storage of all wagons, machinery, and tools, and for the farm workshop. A provision of easily accessible space should

also be available for the temporary shelter of machinery, live stock, or products. Buildings for special purposes, such as the storage of root crops and ensilage and the manufacture of maple products, are necessities on some of the farms.

In studying this problem the size and plan of each building was noted, together with the enterprises to which each building was devoted at the time. The extent of floor and cubic space devoted to the various enterprises has thus been approximated. The thickness of walls and partitions was not considered. While averages of the 21 farms do not include enough cases to justify the drawing of general conclusions, the data contained in Tables IX and X afford a rough working basis. Table IX includes data concerning enterprises the space for which depends to a considerable extent upon the size of the farm. The term "General farm" includes all space devoted to machinery storage, workshop, driveways, and other spaces devoted to the farm as a whole. "Hay storage" includes the area and volume of mows and lofts, the volume being greater than the space ordinarily filled with hay or other roughage. The proportion of the entire volume of mows which could actually be filled by the ordinary methods could not be satisfactorily determined at the time, and the space usually filled was extremely variable; hence, the total volume was used in this table. "Grain storage" includes separate cribs and granaries, also all bins and storage places for grain and seed in other buildings.

TABLE IX.—*Average area and volume of space devoted to the storage of products, machinery, etc., in buildings on 21 Ohio farms.*

Enterprise.	Average per farm.		Average per acre.		Average per acre of crops.	
	Area.	Volume.	Area.	Volume.	Area.	Volume.
	Square feet.	*Cubic feet.*	*Square feet.*	*Cubic feet.*	*Square feet.*	*Cubic feet.*
General farm	2,038	24,732	12.3	149.0	23.7	288.5
Hay storage	2,752	46,558	16.5	280.6	32.1	543.2
Grain storage	505	5,192	3.0	31.3	5.8	60.5

The average space per acre shown in Table IX would tend to vary inversely with the size of the farm. On the smaller farms the amount of waste space would be greater for each enterprise and the space devoted to certain general farm purposes would remain practically the same as for the larger farms.

Table X shows averages in connection with the space devoted to live-stock enterprises. In order to obtain comparable units all young stock except colts was reduced to the basis of mature animals. Two head of young cattle, 2 shotes, or 5 pigs were regarded as equivalent

to 1 mature animal. Since young lambs are later included with the ewes in Table XIII, no correction for them was necessary. The space in harness rooms is included in that shown for horses, and space devoted to milk rooms, etc., in that shown for cattle. For sheep the space includes both floor and rack room, with very little waste. For swine the space shown includes feed alleys, etc., in hog houses. The average space per head is of course much too small for the entire herd of swine. Only 11 out of 21 farms show a definite space devoted to swine, and on the other farms swine usually occupy a portion of the "All stock" space during part of the year. Portable houses for the brood sows are in common use. Such portable houses, averaging 4.1 per farm, were included with the miscellaneous items of equipment rather than with permanent farm buildings.

TABLE X.—*Average area and volume of space per farm and per head devoted to live-stock enterprises in buildings on 21 Ohio farms.*

Enterprise.	Approximate number of animals per farm.	Average space per farm.		Average space per head.	
		Area.	Volume.	Area.	Volume.
		Square feet.	*Cubic feet.*	*Square feet.*	*Cubic feet.*
Horses	7	613	5,242	87.5	748.8
Cattle	13	1,084	9,210	83.4	708.4
Sheep	41	475	4,141	11.6	100.9
Swine	17	327	2,912	19.2	171.3
All stock		448	3,925		

SIZE OF FARM BUILDINGS.

It is possible to plan a practicable set of farm buildings which will almost exactly fit the conditions of the average farm under consideration. The size and nature of the buildings must of course be varied to fit any individual conditions, but assuming that the data in Tables IX and X give the requirements for this particular size and type of farm, the size of the separate buildings is the next item to be determined.

Basement barn.—Of the barns on the 21 farms about half were basement or "bank" barns, and in most of the others the space equivalent to a basement was provided by attaching to the barn unsightly sheds of the lean-to type. On most farms a convenient site for a basement barn can be had without excessive grading, and the advantages of this type are such that they will be provided for in the barn to be planned.

Horses, cattle, and sheep are often sheltered in the basement of a barn. Such a barn 36 by 60 feet provides 2,160 square feet of floor space (outside measurement), while the requirements for the

three classes of stock total 2,172 square feet, these requirements also being calculated on outside measurement. A section 16 by 36 feet at one end will provide 576 square feet for horses, and an additional space 4 by 9 feet would utilize the average space allotted for harness. The 16 feet would be reduced by the thickness of the wall, but would leave ample room for manger, stall, and alley behind the horses. The 7 horses could easily be accommodated in the width remaining after the thickness of one wall is deducted from 36 feet. As a rule, in barns of this kind the basement wall is provided only on the two ends and the long side next the bank.

A section 30 by 36 feet would provide 1,080 square feet for cattle where 1,084 are required. This would afford ample space for the average of nearly 8 cows per farm, for the young and miscellaneous stock, and for a milk room if desired. While there is thus abundant space provided for this number of cows and young stock, it must not be understood that such an arrangement is in any way ideal from the standpoint of a modern dairy barn, as it would be difficult to secure sufficient light and other sanitary arrangements. Experts in sanitation also would object to having the milk room in the cow stable. If it were a beef farm there would be less objection and the space provided would afford room for the miscellaneous stock on a beef farm and feeding room for a small carload of steers. The sheep would preferably be lodged in the center space, in which the harness room and a stairway could be located. Deducting the area of the harness room from the remaining space, 14 by 36 feet, 468 square feet are left for sheep, the average requirement for sheep being 475 square feet. A height of $8\frac{2}{3}$ feet would supply 18,720 cubic feet in the basement, where 18,593 cubic feet is the average requirement. In this plan both horses and cattle are provided with more and sheep with less cubic space than is called for by the average. A basement somewhat similar to the one just described was found on farm 3.

The upper part of this barn is adapted from that of a barn 40 by 60 feet on farm 14. A central driveway 14 feet wide extends through the center of the barn, making a floor space 14 by 36 feet available for general farm purposes. To the left of the driveway is a stairway to the basement, the remainder of this end of the barn being devoted to hay storage. On the right of the driveway a grain room 10 by 23 feet and a space 26 by 23 feet for storage of wagons and machinery occupy the floor space. A mow floor extends over these spaces at a height of 8 feet, and over the driveway at a height of 12 feet. The barn is 18 feet from the top of the basement wall to the corners, or to the "square," and a roof of one-third pitch gives an additional height of 12 feet to the point of the gable. This provides 2,160 feet of

floor space for hay and 230 for grain storage; but, since volume is rather the essential, it provides 39,168 cubic feet for hay and 1,840 cubic feet for grain, leaving balances of 7,390 cubic feet for hay and 275 square feet and 3,352 cubic feet for grain to be provided elsewhere. In the driveway 14 by 36 feet, and storage space 26 by 23 feet, an area of 1,102 square feet and a volume of 10,832 cubic feet are provided for general farm purposes, leaving a balance of 936 square feet and 13,900 cubic feet to be provided for general purposes in other buildings.

The cost of this barn will vary with many factors and can more easily be estimated by the contractor than the necessary size; hence, dimensions only are emphasized in this study. A study of cost items of four comparatively new barns of similar type indicates that about 2½ cents per cubic foot inclosed will cover the cost of a barn of this size and type. Ohio farmers who have timber available commonly utilize lumber sawed on the farm, the exact value of which it is difficult to estimate. This barn contains 70,560 cubic feet; at the rate given it would cost close to $1,800, but this is probably a low estimate.

Hay barn.—Where a basement barn is not practicable a second building is usually provided for the storage of hay and the shelter of a part of the live stock. On some farms such hay barns are made large enough so that sheds attached to the barns are dispensed with. In order to provide for the additional space (448 square feet and 3,925 cubic feet) required for "All stock" and for the additional storage of hay, a building of this sort is here planned for the average farm supplemental to the above-planned farm. To combine the cubic space required for both purposes with the floor space required by "All stock" would result in a building of unusual proportions, hence the ground area is increased from 448 to 512 feet as shown in Table X. A building 16 by 32 feet, 16 feet high to the "square," with roof given one-half pitch will give an excess of 64 square feet and 171 cubic feet for "All stock." If the second floor is placed 8 feet above ground it will also provide 6,144 cubic feet for the hay storage, as compared with the remaining requirements of 7,390 cubic feet. A further increase of floor space accompanied by a decrease in height would improve the proportions of the building, though they are not unusual. This building may be of cheap construction; $150 should cover the cost.

Wagon shed, crib, etc.—The grain room in the basement barn failed to provide for a large part of the space required for grain storage. The ratio between floor and cubic space remaining suggests a high crib or granary. A popular building is a double crib, or a combination of crib and granary, with a driveway between, which, when inclosed by doors at either end, may be used as a convenient

wagon or buggy shed. A building 20 by 28 feet on the ground and 10 feet in height, with an 8-foot gable, is suggested. Two cribs, each 5 feet wide, and a driveway 10 feet wide, all extending the length of the building, would occupy the floor space. For grain storage this building would provide 3,360 cubic feet and 280 square feet, or almost exactly the remaining balance required (275 square feet and 3,352 cubic feet) as shown on page 33. Including the loft above the driveway, which could be used for the storage of light implements, ladders, etc., 3,920 cubic feet would be provided for general farm purposes and 280 square feet of ground space. This building, with the average finish, will probably cost $200 to $250.

Machinery shed and workshop.—In the foregoing plans for a basement barn and a combined wagon shed and crib, an area of 1,382 square feet and a space of 14,752 cubic feet for general farm purposes were provided. Balances of 656 square feet and 9,980 cubic feet are yet to be provided for these purposes, if the requirements as set forth in Table IX are complied with. The storage space for a part of the farm machinery and a building for the farm workshop have not been provided; hence, a building 22 by 30 feet and 12 feet in height to the eaves is designed to meet these needs. If the workshop is finished properly the building will probably cost $250 to $300.

Hog house.—Only part of the farms have separate permanent hog houses. The average floor space devoted exclusively to hogs on the 21 farms was 327 feet. A house 12 by 27 feet would meet this requirement and also provide for a 4-foot feed alley the length of the building and 4 pens 8 by $6\frac{3}{4}$ feet. With this building, several portable houses, and the occasional use of space in other buildings, the approximate average herd shown in Table XIII (1 boar, 6 brood sows, 22 shoats, and 21 pigs) could be accommodated. If the house were made 10 feet high in front and 8 feet in the back, with a shed roof, the average requirement of cubic space would be met. The probable cost of the hog house is $60 to $100.

Poultry house.—Poultry houses on 5 farms other than the group of 21 which has been under discussion are considered in the following averages. The average flock on these farms was equivalent to 106 hens, or a trifle larger than on the 21 farms. The floor space per hen varied from $1\frac{1}{2}$ to 11.7 square feet on different farms. Excluding the one farm having excessive allotment of space, the mean was 3.46 square feet per hen; and assuming 7 feet as the average height of houses, the mean volume of space per hen was 24.4 cubic feet. On 40 per cent of the farms the area per hen was between $1\frac{1}{2}$ and $2\frac{1}{2}$ square feet; on about 40 per cent of the farms the area of the poultry house was between 150 and 250 square feet, and on 60 per cent of the farms the number of fowls kept in one house was between 60 and 120.

The remaining farms show wide variations. To house 106 fowls at the mean rate of floor space per fowl requires an area of 367 square feet of floor space, which would be provided approximately by a house 12 by 30 feet. Five square feet of floor space per hen is often recommended by poultry authorities, and 4 square feet per hen should be considered as a minimum in good farm practice; but 60 per cent of this is apparently closer to actual conditions on most farms, and a house 12 by 20 feet is probably nearer the average than one 12 by 30 feet. A house for the accommodation of the flock of average size should be not less than 12 by 36 feet, or 16 by 27 feet; or, better still, two houses, each 12 by 20 feet. With two houses the 1-year-old fowls could be kept in one and the 2-year-olds in the other, and the difficulty of separating the old from the young would be obviated. The poultry had free range on practically all the farms. The poultry house on the average farm will represent an outlay of $50 to $75.

Silo.—Silos are usually associated with the cattle enterprise. Six wooden silos of 100 to 120 tons capacity were found, 4 in connection with dairy cattle and 2 with beef cattle. The cost, depending on the size and material, was $150 to $250 each, in place.

Sap house.—Where a "sugar bush" is turned into revenue a separate building is usually found advisable. This building often consists of a room for the evaporator, etc., and a woodshed. It is ordinarily built of old or rough lumber and as cheaply as possible. A building 18 by 32 feet, 8 feet high, with roof one-third pitch, is close to the average of 3 sap houses found on these farms.

Miscellaneous buildings.—On many farms there are buildings for special purposes not already discussed. On farm 9 is a potato cellar costing about $75. On farm 29 there is a beehouse for storing the bees, hives, etc., during the winter. An occasional well house is included under water supply. An investment of $75 per farm would probably be an average for silos, sap houses, and other farm buildings of a miscellaneous character on the 21 farms.

SPACE UNITS IN FARM BUILDINGS.

The forgoing discussion makes apparent the great need for definite space units to be used in the planning of farm buildings. The usual division of crops on the 21 farms studied makes it necessary to provide for storing the yields of 25 to 30 acres each of corn, small grain, and hay. Yields of 50 bushels of corn to the acre from 28 acres, 20 bushels of wheat from 14 acres, and 40 bushels of oats from 14 acres would require approximately 4,550 cubic feet of space, which is more than provided for on the average, since some of the corn is used for silage and some of the grain is sold immediately. Maximum yields, however, would encroach on the "general" space. A hay yield of

2½ tons to the acre from 28 acres would tax the capacity of the mows provided on the average farm (165.88 acres), and straw would ordinarily have to be stacked outside, especially if corn stover were shredded.

The units of space for field products are well understood, however, in comparison with those for live stock and general farm purposes. The averages presented are simply those of actual conditions on a small number of farms, and it is a matter of common observation that most farm buildings can not be regarded as models of economy and convenience. Units of space for each class of live stock, including the area occupied by the animal itself, the racks or mangers, alleys, and the feed of the animal, would be of great assistance in the planning of buildings for economy of space. These units can not be worked out satisfactorily on theoretical grounds, but should be obtained from a careful study of the best farm practice.

FENCES.

The study of the extent of fence on the group of 21 farms yielded some interesting data which are presented in Tables XI and XII. Table XI gives the total rods of fence maintained by each farm, divided into outside (line) fence, inside fence, and road fence. Only the total fence kept up by the owner is represented; hence, the amount of line fence should be doubled in order to get the total number of rods touching the farm. The first cost of fence per acre is affected not only by the character of the fence but by the number of rods per acre. The effect of a large extent of road fence on the number of rods per acre may be seen on farms 7 and 8, having 284.1 and 333.9 rods of road fence, respectively, making the average rods per acre 13 and 10.4, respectively, as contrasted with farms 1 and 2, which have 6.1 and 4.9 rods per acre, respectively, of road fence. The term "road fence" includes river or other outside fence not shared by the adjacent owner. Naturally, the smaller farms show a greater extent of fence per acre than the larger, but this is not necessarily always true. The average of all the farms shows approximately one-half the fence inside, one-fourth on the road, and one-fourth between the farm and those adjacent. A slight discrepancy is shown between the acre value of fences in Tables IV and XI, as in Table IV the total value of fences on each farm was brought to a round number, while in Table XI the actual value is used.

TABLE XI.—*Total rods of line, road, and inside fence maintained by the owners of 21 Ohio farms, with cost, value, and number of rods per acre.*

Farm No.	Area.	Owner's share of line fence.	Road fence.	Inside fence.	Total owner's fence.	Fence per acre.		
						Cost.	Value.	Average.
	Acres.	*Rods.*	*Rods.*	*Rods.*	*Rods.*			*Rods.*
1	116.20	365.8	11.9	330.0	707.7	$2.49	$2.11	6.1
2	164.11	351.2	.....	444.8	796.0	1.76	1.52	4.9
3	104.25	125.2	220.3	710.7	1,056 2	5.88	4.37	10.3
4	108.34	124.8	202.4	405.6	732.8	4.36	2.71	6.8
6	143.32	422.6	180.8	538.4	1,141 8	5.48	3.94	8.0
7	49.61	119.8	284.1	241.4	645.2	9.01	4.50	13.0
8	78.64	140.1	333.9	344.3	818.3	5.20	5.08	10.4
9	147.67	119.8	128.1	224.4	472.7	1.61	.65	3.2
10	100.00	129.6	178.0	803.2	1,110.8	7 64	5.89	11.1
12	156.97	354.9	79.2	609.0	1,043.1	3.36	2.57	6.7
13	198.25	735.0	475.0	1,060.0	2,270.0	7.50	5.72	11.4
14	388.92	285.4	713.6	856.4	2,419 2	3.82	2.29	6.2
15	219.82	258.4	537.6	727.6	1,523.6	4.86	3.65	6.9
16	172.52	268.8	141.2	596.0	1,006.0	4.36	3.63	6.2
17	275.99	416.4	539.2	864.8	1,820.4	5.67	3.86	6.6
18	207.83	324.0	375.2	827.6	1,526.8	4.54	1.51	7.4
19	103.81	.....	.....	.....	997.0	5.06	3.87	9.6
20	185.25	505.6	77.2	1,027.6	1,610.4	5.03	3.56	8.7
21	228.62	176.4	390.8	956.8	1,524.0	4.00	3.60	4.9
22	156.00	329.2	190.8	579.6	1,099.6	3.24	2.35	4.8
23	177.27	291.0	409.1	734.1	1,434.1	5.80	4.06	8.1
For the group of 21 farms:[1]								
Average (acreage unit)	165.88	292.19	273.26	644.11	1,227.93	4.60	3.25	7.4
Per cent of total	.....	24.1	22.5	53.2	100.0	.....	.....	.....
Mean (farm unit)	.....	.....	.....	.....	.....	4.79	3.40	7.67

[1] Nos. 5 and 11 omitted.

The character of fences on the 21 farms is brought out in Table XII, which shows the extent of each of the eight principal kinds of fence and the average cost per rod of all fence on each farm. The total of the eight kinds shown averages 1,204.6 rods per farm, or over 98 per cent of the total, a few miscellaneous kinds being omitted. The cost of the various kinds of fence varies with the difference in the cost of materials in different localities, but even more with the height, number of wires or boards, distance apart of posts, and the labor expended in construction. Woven-wire fence, for instance, may be 5 feet in height without barbed wires in addition, or 3 feet in height with several barbed wires above and one below. It may be made of either heavy or light wire, with posts 10 to 33 feet apart, the posts costing 10 to 30 cents each. Owing to these variations, estimates of the cost of construction can hardly be made general.

TABLE XII.—*Number of rods of each of eight principal kinds of fence maintained by the owners of 21 Ohio farms, with the average first cost per rod of all kinds of fence on each farm.*

Farm No.	Woven wire.	Barbed wire.	Smooth wire.	Board.	Worm rail.	Straight rail.	Picket.	Hedge.	Average cost per rod.
	Rods.	*Rods.*	*Rods.*	*Rods.*	*Rods.*	*Rods.*	*Rods.*	*Rods.*	*Cents.*
1	70.3	448.2	.....	.....	123.5	65.7	.....	.....	40.9
2	.....	140.0	.....	.....	602.0	6.0	48.0	.....	36.4
3	50.8	.....	81.0	34.7	624.5	20.0	245.3	.....	58.0
4	126.4	.....	36.0	28.0	277.6	.....	24.0	240.8	64.5
6	612.4	.....	272.8	80.0	20.0	133.8	22.8	.....	68.7
7	28.5	104.7	.....	113.8	134.0	26.0	.....	47.7	69.4
8	225.0	.....	.....	.....	130.6	.....	462.8	.....	50.0
9	134.8	.....	.....	31.2	306.2	.....	.....	.....	50.0
10	586.0	.....	.....	119.2	371.2	20.8	13.6	.....	68.6
12	348.7	.....	493.6	.....	200.8	.....	.....	.....	50.4
13	720.0	30.0	.....	140.0	1,140.0	93.0	37.0	110.0	65.5
14	605.0	643.6	.....	71.6	464.8	535.8	98.2	.....	61.4
15	302.1	680.8	142.4	268.0	109.2	.....	21.2	.....	70.0
16	186.4	422.8	.....	.....	126.8	.....	270.0	.....	74.8
17	986.0	.....	.....	216.4	310.0	.....	232.8	75.2	86.0
18	214.4	.....	.....	267.6	773.6	.....	271.2	.....	61.8
19	.....	.....	180.0	245.0	408.0	60.0	104.0	.....	52.3
20	305.2	614.8	59.6	202.8	374.8	53.2	.....	.....	57.8
21	356.8	30.4	18.0	68.0	723.6	86.8	240.4	.....	60.0
22	208.8	.....	.....	50.8	811.8	8.0	20.2	.....	46.2
23	211.3	765.6	52.0	88.0	228.3	.....	36.5	52.3	71.7
For the group of 21 farms:[1]									
Average	299.0	184.8	63.6	96.4	393.4	38.5	103.7	25.1	59.7
Per cent of total	24.8	15.3	5.2	8.0	32.6	3.1	8.6	2.0	.....

[1] Nos. 5 and 11 omitted.

The old "zigzag" or "worm" fences are still much in evidence, but are being replaced as they decay, largely by woven wire. A small percentage has been rebuilt as straight rail fences. The use of barbed wire is somewhat restricted by law, but it is popular as a cattle fence. Board fences and picket fences (usually made of wire and pickets) are still used to some extent for tight fencing, but are being replaced by woven wire. The hedge fences (usually of Osage orange) are being destroyed on many farms, not only because of their unsatisfactory character and the labor of keeping them in shape, but because of the ground rendered unproductive on either side of the fence row. The smooth-wire fences include various types representative of the effort to supply a fence safer than barbed wire and easier to put up than woven wire.

Regarding the cost of construction at the present time, it may be said that this applies almost entirely to board and barbed or woven wire. Hedge fences were formerly installed at about $1 per rod and entail an expense of 5 to 10 cents per rod each year for trimming. Reference has already been made to the cost of building old rail fences. The labor cost probably ranged between 30 and 50 cents per rod. The material was not valued, and in fact often had no market value at the time the fence was built. The rebuilding of rail fences costs 20 to 30 cents per rod for labor, and if the rails are fastened to posts

one post will be required for each 11-foot rail length. Picket fences require 1 to $1\frac{3}{4}$ posts per rod. The pickets, wire, etc., cost 60 cents to $1 per rod, and the labor of erecting 15 to 20 cents per rod. None of these types are now built to any great extent.

Barbed-wire fences for cattle usually consist of 3 or 4 wires at a cost of 3 to 4 cents per rod for each wire. Posts are usually set 11 to 22 feet apart, costing 5 to 8 cents per post for setting. They are of oak, chestnut, catalpa, Osage orange, locust, and cedar, principally, costing anywhere from 10 cents up. The corner and brace posts cost from 50 cents up for the posts, and from 50 cents to $1 for setting.

Woven wire costs 25 to 75 cents per rod for the usual heights and grades, the lower heights usually taking several strands of barbed wire in addition. As a rule posts are set 11 to 33 feet apart. Setting of posts for woven-wire fences costs about the same as for barbed wire, but the end posts must be heavier and more firmly braced, costing as high as $3 on some farms for post and setting. The labor of erecting wire fences, outside of setting posts, is estimated at 5 to 10 cents per rod, but accurate figures are not easily available. This refers, of course, to ready-made fence, i. e., not woven on the ground.

Board fences usually require two or more posts and 25 to 40 feet of lumber per rod. The rise in price of fence lumber has practically restricted board fences to the lots about the farmstead. While the estimates must be varied to suit conditions, it is probable that 45 to 60 cents per rod for barbed wire, 60 to 90 cents for woven wire, and from $1.25 up for board fences will cover the cost.

DRAINAGE.

The investment in artificial drainage shown in Table VII (p. 26) represents the cost of installing such improvements. Only a few farms have practically all fields drained. Figure 2 represents the drainage system on farm 10, as shown on the owner's map, all of the farm except the wood lot being tile-drained. The owner's map shows the size, depth, and location of all tile, this being very convenient when drains are to be cleaned or new ones installed. The cost of the drainage on this farm was $17.70 per acre for the whole farm and about $18.60 per acre for the area drained.

The average of the group of 21 farms showed an investment of $366.43 per farm for drainage. At the rate prevailing on farm 10, this would tile about 20 acres thoroughly. In practice, however, "strings" of tile are found only in the low places, and a much larger area could be drained. The work of digging the ditches and laying the tile was often done by contract at the rate of 6 to 10 cents per "rod-foot" for small tile, i. e., a ditch 1 rod long, 1 foot deep, and wide

enough to admit tile 2½ to 5 inches in diameter. At the present time the cost of laying tile is considerably greater. For central Iowa in 1910 the prices for laying tile were about as follows: For 4, 5, and

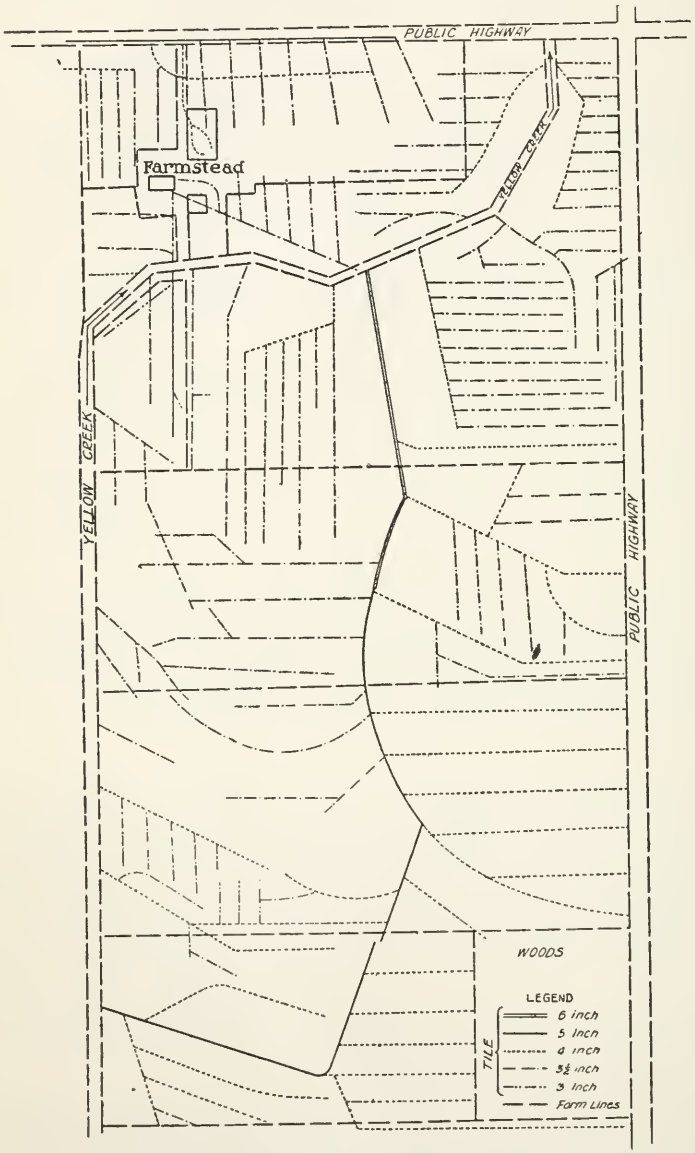

FIG. 2.—Plan showing the drainage system of farm 10, in Putnam County, Ohio.

6 inch tiles laid 3 feet deep, 44 cents per rod, with 1¼ cents additional for each inch over depth, the owner filling the ditch; for 8, 9, and 10 inch tile, 62½ cents per rod, with 2½ cents per inch additional for

every inch over depth; for 15-inch tile, 95 cents per rod for a 3-foot ditch, with 6 cents additional per inch for over depth. Practically no tile as small as 2½ inches in diameter is being used on farms at the present time, and many factories do not make sizes less than 4 inches in diameter. Filling the ditches is usually done by a team and plow at very slight cost.

The tile varies in price with locality. The average prices of a number of firms in the Central West in 1910 were about as follows: 3-inch tile, $12.50 per thousand tiles, each tile being 1 foot long; 4-inch tile, $17; 5-inch tile, $23.50; 6-inch tile, $32; 7-inch tile, $42; 8-inch tile, $53; 10-inch tile, $81; and 12-inch tile, $104 per thousand.

WATER SUPPLY.

Owing to the wide variation in the character of water systems, it will hardly be possible to make even an approximate list of the essentials for the average farm. The average present value of the water system, appraising wells at the cost of installation, and pumps, tanks, etc., at their present value, is seen to be $171.76 (Table VII, p. 26) for the group of 21 farms. Allowing for depreciation on the latter items, it is probable that the average cost would reach $225 for the entire system. Between different farms, however, there is a wide range, as shown by Table III (p. 18). The larger number of these farms depend on dug wells 25 to 40 feet in depth and 3 to 4 feet in diameter. Such a well, for digging and walling, costs $1 to $1.25 per foot in depth. A hand pump, costing from $5 to $10, is usually installed in such well. Some of the farms have drilled wells 90 to 150 feet deep. These cost about $1 per foot for drilling and casing and require a more expensive pump, costing $15 to $25 for the pump, piping, and cylinder. One or more cisterns are usually found, ranging in size from 20 to 150 barrels and costing $10 to $35. A cistern pump complete usually costs $4 to $6. Where water is conveyed to tanks or troughs at some distance from the well, 1-inch or 1¼-inch piping is ordinarily used, at a cost of 8 to 12 cents per foot. Small wooden troughs, holding 1 to 3 barrels and costing $3 to $5, are often used in connection with wells or cisterns near the barn, but tanks holding 10 to 50 barrels are commonly used in feed lots. These cost from $10 up in wood, and a trifle more in concrete. Many permanent concrete tanks are being installed by farm labor at a cost of $15 to $40 for sizes ranging from 20 to 80 barrels. Windmills costing $50 to $150 are often found economical. The usual height of the tower is 25 to 30 feet, with a wheel 6 to 8 feet in diameter. A tower costs about $60 to $70. Gasoline engines used only for pumping are occasionally found. These are usually of 2 or 3 horsepower and

cost $75 to $150. Reservoirs are sometimes found necessary in connection with deep wells and windmills. These store up a surplus of water at a depth from which it can be easily pumped by hand when lack of wind cuts off the supply from the well. The cost of construction is about the same as for cisterns.

PERSONAL PROPERTY ON THE AVERAGE FARM.

The requirements of the average farm as to live stock and machinery are discussed in the following pages, including Table XIII, which was compiled from the inventories.

HORSES.

In Table XIII the horses and mules on the group of 21 farms are divided into 5 classes with respect to use. The general-purpose, draft, and draft-and-brood classes might be grouped as work animals, with an average of 4.48 per farm, but the subdivision indicates a little more clearly the character of the animals. The draft-and-brood animals are mares regularly worked rather than mares kept for breeding purposes only. The general-purpose animals are those used for both work and driving on several small farms. The data indicate that 4 work horses, 2 head of young stock, and either a driving horse or brood mare, which may occasionally be worked, are about the average requirements as to horses.

The 94 horses used partly or wholly for heavy work on the 21 farms averaged 1,250.3 pounds in weight. From Table II (p. 12) it will be seen that these farms averaged 85.71 acres of harvested crops. This would mean an average of 19.13 acres of crops per work animal. The acres of crops per work animal varied from between 10 and 11 acres on farms 3, 7, and 22 to 31.1 acres on farm 17. On 55 farms visited by Mr. Thompson and the statistical cooperators, 8.4 horses were found to be the average per farm. On 54 of these farms, from which data were more complete, averaging 199.55 acres in size and 125.54 acres in harvested crops, an average of 5.39 work horses per farm was found, and the acreage of harvested crops per work animal averaged 23.3. On one group of 27 farms, averaging 153.65 acres in size, the acreage of crops per work animal averaged 18.9, and on a group of 17 farms averaging 272.44 acres the average crop area was 27.5 acres per work animal.

The farms in Ohio visited by Mr. Thompson were mostly in the southwestern part, the level, "large-farm" area. On 17 farms visited in 1907 and 1908 by him 119 work horses were kept, averaging 1,368 pounds in weight, with an average value of $158.91 and an average

age of 8.98 years. On farms 20 to 23, inclusive, in the "hill section," 17 work animals, averaging almost exactly 7 years in age, and 1,170 pounds in weight, were valued at $146.41 each. These 4 farms average 186.79 acres in size, but average only 65.4 acres in crops, or 15.4 acres per animal. On 52 farms, including those of cooperators, 275 work horses were kept, averaging 1,306 pounds in weight.

The work stock, like machinery, is seldom utilized to its full capacity on small farms or where conditions cut down the crop area. The number of work animals needed depends not only on the acreage of crops, but upon the total area of the farm, the kind and extent of live-stock enterprises, the kind of crops, the topography, the distance of the farm from town, and numerous other factors which can not be studied in detail at this time. On most farms the number of work animals is determined by the minimum power requirements during the two busiest seasons—seed time and harvest time.

CATTLE.

The values for cattle on this group of 21 farms in the spring of 1909 are approximated in the column of "Value per unit" (Table XIII). These will of course fluctuate with the market, and round numbers (based on averages, except as otherwise stated) are used for convenience. The value of $100 has been arbitrarily set as fair for a good bull of either a beef or dairy type, and $40 has been taken as nearer the usual value of a beef cow than the actual average on two farms reporting. On one of these farms 14 Shorthorn cows were valued at $100 or more each, and on the other 4 grade cows were valued at $35 each. Steers were figured on the prices of 4 to 4½ cents prevailing at that time, and young beef stock at about the average value per head.

On farms 1, 2, 6, 9, 21, and 23, on which dairying is the principal enterprise, 95 milch cows were kept, averaging $40.80 per head. These included some pure-bred cows. On 10 other farms there were 29 milch cows, averaging $37.72 per head. The average value of 124 cows on 16 farms was $40.18 per head. The 6 dairy farms averaged $648 worth of milch cows per farm, and the 10 other farms $109.40 per farm. On the 6 dairy farms there were 44 head of young stock, or nearly 1 head for each 2 milch cows. The figure for the value of young stock is close to the average for all calves and heifers found on these farms.

SHEEP.

The value of $10 per ram is a trifle higher than would be true of many farms, owing to the presence on farm 17 of a number of rams which were raised for sale as breeding animals at $12.50 each. The

figure given, however, is not too high for good results. All lambs at foot are included in the value of the breeding ewes. Feeding wethers, lambs, and ewes are grouped under "Wethers, etc."

SWINE.

Swine are quoted at a round figure approximating the average value on these farms at that time as follows: Boar, $15; sow, $14; shoat, $5; pig, $2.50. Several fat hogs are included under "shotes," and the dividing line between "shotes" and "pigs" is not well defined. About 5½ cents per pound was the farm value of hogs at the time the inventories were taken.

MACHINERY, TOOLS, ETC.

As stated elsewhere,[1] the first cost of the great number of minor articles of farm equipment not mentioned in Table XIII would probably be from $200 to $300 by the time the outfit was complete for the average Ohio general farm of 160 acres. This figure, however, would include an appropriation of $50 or more for repair materials, which in this report are invoiced with "Produce, supplies, etc." Taking all the minor items other than repair materials for 33 farms, using the ordinary retail prices and dividing by the number of farms, the first cost of minor items for the average farm of 167 acres was found to be about $190. In taking an inventory of the small items many were doubtless omitted, and $200 is probably a figure low enough to allow for the average equipment of this sort.

The values for harness, machinery, etc., in Table XIII are as nearly as can be ascertained, the usual retail prices prevailing in Ohio for new articles. Both farmers and merchants were consulted in the effort to obtain these prices, but, of course, the figures given are merely suggestive. The "First value" cost shown in Table XV includes both first and secondhand prices and may be regarded as indicative of the usual farm practice.

In making up a list of machinery for the average farm so many factors enter into consideration that a generalization would be of little value. The number of any single item reported for all the farms, the average for all farms, the percentage of farms reporting the article, and the number of articles per farm reporting are all to be regarded as useful in separating the necessary items from those only occasionally or rarely used. A careful study of Table XIII is recommended as of more value than a suggested list, especially with the major items of equipment shown. For the purpose of this study it is desired only to obtain an average figure for the total first cost of

[1] Circular 44, Bureau of Plant Industry, U. S. Dept. of Agriculture.

machinery and tools; hence, the total value is computed by multiplying the average number of each item on the 21 farms by the usual cost per unit.

TABLE XIII.—*Major items of personal property found on 21 Ohio farms, with the average number of each item for all farms and for each farm reporting the item, the approximate value of each item, and the average value of each item for each of the 21 farms.*

Designation of item.	Number.[1]				Value.	
	Reported.	Farms reporting.	Average per farm reporting.	Average per farm, all farms.	Per unit.	Total per farm, all farms.
Horse, general purpose	6	3	2.0	0.29	$140.83	$40.24
Horse, driving	17	10	1.7	.81	104.12	84.29
Horse, draft	73	18	4.06	3.48	145.82	506.90
Horse, draft and brood	15	6	2.5	.71	131.00	93.57
Colts	38	13	2.92	1.82	92.11	166.66
All horses	149	21	7.1	7.10	125.64	891.66
Double work harness	52	21	2.5	2.48	35.00	86.80
Single work harness	2	2	1.0	.10	20.00	2.00
Double light harness	11	11	1.0	.52	25.00	13.00
Single light harness	41	21	2.0	1.95	15.00	29.25
Bull	10	10	1.0	.47	100.00	47.00
Milch cows	163	21	7.8	7.76	40.00	300.40
Young dairy stock	75	15	5.0	3.57	16.00	57.12
Beef steers	43	2	2.6	2.04	44.00	89.76
Young beef stock	40	4	10.0	1.90	18.00	34.20
Ram	21	8	2.6	1.00	10.00	10.00
Ewes, breeding	361	9	40.1	17.19	6.25	107.44
Wethers, etc	482	4	120.5	22.90	3.50	80.15
Boar	8	9	.9	.38	15.00	5.70
Brood sow	90	15	6.0	4.28	14.00	59.92
Shote	288	13	22.1	13.71	5.00	68.55
Pig	226	11	20.5	10.76	2.50	26.90
Hens	1,768	21	84.2	84.19	.55	46.30
Rooster	113	20	5.7	5.38	.55	2.96
Other poultry	44	9	4.9	2.09	1.00	2.09
Bees (stands)	34	8	4.3	1.61	2.50	3.14
Walking plow	40	21	1.9	1.90	10.00	19.00
Sulky plow	4	4	1.0	.19	35.00	6.65
Gang plow	6	6	1.0	.28	65.00	18.20
Spike-tooth harrow	27	21	1.3	1.29	15.00	19.35
Spring-tooth harrow	7	6	1.2	.33	16.00	5.28
Acme harrow	1	1	1.0	.05	18.00	.90
Disk or cutaway harrow	18.5	19	1.0	.88	33.00	27.94
Roller or crusher	13.5	14	1.0	.64	25.00	16.00
Planker	11	10	1.1	.52	3.00	1.56
Weeder	14	15	.9	.66	10.00	6.60
Shovel plow	15	14	1.1	.71	2.50	1.78
Manure spreader	11.5	13	.9	.54	125.00	67.50
Cornstalk cutter	1	1	1.0	.05	25.00	1.25
Farm wagon and box	28	21	1.3	1.33	75.00	99.75
Truck or "handy" wagon	11	11	1.0	.52	30.00	15.60
Spring wagon	11	10	1.1	.52	75.00	39.00
Road cart	6	5	1.2	.28	25.00	7.00
Handcart	4	4	1.0	.19	5.00	.95
Carriage	14	13	1.1	.66	100.00	66.00
Buggy	33	20	1.6	1.57	75.00	117.75
Sled	20	15	1.3	.95	30.00	28.50
Cutter or sleigh	9	9	1.0	.42	30.00	12.60
Road drag	3	3	1.0	.14	3.00	.42
Stone boat	15	10	1.5	.71	2.00	1.42
Stock rack	5	4	1.3	.23	10.00	2.30
Gravel or dump bed	3	3	1.0	.14	6.00	.84
Scraper or slip	3	3	1.0	.14	5.00	.70
Gasoline engine	5	5	1.0	.23	200.00	23.00
Babcock tester	2	2	1.0	.09	5.00	.45
Aerator	1	1	1.0	.05	5.00	.25
Refrigerator	1	1	1.0	.05	15.00	.75
Cream separator	8	8	1.0	.38	65.00	24.70
Combination churn	1	1	1.0	.05	30.00	1.50
Corn planter, 1-horse	3	3	1.0	.14	18.00	2.52
Corn marker	6	6	1.0	.28	2.00	.56
Corn planter, 2-horse	8	10	.8	.38	50.00	19.00
Cultivator, 2 or 3 horse	30	17	1.8	1.43	28.00	40.04
Cultivator, 1-horse	27	18	1.5	1.28	5.00	6.40
Corn binder	4.75	6	.7	.21	125.00	26.25
Sled harvester	2	2	1.0	.09	25.00	2.25

[1] Machines owned in partnership account for the fractional numbers in the first figure column.

TABLE XIII.—*Major items of personal property found on 21 Ohio farms, with the average number of each item for all farms and for each farm reporting the item, the approximate value of each item, and the average value of each item for each of the 21 farms*—Contd.

Designation of item.	Number.				Value.	
	Reported.	Farms reporting.	Average per farm reporting.	Average per farm, all farms.	Per unit.	Total per farm, all farms.
Corn shocker	2	2	1.0	0.09	$120.00	$10.80
Corn shredder	.6	2	.3	.03	175.00	5.25
Ensilage or fodder cutter	6	6	1.0	.28	40.00	11.20
Corn sheller	14	13	1.1	.66	6.00	3.96
Circular woodsaw	5	5	1.0	.23	8.00	1.84
Grain binder	16	18	.9	.76	125.00	95.00
Grain drill	17	15	1.1	.80	65.00	52.00
Fanning mill	11.5	12	1.0	.54	25.00	13.50
Reaper	1	2	.5	.05	45.00	2.25
Hay loader	5.5	6	.9	.26	55.00	14.30
Mower	23.5	21	1.11	1.11	45.00	49.95
Hayrack	22	18	1.2	1.04	10.00	10.40
Hayrake, sulky	17.5	19	.9	.83	20.00	16.60
Hayrake, wooden	1	1	1.0	.05	5.00	.25
Wheelbarrow seeder	2	2	1.0	.09	8.00	.72
Tedder	9.5	10	1.0	.45	38.00	17.10
Potato cutter	.5	1	.5	.02	6.00	.12
Potato planter	3.5	4	.9	.16	55.00	8.80
Potato sprayer	3.5	4	.9	.16	25.00	4.00
Potato plow (digger)	4	4	1.0	.19	15.00	2.85
Potato digger	2.5	3	.8	.11	90.00	9.90
Potato sorter	2	2	1.0	.09	20.00	1.80
Sap evaporator	6	6	1.0	.28	100.00	28.00
Sap-gathering tank	4	4	1.0	.19	8.00	1.52
Sap-storage tank	8	5	1.6	.38	12.00	4.56
Sap sled	3	3	1.0	.14	3.00	.42
Orchard sprayer	5	6	.8	.23	20.00	4.60
Cider mill	3	3	1.0	.14	10.00	1.40
Fertilizer spreader	1.5	2	.8	.07	25.00	1.75
Feed grinder	3	4	.8	.14	40.00	5.60
Fruit evaporator	2	2	1.0	.09	50.00	4.50
Litter carrier	1	1	1.0	.05	30.00	1.50
Beet cutter	1	1	1.0	.05	25.00	1.25
Beet lifter	1	1	1.0	.05	15.00	.75
Tread power	1	1	1.0	.05	40.00	2.00
Incubator	4	3	1.3	.19	10.00	1.90
Brooder	2	2	1.0	.09	7.00	.63

COST OF EQUIPPING A FARM.

From the foregoing discussion it will be possible to make a summary showing more or less accurately the first cost of equipping the average farm under consideration. In Table XIV the actual inventory valuations of live stock, produce, supplies, etc, are taken from Table VII (p. 26), rather than approximations which might be obtained from Table XIII.

TABLE XIV.—*First cost of equipping an average farm in Ohio.*

Items of equipment.	First cost.	
Real estate (78.15 per cent):		
Land, 165.88 acres at $46.25 (average)	$7,676.42	
Farm buildings	2,700.00	
Household buildings	2,500.00	
Fences	763.74	
Drainage	366.43	
Water supply	225.00	
		$14,231.59

Items of equipment.	First cost.	
Personal property (21.85 per cent):		
Work animals	$640.71	
Colts and driving horses	250.95	
Cattle	582.26	
Sheep	201.05	
Swine	158.34	
Poultry[1]	52.60	
Bees	3.23	
Harness	131.05	
Machinery	1,125.48	
Minor articles	200.00	
Produce, supplies, etc	631.93	
		$3,977.60
Total for both real estate and personal property		18,209.19

In actual practice innumerable factors tend to reduce the cost of equipping farms. Few farms in the older sections of the United States like Ohio are equipped outright with new buildings, fences, and machinery, and the foregoing summary would, of course, apply only to these few farms; but the table is of interest in showing the amount of money spent during a series of years in bringing the equipment up to a profitable working basis. The 21 farms studied in such detail are not in any sense exceptional or "model" farms. They represent a large class, probably more successful than the average, and no doubt the detailed estimates of their average equipment cost will be found helpful as a guide in planning the proper distribution of capital.

UNIT COST OF FARM EQUIPMENT.

The third phase of this study was made less prominent than the two already discussed. This phase is that of current equipment charges on farm operations, including machinery costs per acre of crop, building charges per head of live stock, and storage or building charges per unit of products. From the circulars sent out to Ohio corn growers, from Mr. Thompson's notes, and from the inventories on the farms of cooperators considerable data have been gathered regarding the machinery costs, but the determination of annual and unit costs of buildings, fences, etc., has not been attempted because of the meager information at hand.

That there is a distinct cost each year for buildings, fences, and other improvements is undisputed, but the exact amount is difficult to ascertain, owing to the lack of information concerning the rate of depreciation on such equipment. The depreciation on the modern

[1] As the practice in housing poultry on the average farm is not good, this figure might be slightly increased, although much more serviceable poultry houses might be constructed as economically as the average ones used.

steel-wire fences is rapid, and often excessive, while many of the old rail, wire, and picket fences are in good condition after years of service. The ordinary farm usually has from 3 to 10 kinds of fence, hence, the gathering of data of this sort was found to be too complex for the present study. Building depreciation varies with the construction and subsequent care, as well as with the use to which different structures are put. The increase in the cost of construction during the last generation has equaled if not exceeded the depreciation from the original value; hence, the determination of interest and depreciation involves more study than could be given at this time. The annual deterioration in condition probably ranges from 2 to 5 per cent of the original standard in buildings and from 6 to 20 per cent in fences. If no change occurs in the cost of construction the annual depreciation, repair, and interest charges could be added and the total charge apportioned to the various units. But further investigation is necessary before averages can be presented in this connection.

Regarding machinery costs the problem is simpler. Prices have not changed so materially, the annual rate of depreciation is more easily obtained, and the proportion of use each year more easily reduced to a unit basis. Table XV shows in detail the data on machinery costs, either on the annual or acre basis. The number of machines included in the final average is first shown. On many farms unit costs were clearly out of the usual range of probability, and these were discarded in taking the average. The "First value" at time of purchase by the farmer reporting is shown, this average including many secondhand machines. The "Second value" is that of the inventory rather than the sale value. The "Average investment" is computed by averaging the first value and the value at the beginning of the last season, which is obtained by adding to the value at the close of the last season, as shown by the inventory, the average depreciation. This method produces the same result as would be obtained by assuming that the rate of depreciation was constant throughout the period of use of the machine up to date and averaging the values at the beginning of each season. The method involves a slight possibility of error, due to the fact that the repairs are not put on at a constant annual rate; the actual difference in inventory would be somewhat affected, but the discrepancy would be negligible. The average "Years in use" up to the last date of inventory is shown, and from this and the difference between the first and second values the annual rate and percentage of "Depreciation" are obtained, the percentage being based on the first value. The "Repairs" are from actual records or careful estimates. "Interest" is calculated at 5 per cent on the average investment. The

"Total cost" is the sum of depreciation, repairs, and interest. The lowest and highest acre costs for different machines are shown, though these are not always included in the average. The "Low" figure is usually for a secondhand machine used on a large acreage or for a long period, while the "High" figure is usually for a new machine given very little use. Extra machines on any farm show a much higher cost than those in ordinary use. The interest charge is the greatest factor in the cost of little-used machinery, emphasizing the advantage of utilizing machines to their maximum capacity. All data in Table XV are averages of the entire group of 21 farms, and not a mean between individual costs.

TABLE XV.—*Cost per annum and per acre of machinery on a group of 21 Ohio farms.*

Kind of machine.	Number.	Cost per machine.			Years in use.	Cost per annum.					Acres worked per annum.	Cost per acre per year.		
		First value.	Second value.	Average investment.		Depreciation per annum.		Repairs.	Interest.	Total cost.		Low.	High.	Average.
						Amount.	Per cent.							
Walking plow	115	$13.60	$6.95	$10.62	9.6	$0.69	5.1	$0.71	$0.53	$1.93	27.1	$0.018	$0.359	$0.072
Riding (or gang) plow	42	47.22	33.05	40.17	5.6	2.54	5.4	.96	2.01	5.25	28.8	.017	.42	.183
Harrow, spike	74	12.47	6.83	9.99	8.3	.68	5.5	.29	.50	1.47	79.2	.005	.108	.019
Harrow, spring	16	17.00	7.72	12.88	9.0	1.03	6.0	.21	.64	1.89	38.8	.009	.17	.027
Harrow, disk	62	26.90	14.93	21.62	7.4	1.62	6.0	.27	1.08	2.97	60.4	.005	.317	.049
Roller	23	22.50	14.09	18.67	11.3	.75	3.3	.03	.93	1.71	84.2	.004	.092	.020
Planker or drag	13	2.94	1.42	2.30	6.5	.24	8.0	.....	.11	.35	45.4	.002	.035	.008
Weeder	19	10.79	5.76	8.29	7.2	.70	6.5	.....	.41	1.11	34.4	.013	.173	.033
Corn planter	60	35.45	18.29	27.97	7.8	2.20	6.2	.47	1.40	4.07	50.1	.020	.299	.081
Cultivator, 1-horse	12	4.79	2.58	3.81	8.5	.26	6.3	.07	.19	.52	12.1	.018	.068	.043
Cultivator, 2 or 3 horse	102	24.51	12.00	19.04	7.9	1.57	6.3	.34	.95	2.86	69.7	.009	.418	.041
Corn binder	28	105.32	51.78	82.79	6.3	8.48	8.0	1.60	4.14	14.26	38.5	.199	2.22	.369
Corn shocker	6	120.83	69.17	101.46	4.0	12.92	10.7	.79	5.07	18.78	22.3	.248	2.78	.842
Grain binder	24	117.11	46.96	86.10	8.6	8.13	7.0	1.10	4.31	13.54	51.1	.128	.688	.264
Grain drill	40	59.69	35.35	48.75	8.7	2.81	4.7	.33	2.44	5.58	43.0	.018	.397	.130
Hay loader	12	57.75	30.29	45.76	7.9	3.47	6.0	.65	2.89	7.01	28.3	.130	.488	.248
Mowing machine	45	41.64	21.67	32.94	7.8	2.56	6.1	.93	1.65	5.14	49.1	.040	.558	.105
Hayrake	35	19.21	9.86	15.09	8.5	1.11	5.8	.26	.75	2.12	38.8	.005	.347	.055
Tedder	20	31.70	18.60	25.96	8.0	1.63	5.2	.40	1.30	3.73	22.5	.015	.427	.164
												Annual cost per machine.		
Manure spreader	46	112.25	82.93	102.24	3.2	9.30	8.3	1.88	5.11	16.29	....	7.81	49.38	16.29
Fanning mill	11	20.81	13.72	17.64	9.3	.76	3.7	.....	.88	1.64	....	.41	2.58	1.64
Wagon	76	62.72	28.26	46.99	11.5	3.00	4.8	1.20	2.35	6.55	....	1.23	11.21	6.55
Corn shredder	5	474.30	344.80	431.14	3.0	43.17	9.1	.98	21.56	65.71	....	37.25	84.50	65.71
Ensilage cutter	11	111.04	71.36	94.36	6.3	6.32	5.7	.83	4.72	11.87	....	2.21	36.80	11.87
Corn sheller	11	9.74	5.34	7.73	11.5	.38	3.9	.04	.39	.81	....	.22	2.26	.81

The wide variation in acre cost of all machinery suggests the necessity for considering the acreage per year as an extremely important factor. For instance, 60 corn planters averaged 50.1 acres per year at an acre cost of 8.1 cents; 24, averaging 63 acres, cost between 4 and 8 cents per acre; and 15, averaging 34 acres, cost 10 to 13 cents per acre. This separation of planters into two groups was suggested

by the appearance of curves plotted to show the frequency of different acre costs for all the machines. Extremely high costs on a few farms were sufficient to raise the averages considerably above the cost occurring most frequently. The curve of planter costs showed two distinct groups, with the average midway between. It is evident that machinery costs should be studied for different acreages, especially since the annual cost of the same machine on different farms varies much less widely than the acre costs.

Only 9 out of 130 walking plows cost over 20 cents per acre, and these were excluded from the average. The question of plow costs in the hill section was raised. Twenty plows in this section showed an average of 6.1 cents per acre and a mean of individual costs of 7.2 cents. The first value was $13.20; second value, $6.80; average investment, $10.40; years used, 9.15; annual depreciation, 71 cents; percentage of depreciation, 5.3; acres per year, 26.3. The approximate uniformity of these figures with the average for the whole number was surprising, especially in view of the low percentage of crop area on many farms in this section.

The cost shown for cultivators, harrows, rollers, plankers, and weeders is on the basis of 1 acre covered once, or the "acre-time." Since in the tillage of an acre of land the same implement may be used a varying number of times, the acre-time is considered a more logical unit than the acre. One spring-tooth harrow covering a total of 250 acres per year at 0.7 cent per acre-time and one covering 10 acres per year at 17 cents per acre-time are omitted from the average. The roller operating at 0.4 cent per acre-time was used 300 acre-times per year. Excluding this one, the cost per acre-time was 2.4 cents. About four-fifths of the rollers cost between 0.5 and 5 cents per acre-time. The wooden planker, drag, or float, as it is variously called, is usually homemade, hence the low first cost. Many homemade wooden rollers are also found. Weeders range rather uniformly from 2 to 12 cents per acre-time. One which covered the equivalent of 300 acre-times per year at a cost of 0.3 cent was omitted from the average.

No records are at hand as to the acres covered by many of the manure spreaders, and of course the cost of fanning mills, wagons, corn shredders, ensilage cutters, and corn shellers can not well be reduced to an acre basis. Annual costs are given for all such. The mean acre cost of 12 spreaders was 87 cents, and the mean cost (or machinery charge) per load for 12 other spreaders was 5.9 cents. It is interesting to note that the average years in use for spreaders is much lower than that of most machines. The majority of spreaders in use are probably innovations on the various farms; hence the cost data are more difficult to obtain than those for machines introduced earlier.

Excluding secondhand implements, the cost per acre-time for 1-horse cultivators ranges from 2.6 to 6.8 cents, with the greater number between 4 and 5 cents. A few 3-horse (double row) cultivators are included with the 2 horse. Only 3 of the 2 or 3 horse cultivators cost over 13 cents per acre-time. One of these was an extra cultivator, bought secondhand and used on only 15 acres in four years. The cost of use for most of them ranged between 1 and 10 cents per acre-time, 35 out of 102 being between 2 and 4 cents, 24 between 4 and 6 cents, and 12 below 2 cents.

The acre cost of corn binders varies greatly, but on about half the farms where used it was between 25 and 45 cents per acre. Two sled harvesters cost less than 10 cents per acre. The corn shockers reported were used on a much lower acreage than the corn binders, with a much higher acre cost. The wide variation in size and first cost of ensilage cutters makes the average of doubtful value. Two cutters cut about 120 tons each per year at costs of about 7 cents per ton, while another cut about 1,250 tons per year at a cost of 2.9 cents per ton. Three 2-hole corn shellers had a mean cost of $2.01 per year, while seven out of eight 1-hole shellers cost less than 60 cents per year. The shortness of the period of

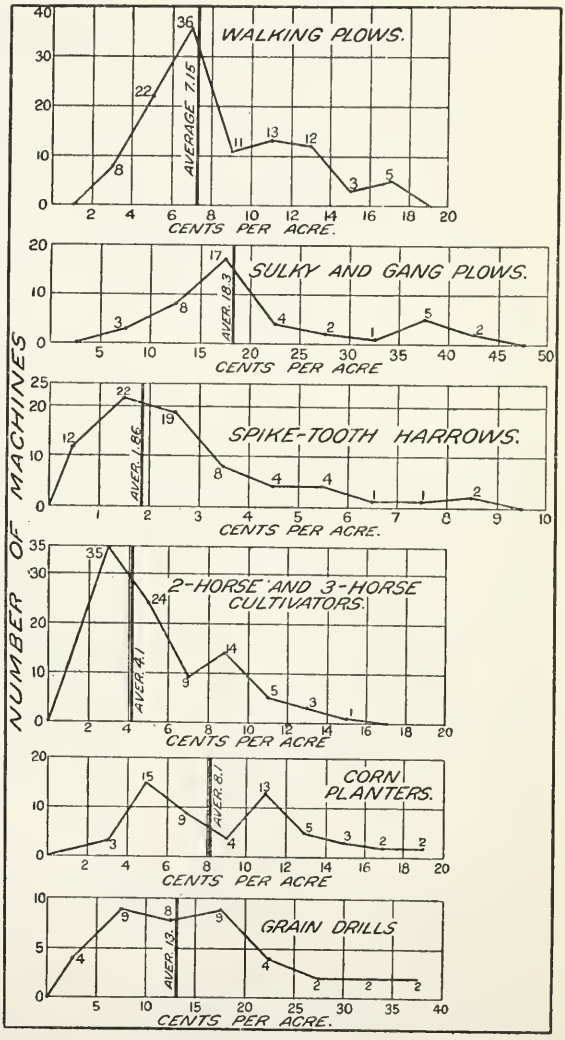

FIG. 3.—Diagram showing the acre cost of individual plows, harrows, cultivators, cornplanters, and grain drills.

years in use undoubtedly accounts for the remarkably low repair cost of the corn shredders. The cost of 14 grain binders ranged between 15 and 30 cents per acre. Grain drills ranged very uniformly between annual costs of about $1 and $10 and acre costs of 6 to 20 cents.

The acre cost of mowing machines varied uniformly between 4 and 18 cents, 35 out of 45 machines being within these limits. The annual cost of 20 out of 35 hayrakes was between $1 and $2.50. The cost of these 20 rakes ranged from 2.4 to 16.8 cents per acre-time, with a mean of 7.3 cents. This is probably a better figure

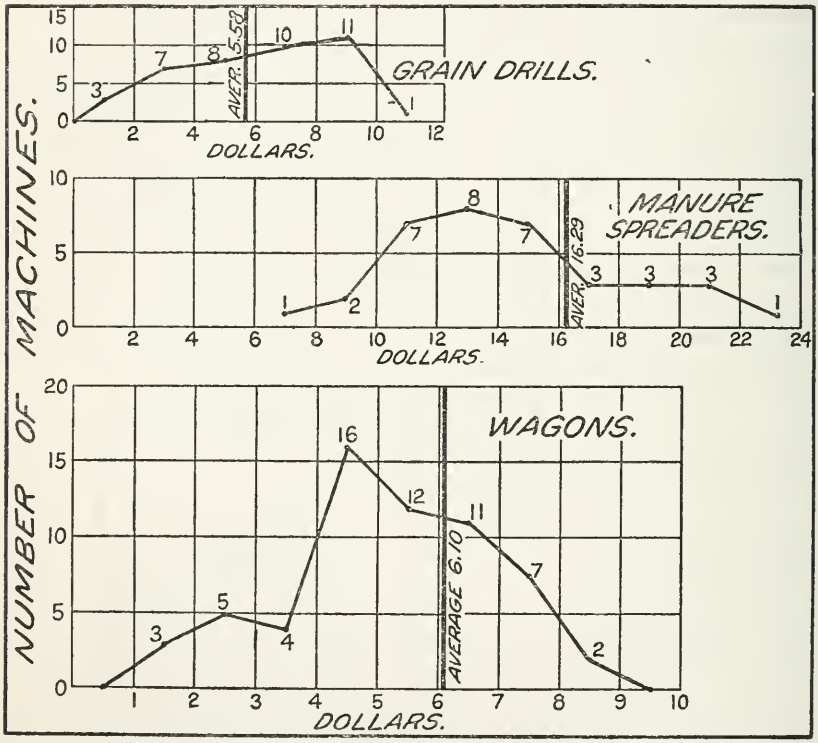

FIG. 4.—Diagram showing the annual cost of individual grain drills, manure spreaders, and wagons.

than the average (5.5 cents) given in Table XV, in which are included a number of revolving wooden rakes and secondhand steel rakes at a cost of 0.5 to 2.5 cents per acre, and two side-delivery rakes at 17.1 and 29.4 cents, respectively. The cost of 13 out of 20 hay tedders was between 15 and 25 cents per acre. The lowest figure is for a secondhand machine and the highest for a machine tedding an average of 5 acres each year. The lowest annual wagon costs are due to truck or "handy" wagons and to those not purchased new. Sixty per cent of wagon costs are between $4 and $8 per year.

Figures 3 and 4 illustrate diagrammatically the various acre and annual costs for different machines. The height of the points on each curve indicates the number of machines with costs within the range indicated by the figures on the base line. Of walking plows, for instance, 8 cost between 2 and 4 cents per acre, 22 between 4 and 6 cents, and so on. The average cost for the group is shown to be usually higher than those acre or annual costs which are most frequent, owing to the influence of abnormally high costs. Implements with annual costs widely separated from the others, as 1 manure spreader with an annual cost of $49.38 and 3 wagons costing over $11 per year, are not considered. The curves show more clearly than the average the cost of the greater number of machines, but the average is valuable because of the consideration given to the most and least as well as the normally expensive ones.

While the lack of numbers makes the data suggestive rather than conclusive, these figures present a fair basis for estimates of the machinery cost of producing crops.

SUMMARY.

Proper organization, a prerequisite to successful farm management, refers not only to the cropping system, live-stock management, etc., but to the distribution of capital and the selection of equipment. This study of a number of Ohio farms does not afford sufficient data from which to draw general conclusions, but illustrates by concrete example many of the factors to be taken into consideration in equipping farms. Further study along the lines indicated should provide data of great value to the farm manager. This outline of some of the economic problems involved in the equipment of farms is presented as worthy of the attention of students of farm management and of farm economics in general.

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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 213.

B. T. GALLOWAY, *Chief of Bureau.*

CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY.

BY

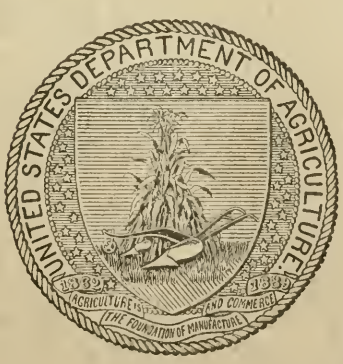
ERWIN F. SMITH, Pathologist in Charge of Laboratory of Plant Pathology,

NELLIE A. BROWN, Scientific Assistant,

AND

C. O. TOWNSEND, formerly Pathologist in Charge of Sugar-Beet Investigations.

Issued February 28, 1911.



WASHINGTON:

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1911.

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U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 213.
B. T. GALLOWAY, *Chief of Bureau.*

CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY.

BY

ERWIN F. SMITH, Pathologist in Charge of Laboratory of Plant Pathology,
NELLIE A. BROWN, Scientific Assistant,
AND
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Issued February 28, 1911.

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BUREAU OF PLANT INDUSTRY.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., February 6, 1911.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 213 of the special series of this Bureau the accompanying technical paper by Dr. Erwin F. Smith, Miss Nellie A. Brown, and Dr. C. O. Townsend, entitled "Crown-Gall of Plants: Its Cause and Remedy."

This paper deals with an infectious disease of fruit trees and many other economic plants which, because of its infectious character, has spread to many parts of the United States. It is known to occur also in Europe and Africa.

The importance of this disease is evident from the frequency of appearance of references to it and the amount of literature already published regarding it. Various theories have been advanced as to its cause, many of these by men of high standing in pathological work, but none have been able to establish their theories conclusively. The disease has been ascribed to frost injuries, to fungi, to slime molds, and to various small animals found infesting the older galls. By practical orchardists and by most pathologists crown-gall is generally regarded as a dangerous and destructive disease; by some others it has been considered nonparasitic and of little economic importance.

The investigations here reported upon have covered a period of six years, during which the nature of the disease has been determined, its cause discovered, and its broadly infectious character established through hundreds of carefully conducted experiments. Its ready communicability by inoculation from plants of one natural family to another is thoroughly established and indicates its importance to the farmer and the horticulturist.

As the problems involved are varied and important, with very practical bearing on the production of a wide range of crop plants, it is important that the well-established evidence accumulated in

these investigations be presented in full. With the cause of the disease once generally accepted as established, the practical questions relating to its control will be much simplified.

The illustrations submitted are essential to a proper understanding of the text.

Respectfully,

WM. A. TAYLOR,
Acting Chief of Bureau.

Hon. JAMES WILSON
Secretary of Agriculture.

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CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY.

HISTORY IN BRIEF.

EUROPE.

This disease has been known in Europe on various plants for many years (50 or more in literature) and has been generally ascribed to frosts or to mechanical injuries (Goethe, Viala, Sorauer, Briem, Prillieux, and others); but to bacteria by Corvo? (Phylloxera of grape) 1885; Cuboni (grape) 1889; Comes (grape) 1892; Cavara (grape) 1897 and 1900, (peach? and juniper?) 1898; Scalia (rose) 1903; and Brizi (poplar) 1907.

Cavara was the first to demonstrate the bacterial nature of the disease (on vines) by means of inoculations from pure cultures. His studies, as well as those of other writers of southern Europe, having been generally overlooked in this country, it appears to be worth while to summarize the leading French and Italian papers somewhat carefully, in so far as they have ascribed this disease to bacteria.

The labors of Corvo and Cuboni being early, they are not bacteriological in any modern meaning of that term. Their views as to the cause of the disease appear to the writers to have been happy guesses based on the resemblance of the tumors of the grape (rogna) to the tubercle of the olive, then much talked about, rather than opinions based on convincing proofs, for the reason that the bacteria seen by them appear to have been common saprophytes lodged in cracks and crevices of the dead parts of the older galls, quite after the manner of the bacterial occupation of the olive tubercle, whereas the actual gall-forming organism in this case, as Cavara first pointed out, occurs in small numbers scattered through sound-looking tissues, and is very difficult to see even when stained. This will be better understood after reading the following summary:

In 1885 Corvo published a note (C. R. des Sé. de l'Acad. des Sci., Paris, T. 101, p. 528) in which he maintained that there is no such disease as Phylloxera of the vine, strictly speaking, but only a tuberculosis due to bacteria which are transmitted by the insects, but

which may also induce infections independently of them. Corvo's statements do not appear to have received any attention from students of Phylloxera in France or elsewhere, and his bacteriological technic is wholly negligible.[a] The organism is supposed to be a bacillus. It was obtained [sic] by putting the brown slime and fragments of tissue into flasks of vine juice diluted with distilled water, no mention being made of any surface sterilization of the fragments or of any preliminary sterilization of the culture fluid. The fluid clouded after a few days, and the infections were then obtained by plunging split vine shoots into this yellowish rotting fluid; again it would appear without surface disinfection. The results obtained appear to have been limited to a yellow-brown stain in the tissues, which is said to be the striking characteristic of the disease. In this stained portion, naturally, organisms were found.

In 1889 Cuboni published a note on the subject in the Atti della Reale Accademia dei Lincei (Rendiconti), Rome, vol. 5, p. 571. He reported finding an abundance of bacteria in the tubercles of the vine, but they appear to have been limited to dead portions. The important part of his paper is included in the following citation:

> The examination of microscopic sections made from scabby shoots collected the previous year and preserved in alcohol has demonstrated that in fact in all the tubercles are to be found masses of bacteria wholly identical with those which are to be observed in the tubercles of the olive. These bacteria are united into zoogloeæ by a mucilaginous substance insoluble in alcohol, filling the small canals or lacunæ which are found scattered irregularly throughout the tubercle. The dimensions of the bacteria vary from 1 to 1.5 μ and are about 0.3 μ broad. In the unstained sections placed in glycerine these bacteria are strongly refringent to light; treated with methyl violet they stain quite feebly.
>
> The cells which surround the lacunæ occupied by the bacteria are dead and in great part corroded. The walls of the cells remaining are of a yellowish-brown color, so that the naked eye is able to recognize in a section the nodules and the little canals where the colonies of the bacteria occur. Surrounding the lacunæ, i. e., beyond the zone of dead cells, are found parenchyma cells full of protoplasm with nuclei, many cells filled with starch granules, and also here and there strata of suberized cells alternating with strands of large bast fibers, and finally the woody elements, especially tracheids, contorted in an odd fashion and the whole arranged in an irregular manner, so that it is often difficult to orient one's self as to the genesis of the various elements.

No mention is made of any cultures or inoculations.

In 1889 Trevisan published for the above-indicated bacteria the name *Bacillus ampelopsorae*, drawing his account entirely from the notes by Cuboni and Savastano's reference to Corvo. At least there is no evidence that Trevisan himself took the trouble to make even a cursory microscopic examination, his habit being to name everything left unnamed by others, without troubling himself to

[a] Petri makes no mention of Corvo, but states (Annales Mycologici, June, 1909) that in the galls of Phylloxera he found scarcely any bacteria: "The bacteria were very rare, and never have I been able to isolate from the galls the *Bacillus vitis*."

make studies. Inasmuch as this name was given without inoculations or study of cultures, and as various bacteria occurring in the tumors are indistinguishable microscopically, and the saprophytes often more abundant than the parasite, and particularly as the parasitic organism does not, so far as we know, congregate in zoogloeæ masses in cracks or lacunæ of the dead tissue in the manner described for this organism, his name is not here used. His description in full is as follows:

Bacillus ampelopsorae Trev. in add. ad Gen. pag. 36, Batterio della rogna della vite Cuboni in Rendiconti Accad. d. Lincei, Ser. IV, 1889, Vol. V., p. 571, Bacterie de la tuberculose de la Vigne Andrade Corvo in Savast. Compt. Rend. Paris, 1886.—Baculis cylindraceis, 1 to 1.5×0.3 μ, in colonias canaliculos lacunasque tumefactionis implentes congregatis.

Hab. in tumoribus Vitis.—Dilute coloratur per colorem violaceum methylicum. *B. oleae* analogus.

In 1897 Cavara described the tuberculosis or rogna of the vine from material obtained near Venice (Stazioni Sperimentali Agrarie Italiane, Modena, vol. 30, p. 483) and partially removed the subject from the region of uncertainties by making pure cultures of the right organism and successful inoculations therefrom. His experiments, however, were so few in number that they did not convince anyone and were generally overlooked. He had published a brief preliminary note in 1895, but the following citations are made from the later and fuller article:

The attacked plant presents the following characters: Rachitic development of the leaves; color of the leaf blade greenish yellow. The leaf blades are bent on the margins and acquire a waxy aspect quite analagous to leaves of the peach attacked by *Exoascus deformans*. The branches of the year may be of a yellowish color and enlarged, and, abstraction made of the tubercular part which forms at the nodes, assume a size sometimes double or triple the normal size. The tubercules on the young shoots appear first at the nodes, in correspondence with which the periderm is lifted up in tense bridles; but then they extend to the internodes, the bark of which cracks open in a longitudinal direction. The old shoots show ample crevices, the margins of which are lined with close-set and small tubercles which, as they become old, decompose and show a browned, decayed surface. * * *

I cultivated this bacterium in gelatin prepared with green shoots of the vine, and the Petri-dish poured plates gave circular, flat colonies, mother-of-pearl color, which did not liquefy the surrounding gelatin. In tube culture, transferring from the plate culture by means of the needle, the infections gave a colony pure mother-of-pearl color with disappearing edges, sunken a little in the center while assuming a granular aspect along the line of the stab. The bacterium did not liquefy the gelatin, and behaved like an aerobe. In agar agar it was cultivated also very well, but the development was slower. Stained with methylene blue, the organisms obtained from various cultures and observed under the microscope showed a cylindric form with the extremities rounded, without any refringent particles, and having a form rather of a bacterium than of a bacillus, the dimensions being 1.5 to 2 × 0.5 μ.[a]

With cultural material many times renewed by transfer, I made inoculations in the Botanic Garden of Pavia, where in the garden plot of the Ampelopsidæ were

[a] Compare our own measurements, pp. 105, 128.

planted various species of vines, some of which were American, others Asiatic, along with two varieties of *Vitis vinifera*. There were six shoots which, by means of T-shaped incisions made after the necessary sterilization, were inoculated either in the lower internodes of shoots of the year or in upper internodes, while as many were simply incised without inoculation of the virus, for reasons of control. All were then wound with taffeta and with paper and tied with thread at the point of incision.

This inoculation experiment was made in July, therefore in a stage too advanced perhaps to have hope of any success whatsoever. Weeks and months passed away without there being shown in any of the vines any traces whatsoever of localized and diffused hypertrophy. So I lost all hope and ceased to visit them any more.

Toward the end of the winter the head gardener of the Botanic Garden, Signor Giacomo Traverso, in pruning the vines of the above-mentioned garden plat, was surprised by certain nodules which one of the European vines showed, and as he had assisted me in the experiment, came to inform me in the laboratory with a specimen which he had cut off, and which bore just two of the characteristic tubercles in correspondence to the node and with the periderm raised up in bridles in a manner exactly identical with that which had been observed on the vine shoots collected at Udine.

Of the two varieties of European grape, one was inoculated in four of its branches, the other left with simple incisions for control. All of the four inoculated branches gave tubercles, not of large dimensions, it is true (1 to 1.5 cm. in diameter); but, I repeat, of the same form and structure of those of the vine from Udine. The foreign vines were not attacked.

The microscopic examination of the shoots bearing the tubercles likewise revealed bacteria scattered in the vessels and in the tissues of the bark of the small tubercles, and I was able to establish that they do not form distinct foci as in the case of the olive and the Aleppo pine, but are found scattered here and there in the various tissues.

In order better to ascertain that bacteria of the rogna were really in question I made some cultures with material of the same origin, infected in the Botanic Garden, and obtained both on plates and in test-tube cultures the same mother-of-pearl color colonies, and the same bacteria which had served for the inoculation experiment.[a]

In 1890, Cavara published a second paper on the subject, but this is rather of a popular nature and adds nothing to the preceding paper, except notes on the occurrence of the disease in Sardinia, and a very good lithographic plate in colors, showing vine shoots bearing the tubercles, which are like those occurring in the United States.

Although Cavara's account is meager, there is little doubt in the light of our own experiments that he had the right organism and reproduced the disease with it as he says he did.

In the same paper (Staz. Sper. Ital., 1897, vol. 30, p. 504) Cavara describes a tuberculosis of the peach occurring frequently on young shoots in a garden at Pavia, and attributes this also to bacteria, which he states he cultivated out. He made no inoculations in this case and his account of the disease renders it uncertain whether he had to do with pathological formations identical with those occurring on the peach in this country and studied by us. Our peach disease occurs more commonly on crown and roots than on branches, but this may be only a matter of environment. Doctor Farneti, who

[a] Had Dr. Cavara obtained additional infections with these isolated bacteria he would have completed his proof.

furnished the material to Doctor Cavara, was good enough to collect similar material for the senior writer in the summer of 1906, but unfortunately poured plates were not made therefrom, and we can not come to any definite conclusion from the appearance of the specimens. In any event, the dendritic, spore-bearing, liquefying organism which Doctor Cavara isolated and described from these tumors under the name of *Clostridium Persicae tuberculosis* is quite distinct from the one causing the crown-gall of the peach in this country, and therefore need not be taken into consideration here.

In 1898 Cavara (Bull. d. Soc. Bot. Ital., p. 241) also described a tumor from juniper, which he attributed to bacteria. Two species were isolated, a micrococcus liquefying gelatin slowly, and a rod which grew as a white mass and rapidly liquefied gelatin. In this case he made inoculations, but not on the same species of juniper, and did not obtain any positive results with either organism. In 1904 Baccarini (Nuovo Giornale Bot. It., p. 49) and in 1910 Severini (Annali di Botanica, p. 253) claimed this tumor to be due to a Ceratostoma.

In 1903 Doctor Scalia, in Sicily, described a tumor occurring on old stems of the rose near the surface of the earth, but also frequently higher up. His paper deals principally with signs of the disease and the anatomy of the healthy and diseased parts. He does not appear to have made any cultures or inoculations, but on the strength of his microscopic examinations he named the organism *Bacillus rosarum.* He states that he discovered it in very thin sections in the interior cells of the hypertrophied tissue and in the brown gum. The bacteria were numerous in the form of small rods with rounded extremities, measuring 1 to 1.5 μ by 0.2 to 0.3 μ. They were stained with methyl violet by putting thin sections into a drop of water (which may have been sterile, although he does not say so or mention any checks), removing the sections after a short time, allowing the drop to dry, and then fixing and staining what remained upon the glass. The diameter of this organism conforms to Cuboni's measurements and is less than that of *Bacterium tumefaciens.*

It is impossible to be quite certain that the disease described by Scalia is identical with the crown-gall of the rose as it occurs in this country, and, of course, without proofs from inoculations or any description of the cultures, a name such as he has given is worthless for scientific purposes and should be regarded as a *nomen nudum.* Owing to the soft nature of the rose gall and the ease with which it disintegrates one might expect to find almost any saprophyte in the brown gum.

Von Thümen in Austria attributed the tuberculosis of the grape to a fungus, Fusisporium, but without offering any convincing

proofs. In the same way Laubert and Köck in Austria have ascribed the rose canker to Coniothyrium (1905).

Stoklasa in Bohemia ascribed the beet gall to nematodes (Tylenchus) and Bubák in Austria to mites (Histiostoma), but nematodes and mites are present in true crown gall, so far as we have observed, only after decay sets in. Possibly Stoklasa had to do with nematode galls which also occur on the beet. (Pl. IV, fig. 1.)

This disease, as it appears on the vine, is known in France as Broussins, in Italy as Rogna, and in Germany as Krebs or Grind. Viala also gives various other names, as Exostoses, Exostoses fongoïdes, Fongosités, Raude, Kropf, Schorf, Ausschlag, Mauke, Hanab, Tubercoli, Malattia dei tubercoli, etc. The gall on the sugar beet is known in Germany as the Wurzelkropf.

This brings the European history of crown-galls, so far as ascribed to bacteria, down to the appearance of the first paper on the subject by the senior writers of this Bulletin in April, 1907 (Science), a translation of which, with some additions, was published in Germany. (Centralb. für Bakt. 2 Abt., Bd. 20, December, 1907, p. 89.)

In 1907, Ugo Brizi, of Milan, described and figured a tumor of poplar, ascribed the disease to bacteria, claimed infections with pure cultures, and named and described a yellow schizomycete (*Bacillus populi*) said to be the cause of the galls (Atti Congreso Naturalisti Italiani, Milan, June, 1907). The Congress was held in September, 1906, but the paper was not published until the following summer.

Brizi figures one infection only and gives no details concerning his inoculation experiments, i. e., where they were conducted; how many failed; how many checks were held; and whether any of the latter contracted the disease. His experiments are probably of the same sort as Professor Toumey's, where one known organism was introduced and another unknown (and unsuspected) organism actually caused the disease.

The *Bacillus populi* of Brizi, which is probably one of the yellow saprophytes common in crown-galls, may be distinguished readily from the organism described in this bulletin by the following characteristics, which are summarized from his paper:

Yellow growth on culture media (agar, gelatin, potato, sugar beet, etc.); production of spores, which are generally in one end, which is swollen and refringent; rapid production of indol (24 hours at 30° C.); rapid coagulation of milk (12 hours at 25° C.) and re-solution of the curd; rapid growth in weakly acid beef broth at 25° C., i. e., clouding after a few hours. Motility occurs, but he did not succeed in demonstrating flagella by means of stains. Inasmuch as he has lost his cultures of this organism (letter of Brizi to Král, Sept. 2, 1910), we were unable to obtain it for comparison.

UNITED STATES.

Most of the experimental work on the disease has been performed in the United States. References in literature begin about the year 1892, but undoubtedly the disease has been present for a long time. The literature is so well known and so easily accessible that it is not necessary to abstract it at any length. The most recent papers are by Dr. George G. Hedgcock, of the Bureau of Plant Industry (Bulletins 183 and 186), and he has given therein a rather full bibliography.

The infectious nature of the peach gall was rendered certain several years ago by a number of experiment station workers who obtained the growths on young trees either by planting them in the vicinity of diseased ones, by mincing the galls and distributing the fragments in the sand or earth near sound trees, or by grafting. Thus Thaxter in Connecticut (1891 or prior), Halsted in New Jersey (1897), Selby in Ohio (1898). Toumey in Arizona (1900) proved in the same ways the infectious nature of the almond gall.

As the result of his experiments on almonds Toumey concluded the disease to be due to a slime mold described by him as *Dendrophagus globosus*, but this statement is not sufficiently supported by infection experiments and is regarded by the writers as wholly erroneous. Toumey made only a few inoculations and none with indubitably pure material, i. e., his inoculating material oozed from the cut surface of galls which undoubtedly contained the bacteria here described. It was never grown in pure cultures. Of his 10 inoculations 3 only yielded galls.

Hedgcock subsequently cross-grafted fragments of galls successfully on some fruit trees and unsuccessfully on others. His general conclusions, however, have differed so materially in his various papers that the reader is referred to his texts.

Under the name of necrosis of the grapevine (Cornell Univ. Bull. No. 263, Feb., 1909), Reddick figured this disease and ascribed it to *Fusicoccum viticolum* n. sp., but on insufficient evidence, as he has since admitted (2d Meeting Am. Phytopath. Soc.).

This disease, which is commonly known as crown-gall, occurs, on one plant or another, in all parts of the United States. Toumey's inquiries in 1900 showed it present in 22 States, to which all the others may now be added.

SOUTH AMERICA.

In Chile, according to Delacroix, this disease as it occurs on the vine has been ascribed by Lataste to the root coccid, *Margarodes vitium*.[a]

Solano has reported the disease as occurring on the grapevine in Peru (1910).

[a] Possibly two diseases are confused. The woolly aphis (Schizoneura) induces small galls on stems and roots of the apple and those on the roots have been confused with crown galls.

SOUTH AFRICA.

According to Dr. Thomas F. Dreyer, of Cape Town (oral communication), crown-gall occurs in Cape Colony on pear trees both in the nursery and in the orchard, and large swellings of some sort also occur on the limbs of apple trees.

According to I. B. Pole Evans, plant pathologist at Pretoria (oral communication), galls of this general character are common in South Africa on rose, peach, willow, etc., appearing on the parts above ground, especially after hailstorms, which are of frequent occurrence.

Since these paragraphs were written we have received from Charles P. Lounsbury, Government entomologist for Cape Colony, an account of these galls (Agricultural Journal, April, 1910) entitled "Giant twig-gall of willow, poplar, peach, apple, and other trees," in which he states that the gall is most common on willow (*Salix babylonica*). The poplar (*Populus alba*), peach, apple, apricot, pear, and rose are said to be attacked also. The largest gall seen by Mr. Lounsbury on the willow was 5 inches in length by 3.5 inches in diameter. He says: "Much larger galls than this are said to occur, but ones under 3 inches in length are far more numerous." They are said to be very abundant on the branches of the willow and to injure it seriously, killing the branches beyond the gall. It has been suggested by some that the galls begin in wounds made by hail, and by others that they start from insect punctures. The disease occurs also in the Transvaal. These galls have been investigated by various experts (Mally, MacOwan, Pole Evans, Lounsbury), but no conclusion is reached as to their cause, except that Mr. Lounsbury says that Mr. Pole Evans has discovered that a very common knot "which occurs throughout the land on quince trees" is "associated with a particular fungus."

Willow galls received from Mr. Lounsbury are reported on later in this bulletin (p. 94). Mr. Lounsbury's figures strongly suggest crown-gall, but in conclusion he says: "It seems improbable that it [the American disease] is identical with the South African trouble under discussion."

We have, however, produced the disease on willow with a schizomycete isolated and subcultured from one of his galls.

EARLIER STUDIES IN THE DEPARTMENT OF AGRICULTURE.

The senior writer's first acquaintance with this disease (as it occurs on peach trees) was in 1892 (Journal of Mycology, vol. 7, p. 378). This was the first work on the disease in the Department of Agriculture. In 1893 he spent about six months on the crown-gall of peach, making microscopical studies and cultures with material received from various places in California, and also with some

from Georgia. He did not then have bacteria in mind, but rather plasmodia and fungi, especially the latter, an effort being made to connect the growths with certain roundish brown chlamydospores found very abundantly in some of the galls. Nothing was published on the subject, because the conclusion was finally reached that neither plasmodia nor fungi were the cause of the disease.

Thereafter none of us (the writers) did anything with the subject of crown-gall for a period of 10 years—i. e., until 1904.

In the interim other workers in the Bureau of Plant Industry took up the subject—i. e., Waite, O'Gara, von Schrenk, and Hedgcock, but without discovering the cause. It may also be added that in beginning work on the daisy gall the writers had no idea that it would reopen the whole subject and lead in all sorts of directions.

STUDIES DETAILED IN THIS BULLETIN.

DISCOVERY OF THE BACTERIA, FIRST ISOLATIONS, AND INOCULATIONS.

In February, 1904, the Bureau of Plant Industry received a number of marguerites or Paris daisy plants (*Chrysanthemum frutescens*), both white and yellow varieties, all of which were affected with gall-like growths on various parts of the stems and leaves. These plants were sent in by one of the large commercial daisy growers in New Jersey, and were accompanied with the statement that both old and young plants were attacked, but that the older ones were more seriously affected than the younger ones. The further statement was made that the disease appeared on the plants in the open in summer and in the greenhouse in winter, and that the galls appeared on stems and leaves without any apparent cause. The galls received varied in size from one centimeter to several centimeters in diameter. The smaller and younger galls were green in color, nearly smooth in appearance, and soft and spongy to the touch. As the galls became older they increased in size and darkened in color externally until they were distinctly brown, the surfaces were rough (corky), sometimes convoluted, and they were firm and hard. All gradations were noticeable, so that regardless of the unlike appearance of the different galls, it was evident that they were all of the same origin. New galls appeared from time to time after setting out the plants in our hothouse.

The various conditions under which the galls formed excluded the possibility of their being due to insect injuries. A careful examination of the galls for fungi resulted in none being found in the interior of the tissues, and only one was found on the surface—a Macrosporium—which occurred on a portion of the knots and which had

every appearance of being a saprophyte. Moreover, when the galls were placed under favorable conditions for the development of fungi which might possibly have been overlooked in the microscopic examination, no fungus appeared.

Bacteria in the interior of the undecayed galls were first detected by the senior writer in some fresh unstained thin sections which had been prepared by Dr. Townsend. Whether these were actually the bacteria we have since isolated is uncertain. These bacteria occurred sparingly in small clumps but were so unmistakable, when once actually seen, that it was agreed forthwith to make the disease a subject of further study. With this end in view Miss Alice C. Haskins, who had been trained in the senior writer's laboratory and who was then an assistant in Dr. Townsend's laboratory, was directed to make agar-poured plates from the interior of suitable galls; and, with the bacteria so obtained, inoculations on healthy daisy plants. This work proceeded for many months without positive results. Bacteria of several sorts were obtained frequently from the interior of the galls, sometimes in abundance, but all the inoculations were negative. Several factors contributed to this result. It was not then known that the true parasite comes up slowly on agar-poured plates (three to six days or more being required), nor that the tissues frequently contain a variety of saprophytes which develop rapidly on agar. Probably most or all of the inoculations of this period were made with saprophytic organisms, i. e., with those first appearing on the poured plates.

In studying the relation of bacteria to these galls, we found little encouragement in the microtome sections, either stained or unstained. The stain used in this connection was carbol fuchsin, with the result that with high powers granules could be seen in and around some of the cells, but these were few in number and did not seem to have the characteristic even outline of bacteria. This material was fixed in alcohol.

The cultural methods used by Miss Haskins were as follows: Galls were crushed in beef broth, from which agar-plate cultures were made. For this purpose, fresh, soft galls of small size were used, as well as galls of larger size and firmer texture. In preparing the galls for these cultures, the common technic of the laboratory was used, i. e., the surfaces were scraped off with a sterile scalpel; the galls were then washed for 30 seconds in mercuric chlorid water (1: 1,000), and then in sterile water. After this they were cut into small pieces with a sterile knife and placed in tubes containing 10 c. c. of neutral sterile peptonized beef broth, one tube being used for each gall. The galls were then crushed as much as possible with a sterile glass rod.

At intervals of two to four weeks during the greater part of two years agar plates were poured from preparations made in this manner. These plate cultures, which altogether amounted to several hundred, were kept at temperatures varying from 20° to 30° C. Both white and yellow colonies of different shapes and tints appeared from time to time, and some of the plates seemed to contain pure cultures, but none of the organisms were constant in all the plates from all the galls. Slant agar cultures were made, however, from a selection of such colonies as appeared, and from these subcultures inoculations were made by means of needle pricks into both old and young stems and leaves of healthy daisy plants. An occasional gall was found at or near the point of inoculation, but they were so few and so uncertain as to their formation that the inoculations were considered as having no significance.

The possibility of these growths being due to bacteria was therefore temporarily abandoned (Dr. Townsend) and various attempts were made to produce them by mechanical injuries practiced upon both young and old plants. Notches varying in number and extent were cut with a sharp knife into the sides of main stem and branches. The main stem was cut off at different distances from the ground. In some instances the entire top was removed, and in other instances only the top of the main stem was cut off. Other injuries of this nature graded between these two extremes. Branches were also cut off at different distances from the main stem, and some were simply clipped without cutting off. Parts of leaves were cut off. The main stems were injured near the base by jabbing with the point of a knife. Some stems and branches were broken off, while others were simply broken and left hanging by a portion of the tissue. In addition to the injuries mentioned, combinations of these were made upon healthy plants in various degrees of severity until we had 20 series of simple and compound injuries. These were all started in the pathological greenhouse upon plants produced from cuttings from healthy marguerites. Abnormal growths appeared on some of the injured plants, but they were not produced with any degree of regularity or certainty, and the growths rarely occurred exactly at the point of injury. Furthermore, the abnormal formation when occurring at the point of injury had more the appearance of callous growths than of the original daisy galls.

About this time (May, 1906) one of us observed, in studying microtome sections stained with anilin compounds having a strong affinity for bacteria, that while no distinct bacteria could be made out, nevertheless that part of the section lying deepest, i. e., bordering on the sound tissues, took the stain much heavier than the rest of the gall,

as though the living bacteria might be lodged most abundantly in this portion. It was suggested, therefore, that for the next series of plates deeper tissues should be used. Six sound daisy galls varying in size from 2 to 20 mm. in diameter were therefore selected by Miss Haskins and carefully washed in distilled water with a firm brush, rinsed in twice distilled water, put into mercuric chlorid water (1:1,000) for 45 seconds, rinsed in sterile water and each knot then placed in a test tube containing 10 c. c. of sterile bouillon. In cutting these galls, a small portion of the stem at the point of attachment of the gall was also removed with the gall. After placing these galls in bouillon they were cut and mashed as much as possible with a sterile knife and a sterile glass rod. Some of the more woody portions it was impossible to crush thoroughly. However, from these six bouillons, each inoculated from a separate gall, 19 agar-plate cultures were made, three from each tube except No. 1. In 48 hours all the bouillon tubes were clouded and a yellow organism developed during the same period in four of the six groups of plates; that is, four of the knots had produced agar-plate colonies in 48 hours, the plates being kept at room temperatures of from 20° to 25° C. On the fifth day after the plates were poured a few small, round, white colonies appeared in each plate in five of the six series. Slant agar and potato cylinder cultures were made from both the yellow and white colonies, also cultures in litmus milk. The indications were that three kinds of yellow colonies had formed, and that all the white ones were alike.

On June 1 inoculations were made from each of the 4 organisms into the stems of young healthy daisy plants growing in the pathological greenhouse. The inoculations were made at the top, middle, and base of the stem in each case. For this purpose young slant agar subcultures were used. The portion of the stem to be inoculated was washed with corrosive sublimate water (1:1,000) and then with sterile water. The growing organism was smeared upon the stem with a sterile platinum needle and pricked into the tissues by means of a sterile steel sewing needle. Control pricks were made with a sterile needle on other daisy plants for comparison.

On June 8 another set of healthy plants, older than the former set, was inoculated with fresh cultures of the four organisms in the manner described above.

On June 18, in the first series (those of June 1) a distinct elevation (knotty growth) was visible at each point where an inoculation had been made with the white organism, but no change had taken place in any of the plants inoculated with the yellow organisms nor in any of the control plants.

In the second series on June 23, that is, 15 days after inoculation, slight elevations were visible at all points where the white organism

was used, but no elevations were discernible at points where the yellow organisms were used nor on the control plants.

Knots of considerable size subsequently developed in some of the pricked spots, but they were not watched and the labels were lost off.

CONFIRMATORY INOCULATIONS AND CROSS INOCULATIONS.

Further inoculations by the writers of this bulletin were then made as follows with the daisy organism and with the same or similar bacteria plated from galls on other host plants, the organism from the daisy being described after a few months as *Bacterium tumefaciens* Smith and Townsend (Science, Apr. 26, 1907, pp. 671–673; and Centralb. für Bakt., 2. Abt., XX. Bd., December, 1907, pp. 89–91.)

EXPERIMENTS WITH THE DAISY ORGANISM.

DAISY ON DAISY.[a]

Inoculations of November 27, 1906 (Brown).

Made 28 inoculations into marguerite daisies, using 4 different organisms plated from a daisy gall found in the greenhouse (probably produced by one of Miss Haskins's inoculations—labels lost off). Inoculated each organism into 7 different daisy plants at the tip. The cultures were 2 days old.

Result.—December 12: All 7 plants inoculated with the white organism (designated *B*) had knobby outgrowths. No protuberances were visible an plants inoculated with the other organisms.

December 18: Galls formed on all those plants inoculated with *B*. The same organism (*B*) was isolated by poured plates from one of these galls and its infectious nature proved by the following inoculations:

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[a] Wherever the word daisy is mentioned in the following pages it means hothouse varieties of *Chrysanthemum frutescens* unless otherwise stated. All of the inoculated plants were grown in hothouses unless otherwise stated. All of the inoculations recorded in this bulletin are pure-culture inoculations made with the bacteria described by us; all were made from poured-plate colonies or subcultures therefrom, usually the latter, and all the figures of galls shown in the plates are the result of such pure-culture inoculations, with the exception of a few figures introduced for comparison, viz, Plate III, upper left-hand figure (oleander from California); Plate IV, figure 1 (nematode galls on sugar beet); Plate XVI, figure 2*b* (nitrogen-fixing root tubercles on alfalfa); Plate XX (crown-gall of rose and nematode galls on Stizolobium); Plate XXIII (poplar gall from New England); Plate XXXI (hard gall on apple from Oregon and gall on blackberry from Wisconsin); Plate XXXV, figure 2 (quince gall from Algeria); Plate XXXVI, figure 2 (lettuce gall from a hothouse in Maryland). The reader who wishes to get at the positive and negative results of the inoculations quickly is advised to consult Tables II and III, beginning on page 133.

In all inoculation headings and also in the plate descriptions in such expressions as *Daisy* on Daisy, *Peach* on Peach, etc., the first word is to be understood as a convenient substitute for a phrase, e. g., "Daisy on daisy" means pure culture of a schizomycete originally isolated from a natural tumor on daisy and inoculated on daisy.

The name of the individual making the experiment is usually prefixed to it, but generally two of us were present when the results were recorded, and the authors of this bulletin are to be held jointly responsible for all statements made in it, except those relating to cancer, for which the senior writer alone is responsible.

Finally, all of our results are reported, whether favorable or unfavorable.

Inoculations of January 8, 1907 (Brown).

Inoculated 8 plants with organism plated December 18 and designated as *B*.

Result.—In 7 days galls had started to form on each plant at the place inoculated.

Inoculations of January 18, 1907 (Brown).

Inoculated 7 old plants on both old and young stems—24 inoculations in all. Agar culture 49 days old (white organism).

Result.—February 6: No galls had formed; cultures probably dead.

Inoculations of February 6, 1907 (Brown).

Inoculated 8 more old daisy plants on young twigs only with a culture 9 days old.

Result.—February 18, 1907: Small galls had formed.

Other inoculations were made with the original cultures, as follows:

December 13, 1906.—Eight daisy plants were inoculated with cultures of the same date as those used November 27 and which had produced galls. These cultures were now 19 days old.

Result.—December 24: Galls were forming at inoculated places.

December 31: All the inoculated plants had galls. On February 23, 1907, photographs were made (Pl. I, fig. 1).

July 10, 1907: The galls had reached a large size and were quite hard (Pl. I, fig. 2).

December 21, 1906.—Eight inoculations and 4 checks.

Result.—December 31: Galls at all inoculated places; checks free; organism plated out of one of these galls and identified.

January 19, 1907.—Inoculated 7 plants with organism plated from gall on December 31.

Result.—January 30: Indications that galls will form.

February 5: Galls formed at each inoculated place.

Inoculations of February 18, 1907 (Smith).

Four vigorous young plants of white-flowered Paris daisy and 6 similar plants of the yellow-flowered Paris daisy were selected. Each plant of the white variety branched at the base into two equal shoots; 7 of these shoots were inoculated and the eighth was held as a check. On the inoculated shoots also check pricks were made an inch or two above the places where the infected needle entered. All of the inoculations were made by needle pricks, using a slant glycerin-agar culture, 7 days old, which had been streaked from another slant agar culture. The organism was derived from a strain which had been passed twice through the daisy by Miss Brown with the production

of tumors (the organism designated *B*). It was probably a third or fourth subculture from the colony. Four of the inoculated white-daisy shoots received 3 needle pricks each; two received 1 prick each; one received 50 pricks. One to three check pricks were made in each case, except on one shoot, which received 50 check punctures.

The yellow-flowered daisies were each about 9 inches high and limited to a single stem. Two of them received 1 infected prick each near the top, with a check prick on each a little higher up on the same side. Two of them received 3 infected pricks each near the top, with 3 check pricks on each a little higher up. One received 30 infected-needle pricks up and down the stem. The sixth plant, held as a check, received 50 punctures up and down the stem with a sterile needle.

Result.—February 23, 1907: There was distinct evidence of infection on each of the 12 shoots at the end of 5 days, the protuberances on some being nearly a millimeter high.[a]

July: The plants were removed at the end of 1 month, 2 months, and later with well-developed tumors. Galls formed only where inoculated. The 122 sterile (check) punctures healed normally. Every infected prick resulted in a larger or smaller tumor.

November 25: During the summer some of the plants developed many secondary infections (metastases).

Inoculations of December 19, 1907 (Brown).

Seven young daisy plants were inoculated by needle pricks on the stem with agar streak cultures 2 days old. The Queen Alexandra and a large yellow variety were used for these inoculations.

Result.—January 28, 1908: No galls were formed. The plants were not in a growing condition, although they were young cuttings.

Inoculations of March 12, 1908 (Smith).

Five plants of the Paris daisy were inoculated with *Bacterium tumefaciens* from daisy on agar streak cultures 48 hours old. These plants were inoculated as controls on the inoculations from the same cultures into olive and oleander (pp. 31 and 34).

Result.—May 21, 1908: All developed tumors promptly. Only 4 of the 5 plants now remain. They have good-sized tumors.

Inoculations of February 11, 1908 (Brown).

The crowns of 6 daisy plants were cleaned and inoculated by needle pricks with a 2-day-old culture. The soil was then replaced over the inoculated places. Three other plants were punctured on the crown with a sterile needle for checks.

[a] On older and slower-growing material inoculated by Miss Brown 12 days before the growth of the tumors was slower and they were still incipient.

Result.—March 30, 1908: Galls had formed on all the inoculated plants. The checks did not contract the disease.

DAISY ON FIELD DAISY.

Inoculations of April 15, 1907 (Brown).

Wild oxeye daisy plants (*Chrysanthemum leucanthemum* var. *pinnatifidum*) transferred from a field near Washington and grown in pots in the greenhouse, were inoculated on the young stems and also on the leaves. Four plants were inoculated in 4 or 5 places on each, and check pricks were made on one plant.

Result.—April 22, 1907: Galls had formed on all of the inoculated stems at the places pricked. None appeared on the inoculated leaves.

May 11, 1907: The numerous galls did not grow to the size of those on the cultivated daisy, the largest ones being only half an inch in diameter. The check plant did not contract the disease.

DAISY ON JAPANESE CHRYSANTHEMUM.

Inoculations of May 6, 1907 (Brown).

Three hothouse chrysanthemum plants were inoculated by needle pricks on the stems and leaves with agar streak cultures 2 days old. Check punctures were made on two other plants.

Result.—July 19, 1907: Galls had formed at all points of inoculation on the stems; none appeared on the leaves. The checks bore no galls.

DAISY ON CHRYSANTHEMUM CORONARIUM AND ON SHASTA DAISY (BURBANK HYBRID).

Inoculations of July 23, 1907 (Brown).

Six plants of *Chrysanthemum coronarium*, and 6 of Shasta daisy were inoculated by needle pricks on the lower parts of the stems with agar streak cultures 5 days old. Checks on both plants were held.

Result.—August 27, 1907: Galls had formed on all the inoculated plants at the places punctured. Those on the Shasta daisy were quite large. The checks remained free.

September 30, 1907: The inoculated *Chrysanthemum coronarium* are all dead, apparently as a result of the inoculation.

DAISY ON THE CORN MARIGOLD.

Inoculations of August 1, 1907 (Brown).

Six plants of the corn marigold (*Chrysanthemum segetum*) were inoculated on the stems by needle pricks with agar streak cultures 2 days old; two checks were held.

Result.—August 27, 1907: All the inoculated plants had galls. The checks were free.

September 30, 1907: All the inoculated plants were dead. The checks were alive.

The disease sometimes occurs naturally on this species. One such plant has been observed bearing 6 galls.

DAISY ON PYRETHRUM.

Inoculations of September 26, 1907 (Brown).

Seven full-grown plants of pyrethrum (*Chrysanthemum coccineum*) were inoculated by needle pricks on the stems with agar streak cultures 2 days old. The stems were woody and had almost ceased growing.

Result.—October 21, 1907: Small knots had formed on all of the inoculated stems. The 3 check plants were free from knots.

DAISY ON ENGLISH DAISY.

Inoculations of August 1, 1907 (Brown).

Eight seedling plants of the English daisy (*Bellis perennis*) were inoculated by needle pricks with agar streak cultures 2 days old. The stems were inoculated just below the surface of the ground.

Result.—August 27, 1907: Five of the inoculated plants bore knots; the two checks had no knots.

DAISY ON SALSIFY.

Inoculations of March 2, 1907 (Townsend).

Twelve plants of *Tragopogon porrifolius* were inoculated near the crown, using agar streak cultures of the daisy-gall organism made February 27. Six controls were made at the same time on other plants in the same relative positions.

Result.—No galls formed on either the inoculated or the control plants. In all probability these were the cultures used successfully on carnation of same date.

Inoculations of February 27, 1908 (Townsend).

Dr. Townsend made a second set of inoculations on salsify with positive results (Pl. XXII, fig. *A*), but the notes concerning this series have been misplaced. We do not know how many plants were inoculated. The one shown in the illustration was removed and put into alcohol May 8, 1908.

DAISY ON TOMATO.[a]

Inoculations of February 18, 1907 (Smith).

About a half dozen needle pricks were made on each of 3 soft terminal shoots of as many tomato plants which were about 18 inches high. The organism used was an agar streak culture 7 days old (used also for the daisy inoculations of Feb. 18).

Result.—Nothing was immediately visible, but after some weeks slow-growing hard tumors developed on each one of these plants in the inoculated part.

DAISY ON POTATO.

Inoculations of March 2, 1907 (Smith).

The stems of 6 potato plants (*Solanum tuberosum*) were inoculated by needle pricks with agar streak cultures 3 days old. The plants were in pots in the greenhouse.

Result.—March 27, 1907: Galls developed at all the points of inoculation and are at this date 1 cm. in diameter. One was cut off and plates were poured from it. Four days later the characteristic colonies appeared, and daisy plants were inoculated with subcultures from these colonies. In 15 days small galls had formed on the daisies.

April 22: Stems cut off and photographed (Pl. II, fig. 5*a*, *b*).

Inoculations of March 21, 1907 (Townsend).

Eighteen plants of *Solanum tuberosum* were inoculated on the stems. Fourteen of these were inoculated near the tip and four near the base. A number of control punctures were made on other plants at the same time.

Result.—Galls were formed in 2 to 3 weeks at all of the inoculated points. No galls formed on the control plants.

DAISY ON TOBACCO.

Inoculations of February 18, 1907 (Smith).

About a half dozen needle pricks were made on each of 3 terminal soft shoots on as many tobacco plants, which were about 3 feet high. The material for inoculation was an agar streak culture, 7 days old (used also on daisies of Feb. 18).

Result.—Nothing was immediately visible, but after some weeks slow-growing hard tumors developed on each one of these plants in the inoculated part and not elsewhere. (For microscopic appearance of a section through one of these tumors see Pl. XXIX.)

[a] Dr. G. P. Clinton, of Connecticut, has reported the finding of crown-gall on bittersweet (*Solanum dulcamara*).

Inoculations of March 20, 1907 (Brown).

Six tobacco plants were inoculated on the stems and leaves by needle pricks with agar streak cultures 2 days old. One plant was held as a check.

Result.—April 12, 1907: Knots a half inch in diameter had formed on all of the inoculated plants on leaves as well as stems. The check remained free.

Inoculations of September 19, 1907 (Brown).

Six tobacco plants were inoculated by needle pricks on the stems with 3-day-old agar slant cultures. Four plants were held as checks.

Result.—October 25, 1907: The inoculated stems all bore knots; there were none on the checks.

DAISY ON OLEANDER.

Inoculations of May 6, 1907 (Brown).

The stems of 3 oleanders were inoculated with agar streak cultures 2 days old; two checks were held. The plants were small and not in very good condition.

Result.—March 4, 1907: Small galls are visible.

July 19, 1907: Galls had formed on the inoculated plants where punctured, but they were not very large, i. e., not over half an inch in diameter; none appeared on the checks.

Inoculations of March 5, 1908 (Brown).

Four young shoots of single white oleander were inoculated by needle pricks with agar streak cultures 2 days old; two checks were held.

Result.—March 18, 1908: Galls had formed on all the places inoculated. The checks remained free from galls.

Inoculations of March 7, 1908 (Smith).

Six oleander plants were inoculated with agar slants 48 hours old.

Result.—June 1, 1908: All produced tumors.

Inoculations of March 12, 1908 (Smith).

Ten plants were inoculated with agar streak cultures 48 hours old of *Bacterium tumefaciens* from Paris daisy. The oleander plants were of three varieties: Madam Peyre, Professor Parlatore, and Single White. They were in excellent condition, 7 out of 10 of them having double shoots from the roots (young wood), 1 shoot lower and younger than the other; the other 3 bore single shoots. Where 2 shoots came from the same root or stem base, the lower, younger one

was inoculated. The inoculations were made into soft stem tissues and leaf tissues at the top of the shoot. The method was to take out a little of the agar slime on a platinum loop, rub it gently over the surface, and prick through it with a delicate steel needle, making a dozen or more light punctures. Five daisy plants were inoculated with the same culture for controls.

Result.—May 21, 1908: The shoots are now about 15 inches to 2½ feet high. Glossy swellings in the pricked areas began to appear after a few weeks and were especially pronounced on the variety known as Single White, so that at first it seemed as though the other 2 varieties would prove immune. But they soon showed distinct, small, ruptured (corky) tumors which grew slowly. The results by plants on this date are as follows:

Plant A, Màdame Peyre: The top of the shoot which was inoculated has grown 5 inches beyond the punctured part. That part now bears 12 tumors, which are small but decided, there being no doubt whatever as to their nature. Their height is about 2 millimeters and their breadth about the same.

Plant B, Madame Peyre: This shoot has grown about 4 inches beyond the punctured part. The tumors have fused so that the exact number can not be stated, but there is a knobby mass where the plant was punctured. The size of the fused portion may be about 6 by 4 millimeters, and the height of it perhaps 3 millimeters, and around this are a few independent small tumors.

Plant C, Professor Parlatore: This plant has grown about 8 inches beyond the punctured part; there are 9 distinct small tumors and a couple of fused ones. Their height is about 3 millimeters, and their diameter 2 to 3 millimeters. Surface somewhat brown and roughened, which is true of all of the larger tumors.

Plant D, Single White: This shoot has grown about 3 inches beyond the punctured area. The punctured part bears about a dozen tumors, some fused; the largest is about 3 millimeters high by 3 to 4 millimeters broad, with a roughened surface. The smallest one still has the smooth, shiny, unbroken skin characteristic of all of them on the start, and characteristic also of the smallest tumors observed on the oleanders received from Fresno, Cal., this spring.[a]

Plant E, Professor Parlatore: This plant has shown less reaction than any of those hitherto examined, probably because the inoculated shoot has made less growth. The growth beyond the punctured area is only about 1½ inches, and there are only three small tumors, each about 1 millimeter high and the same in diameter. Fifteen other needle punctures appear to have given no reaction.

Plant F, Single White: This is a tall plant of a single stalk and is now in blossom. Since the punctures were made it has grown about 10 inches (beyond the pricks) and developed the blossom stalk. In all of those hitherto described the tumors have been on the stem, but 4 of the tumors on this plant are on the base of a petiole and 5 are on the stem. Three additional pricks have not developed anything. The larger of these tumors are estimated to be 3 millimeters broad and about 2 millimeters high, surface roughened. Distinct overgrowth.

Plant G, Madame Peyre: Shoot has grown about 4 inches since it was inoculated, and it bears 10 separate tumors (one fused out of about 4) and 4 punctures that have not given any distinct growth. One of the little tumors is on the base of a petiole.

[a] The organism causing the Fresno disease is probably not identical with the one here described.—E. F. S.

The tumors have a rough, corky surface quite distinct from any wound reaction due to the needle punctures.

Plant H, Madame Peyre: Shoot has grown 3 inches since it was punctured. There are 3 little tumors on the midrib of a leaf, and 5 below this on the stem.

Plant I, Single White: This is one of the tall single-stem plants. It has grown about 10 inches beyond the point of inoculation and is budding ready to bloom. The shoot bears 9 distinct tumors, the largest of which are about 4 millimeters in diameter and about 3 millimeters high. Some are on the stem; some on the base of a petiole. Three needle punctures have failed to give any distinct growths.

Plant K, Single White: This is a tall plant, consisting of a single shoot. It has grown beyond the point of inoculation a distance of about 9 inches. The tumors are on the stem. They have mostly fused into a rough, brownish mass, but there are 2 or 3 separate ones. Their height above the surface of the green stem is perhaps 2 to 3 millimeters.

The last two plants and the one confined to a single stem (Plant F) have made much the greatest growth beyond the point of inoculation, and corresponding to this the tumors also are larger than on any of the others. This fact, taken in connection with the small, imperfect development of tumors on Plant E, which has made the least terminal growth, is very instructive and points without doubt to the conclusion that the amount of tumor growth is dependent upon the rapidity of growth of the shoot itself, i. e., the condition of nourishment of the part—a slow-growing shoot would have slow-growing tumors, but a rapid-growing shoot would develop correspondingly large ones.

These plants have now been inoculated nearly two and one-half months and have developed very good tumors for the amount of time, judging from Clayton O. Smith's statements respecting the slow growth of natural tumors on the oleander.

The Paris daisies inoculated at the same time for controls developed good-sized tumors promptly.

The final photographs (reproduced in Pl. III, figs. B, D) were made October 28, 1908.

DAISY ON OLIVE.

Inoculations of February 14, 1907 (Brown).

Two olive trees about 2 feet tall were inoculated on all the young shoots and a few old stems, with agar streak cultures 7 days old, the third subculture from the poured-plate colonies.

Result.—April 4, 1907: No knots.

Inoculations of March 11, 1907 (Smith and Townsend).

A young growing shoot of olive was inoculated with a virulent agar culture of the daisy organism (used also on this date for successful inoculations of the peach, p. 38).

Result.—Negative.

INOCULATIONS OF MARCH 12, 1908 (SMITH).

Five olive plants were inoculated with the daisy organism from agar streak cultures 48 hours old in soft wood near the growing tip of each shoot. They were held as checks on the second set of oleander inoculations (a set which produced tumors). Five daisy plants were inoculated from the same cultures for controls.

Result.—May 21, 1908: The results by plants on this date are as follows:

Plant L: Terminal shoot has grown only 2 inches beyond the 15 needle pricks; no tumors.

Plant M: Terminal shoot has grown vigorously a distance of about a foot beyond the pricked portion; about 30 needle pricks; no tumors.

Plant N: Terminal shoot has 20 needle punctures, no tumors; slightly raised rough corky places where the pricks have healed. Shoot has grown vigorously a foot beyond the needle pricks.

Plant O: Terminal shoot has about a dozen punctures, slight corky projections where needle entered. These raised portions are perhaps one-third millimeter in diameter and the healed corky portion of the wound itself possibly 1 mm. in diameter. All are alike on this and on the other plants. There are no tumors. The terminal shoot has grown over a foot beyond the point of inoculation.

Plant P: Basal shoot and terminal shoot inoculated; about 12 needle punctures. Shoots have grown about 6 inches beyond the pricks; no tumors.

November 16, 1908: No tumors.

The daisy plants inoculated at the same time all produced good-sized tumors promptly.

DAISY ON VEGETABLES (BEET, RADISH, CARROT, ETC.).

INOCULATIONS OF APRIL 26, 1907 (SMITH AND BROWN).

The vegetables were purchased at the market and taken to the laboratory, where they were washed thoroughly and inoculated. Thirty punctures, in groups of 5, were made on both checks and inoculated plants. Agar streak cultures 2 days old were used for the inoculations. The varieties and numbers of plants used were: Radish, long and round mixed, 10; turnips, 5; rutabagas, 2; parsnips, 6; carrots, 4; red beets, 3. Two to four checks were held of each variety, except the rutabagas.

Result.—June 28, 1907: A radish plant with a large irregular tumor on one side of the root was brought in on this date and photographed.

July 19, 1907: The rutabagas, parsnips, and round radishes were dead—i. e., they did not grow. Galls had formed on the inoculated beets, long radishes, and carrots. On the radishes they were 1 inch to 1½ inches in diameter. Small galls one-fourth inch across were on all red beets except one, which was about 1½ inches across. Small galls not larger than a half inch were on the carrots. The checks were free.

July 24, 1907: Additional photographs were made (Pl. IV, fig. 2).

DAISY ON EUROPEAN GRAPES.

Inoculations of April 3, 1907 (Smith and Brown).

Three small, slow-growing shoots of as many vines were inoculated by needle pricks from a 48-hour agar culture.

Result.—June 27, 1907: One only of the three vines developed a tumor—a small growth about half an inch long, one-fourth inch broad, and perhaps one-eighth inch high (Pl. V, fig. 1). It was on Muscat Hamburg. The failure of the other two (Golden Hamburg and Champion Hamburg) is attributed to poor condition, the plants having made scarcely any growth.

Inoculations of April 11, 1907 (Brown).

A Golden Hamburg and Champion Hamburg were each inoculated in the laboratory with agar streak cultures 5 days old. The plants were small and covered with scales, which were removed before inoculating. The plants were then set out in the greenhouse.

Result.—May 1, 1907: The Golden Hamburg was dead; the other had made no growth and no gall had formed.

Inoculations of May 9, 1907 (Brown).

A dozen Black Hamburg vines were taken from the pots, washed carefully, and 9 of them inoculated with agar streak cultures 4 days old. They were well-rooted cuttings. The inoculated plants were treated all in the same way, each receiving 20 to 25 punctures on the green shoot, underground stem, and young root. The checks were punctured in the same manner with a sterile needle.

Result.—July 20, 1907: Small galls had formed on each inoculated plant, but only on the green shoots. The galls produced were not like the regular grape galls. The checks did not develop galls.

Inoculations of May 14, 1907 (Brown).

Young, well-rooted cuttings 3 to 4 inches tall were taken from the pots, washed carefully, and 9 plants inoculated on both root and shoot, 20 to 25 punctures being made on each. The cultures used were 3-day-old agar streaks. Three checks were held. The vines were afterwards repotted.

Result.—July 20, 1907: Knots had formed on all of the shoots inoculated. The checks were free from knots.

Inoculations of June 9, 1907 (Townsend).

Vine No. 490, Black Hamburg, was inoculated with a 4-day-old agar culture.

Result.—June 27, 1907: The plant died. It was pulled up and no tumor found on the stem.

Inoculations of August 9, 1907 (Brown).

Eight plants were inoculated by needle pricks on the youngest parts of the stems with agar streak cultures 3 days old. Three checks were made. The varieties used were Prince Albert, Black Prince, and White Tokay. The vines were 2 feet tall and had made rapid growth.

Result.—August 27, 1907: Small knots had formed on all but 2 plants. Of the latter, 1 was a White Tokay and the other was a Black Prince. No knots formed on the checks.

Inoculations of March 7, 1908 (Smith).

Three plants of *Vitis vinifera*, 1 each of varieties Prince Albert, Barnes Muscat, and Black Prince, were inoculated by needle pricks in two places on each with 48-hour agar slants of the daisy organism.

Result.—One of the 3 developed a small tumor. The plants made very little growth.

DAISY ON AMERICAN GRAPES.

Inoculations of April 3, 1907 (Smith and Brown).

Four varieties of grape were inoculated with 2-day-old agar streak cultures. Thirty punctures were made on each in groups of 10— on the root, on the underground stem, and at the base of young shoots near the top of the stem. Two plants of each variety were inoculated and 1 of each held as a check, the check receiving the same number of pricks from a sterile needle. The varieties were as follows: Moore Early, Delaware, Concord, Martha.

Result.—May 9, 1907: A small gall was found on the underground stem of a Martha grapevine. No galls found on the others. All were dormant when inoculated.

DAISY ON IMPATIENS.

Inoculations of May 26, 1908 (Smith).

One young growing plant of *Impatiens sultani* was inoculated from an agar streak culture 4 days old. The plant was punctured on the soft stem in several places. A daisy plant inoculated from the same culture was held for control.

Result.—No galls resulted. The plant was under observation for several months. No record respecting the control.

Inoculations of April 4, 1910 (Brown).

Five shoots of a red-flowered plant and 7 shoots of a coarser growing white-flowered sort were inoculated by needle pricks, using one of the actively pathogenic recent isolations from daisy.

Result.—June 24, 1910: All negative.

DAISY ON CLOVERS AND ALFALFA.

Inoculations of March 12, 1908 (Brown).

The roots of 2 white clovers (*Trifolium repens*), 2 red clovers (*T. pratense*), and 2 alfalfa plants (*Medicago sativa*) were inoculated by needle pricks with agar streak cultures 3 days old. Two checks were held on the clovers. The inoculated roots were marked by strings tied around each one below the point of inoculation.

Result.—March 27, 1908: Galls had formed at the inoculated points, but could not be distinguished at this date from the regular tubercles on clover.

May 14, 1908: The plants were dug up and the marked roots examined. Small galls one-fourth to half an inch across and quite distinct in appearance from the nitrogen tubercles were now present on the inoculated roots where the needle pricks were made. One gall had many projecting hairlike roots, making it resemble the apple or peach gall of the hairy-root type.[a]

Inoculations of May 26, 1908 (Smith).

Five plants of scarlet clover (*Trifolium incarnatum*) were inoculated on the fleshy roots with the daisy organism from agar streaks 4 days old. These plants were dwarfed in 3-inch pots, but stood on earth and had rooted into it beneath the pots. The crowns were uncovered, then inoculated, and repotted in 6-inch pots. The plants had each a half dozen or more shoots, and were about 8 or 10 inches high. Those which had not yet blossomed were selected for this experiment. A daisy plant inoculated from the same culture was held for control.

Result.—Negative. Plants growing slowly and probably too old. Their roots were also injured in repotting.

According to Mr. Karl F. Kellerman (verbal communication), a gall of a similar character to that obtained by us occurs naturally on clover in some parts of the United States, and had been a source of confusion to him.

[a] Viala has figured a galled vine shoot (broussins) bearing also aerial roots.

DAISY ON PEACH.[a]

INOCULATIONS OF MARCH 11, 1907 (SMITH AND TOWNSEND).

Received 27 one-year-old peach trees from Arlington Experimental Farm; washed the roots very thoroughly in running tap water for half an hour, with hand rubbing, then rinsed thoroughly twice in distilled water. All were free from crown-gall and otherwise sound.

Held 9 as check plants, making 20 needle pricks in the crown of each one, i. e., in the bleached part of the stem just below the earth surface. Divided the other 18 into two groups. One group was inoculated with a quite viscid 5-day-old culture on ordinary slant agar. The other 9 were inoculated with a 6-day-old culture on slant glycerin agar. Each one of the inoculated plants received 10 pricks (5 on one side and 5 on the other), mostly in the white tissues of the crown of the plant, but a few lower down in the taproot. They were then taken to the hothouse and planted in good earth in 10-inch pots. Young daisy plants were pricked for control (1 from each culture).

One culture was also pricked into a young shoot of olive with negative results, as already recorded (p. 33).

Result.—March 29, 1907: Nos. 33 and 37 were dug and photographed (at end of 18 days).

April 3, 1907: The roots of all the trees were examined, and 15 out of the 18 were found with tumors where inoculated, the largest being about one-fourth inch across. Five of the roots had 2 tumors each. All inoculated with the younger culture, with one exception (No. 32, a dying tree), developed tumors. Of the other 9, 7 showed tumors. What appeared to be incipient tumors were also found on the roots of the 2 trees counted as negative, so that probably all of the inoculated plants, except the dying one, would in the end have shown well developed tumors. On these 18 trees there were no tumors when inoculated, nor afterwards, except where the infected needle entered.

April 5, 1907: The 9 check plants punctured on March 11 as controls for the inoculations were dug and examined. No tumors were found on the roots of any of them. They were repotted and returned to the house. The daisy controls had tumors.

January 29, 1908: After their examination on April 3, the inoculated trees were repotted but made very little growth for a number of weeks. This setback we now know to be very injurious to the development of galls on roots. To-day the trees were dug to be thrown away, and the following conditions were observed:

On No. 36 a large gall just underground, the same being about 2½ inches in diameter and nearly encircling the root (Pl. VI, fig. 1).

[a] See also daisy under "Peach on Peach" (p. 66), check inoculations of December 5, 1907.

Most of the others have smaller galls, some of which have rotted away except at the base. These galls are about 1 inch in diameter. No. 22 had gall about one-fourth inch in diameter. No. 25, gall not larger than last spring. No. 26, gall about one-half inch in diameter (these two are of the lot marked negative in April). No. 30 has two galls about one-fourth inch in diameter. No. 34, scars of small galls. No. 38 has no gall now visible—i. e., it has recovered.

INOCULATIONS OF APRIL 6, 1907 (SMITH AND BROWN).

Sixty-nine peach trees were brought to the laboratory from the Arlington Experimental Farm and washed carefully. All were free from natural galls and were from a soil supposed to be uninfected. They were labeled Nos. 90 to 158, inclusive. The first 21 (Nos. 90 to 110) were held as checks, being punctured on the roots with 20 needle punctures each, in groups of 5. The remaining 48 (Nos. 111 to 158) were inoculated with agar streak cultures. Daisy plants were inoculated with the same cultures for control.

The roots of 24 trees were inoculated by Dr. Smith by means of a needle, giving 15 pricks in 3 groups of 5 each (2 or 3 trees had more). For this purpose he made use of ordinary brown moderately viscid peptone beef agar cultures, 5 days old, and of white glycerin agar cultures, 5 days old, also moderately viscid.

Miss Brown inoculated 24 trees, giving 15 pricks in 3 groups of 5 each on the roots.

Dr. Smith used 6 slant agar tubes (3 of each sort) as above. Miss Brown used 6 slant agar tubes of the two sorts of agar (3 tubes of each), each 48 hours old and not yet viscid. Work done in laboratory and very thoroughly. Plants set in hothouse.

Result.—July 12, 1907: Dug to-day, brought in, washed, and examined all of the peach trees which were inoculated on April 6. They fall into three groups, as follows:

(1) Plants showing no tumors.

(2) Plants on the roots of which small tumors have developed.

(3) Plants on the roots of which larger tumors resembling the ordinary crown-gall of the peach have developed. None of the tumors are over one-half to three-fourths inch in diameter, i. e., they are not full grown.

Of the 13 uninfected plants (showing no tumors whatever) 5 were inoculated from the older cultures, 8 from the younger.

On the 17 plants showing small tumors the galls vary in size from that of a small shot to a small pea. Six of these were inoculated from the older cultures, and 11 from the younger. The tumors are all on the main root, corresponding, so far as can be determined, to

the position of the needle pricks. They vary in number from 1 to 3 on each plant.

Of the 18 plants showing larger tumors 13 were inoculated from the older cultures and 5 from the younger. All the tumors are on the main root, with the exception of one, which is on a small side root, and appears to be a secondary infection. The others seem to be primary infections, but not every group of needle punctures resulted in a gall. The tumors on these plants vary in number from 1 to 4. On plant 137 (which received 30 pricks in 6 groups) only one tumor resulted.

General remarks.—Of these plants 73 per cent show tumors. The plants were neglected on the start, receiving too little water, and for this reason made a very slow growth for several weeks after they were planted in pots in the hothouse. They have also frequently since that date received too little water.

Photographs were made of the best of this material (Pl. XI, fig. 2).

Respecting the group which shows no tumors, it may be stated that there is some evidence to show that some of those marked negative may have developed little tumors which afterwards perished. The bulk of the tumors are still sound, but a dozen or more have decayed more or less, and a few pretty completely; and if the same thing had happened to much smaller tumors on the first group, then there would be now no evidence of infection, although there might have been evidence two months ago.

Considering the slow growth of the trees the results are fairly satisfactory, especially since all the 21 check trees (420 punctures) have remained entirely free from tumors, although the trees made more growth than the inoculated ones which developed the galls. The check trees have been in the same hothouse, but removed about 30 feet from the inoculated ones. The soil was the same.

DAISY ON ALMOND.

Inoculations of March 7, 1908 (Smith).

Eight seedling hard-shell almonds were inoculated at the crown with 48-hour-old agar slants of the daisy organism.

Result.—March 18, 1908: One of the inoculated almonds was dug, and a small, well-developed tumor found at the entrance of the needle.

March 28, 1908: Two more plants dug. Nothing definite found.

March 31, 1908: The remainder of the plants were dug; 3 were found with small tumors; 2 without. The plants have stood from the time they were germinated in clean sand, and only when they were inoculated was an inch of gardeners' earth put on top of the

sand. They have, therefore, been under conditions such as would not produce a rapid growth. The inoculated tissues were rather woody, and this probably explains the small number of infections (50 per cent).

DAISY ON RASPBERRY.

Inoculations of April 12, 1907 (Smith).

Thirty-two raspberry plants, 16 of the Cuthbert variety and 16 of the King variety, were inoculated with agar streak cultures 6 days old, 15 to 20 punctures being made on each plant. Eight plants of each variety were held as checks. The inoculations were made in the laboratory and the plants immediately afterwards set out in pots in the greenhouse.

Result.—June 27, 1907: These plants were from a nursery in Virginia. They were small and not very satisfactory to work with, and they grew badly when potted, partly because they were of inferior stock and partly because of insufficient water at times. For these reasons a good many of them died on the start. The conditions, therefore, were unfavorable to the success of the inoculations.

Thirteen plants of the King variety and 6 of the Cuthbert produced no tumors. Ten plants were infected (2 King, 8 Cuthbert) with 32 tumors, many at the point of inoculation. Three plants were missing.

The tumors on all these plants are white and growing, except one or two which are decaying or dead. The largest at point of inoculation were 10 by 10 by 10 mm., 15 by 15 by 10 mm., 18 by 14 by 14 mm., and 20 by 15 mm.

July 2, 1907: The checks were brought in and examined. They had been in another hothouse adjoining the one where the inoculated plants were kept. They proved to be badly infected, so that no conclusions could be drawn from the experiment. There was no possible danger of infection from the cultures used, because they were not opened, nor any inoculations made, until after the check plants were punctured with a sterile needle and set out in the other house. The checks have grown more than the inoculated plants and the infection was probably brought along with them from the nursery, because one or two knots were found on the roots of these plants when they were purchased.

The details on checks are as follows: Eight Kings, 4 diseased with 5 tumors; 7 Cuthberts, 5 diseased—on No. 298 whole root occupied, about a dozen tumors as big as peas and others like filberts in clusters. On the others, 13 tumors. One of the two plants free from galls was dead.

DAISY ON BLACKBERRY.

INOCULATIONS OF APRIL 11 AND 12, 1907 (SMITH AND BROWN).

Thirty-four blackberry plants, 17 of Ena variety and 17 of the Rathbon variety, were inoculated with agar streak cultures of the daisy organism, the former with 5-day-old cultures, the latter with 6-day-old cultures, each plant receiving 15 to 20 punctures. Seven checks were held of each variety. The surface of the streak cultures used for these inoculations was smooth and wet-shining; they had not spread very widely over the surface of the streak—widest, however, near the fluid in the V. The fluid itself was thinly clouded, except at the top, which had stringy white masses of bacteria. The inoculations were made in the laboratory and the plants immediately afterwards set out in pots in the greenhouse. The plants were obtained from Virginia.

Result.—June 27, 1907: Three of the Rathbons dug; no tumors. One was dead when dug.

July 3, 1907: The remainder of the inoculated blackberry plants were dug and the roots examined. No tumors were found. Twenty of the plants were dead. The others had made a moderate growth.

DAISY ON APPLE.

INOCULATIONS OF APRIL 13, 1907 (SMITH).

Three varieties of apple (2 trees of each) were inoculated with the daisy organism, each tree receiving 30 pricks, in groups of 5. The varieties were as follows: Baldwin, Early Harvest, Ben Davis (No. 386 had hairy knots on its roots, which were pruned off).

Six trees were held as checks, each receiving 30 pricks in groups of 5, as follows: 2 Baldwin; 2 Early Harvest, 2 Ben Davis.

Result.—June 28, 1907: Early Harvest, No. 382—A few slight calluses on the cut surface of the root; no indication of galls in the pricked areas or elsewhere. Baldwin, No. 358—Similar to the preceding; nothing suggestive of tumors. Ben Davis, No. 386—Much more decided evidences of tumors; nearly every root which was pruned back has an abnormal amount of callus, resembling a gall, on its cut surface, and there are also some little galls on one root; no evidence of any tumors where the needle entered—in fact, it is difficult to find where the needle did enter.

July 13, 1907: The remainder of the apple trees inoculated April 13, 1907, were dug, the roots washed and examined for galls. Condition of roots as follows: Early Harvest, No. 381—Badly overgrown calluses. Ben Davis, No. 387—Very badly overgrown calluses, seven of them. Baldwin, No. 357—Overgrown calluses.

Remarks.—No positive conclusions can be drawn from this experiment, since at least some of the trees came from infected soil, as shown by the hairy root.

Inoculations of May 26, 1908 (Smith).

Five vigorous shoots of Wealthy apple (in the hothouse) were inoculated near the tip with the daisy organism from an agar streak 4 days old. As a check on these a daisy plant was inoculated in two places from the same tube used to inoculate the apple shoots.

Result.—June 1, 1908: The shoots are vigorous—about 3 feet long. The daisy is developing tumors in both the places inoculated. Apple tumors, therefore, should be obtained later. Nothing definite now.

September, 1909: No tumors appeared on the apples.

Inoculations of July 20, 1910 (Smith and Brown).

Trees 1 and 2 years old were inoculated on shoots and on crowns by needle pricks from young agar cultures of a recent isolation.

Result.—October 22, 1910: Negative above ground and uncertain below. Numerous galls were present on the crown and roots of a number of the trees, but *Schizoneura lanigera* was present.

DAISY ON ROSE.

Inoculations of March 27, 1907 (Brown).

Very young rose shoots were inoculated by needle pricks with slant agar cultures 2 days old. The shoots of 3 plants were punctured with a sterile needle for checks.

Result.—April 19, 1907: Small knobbed protuberances were formed at each inoculated place; the checks were free from knobs.

Inoculations of April 3, 1907 (Smith and Brown).

Eighteen rosebushes were inoculated with agar streak cultures 2 days old. The plants, including the 6 healthy checks, were washed thoroughly in running water. The varieties were Bridesmaid and Bride. Nine plants of each variety were inoculated and 3 checks held of each variety. Each plant received 10 to 20 punctures. Some were inoculated at the base of the shoot on the main stem, some on the stem below ground, and some at the base of young shoots. All were growing slowly. The plants were in pots in the greenhouse. Daisy plants were inoculated for control.

Result.—May 9, 1907: No galls on the rosebushes. The daisy controls developed galls.

DAISY ON VARIOUS ORCHARD TREES.

INOCULATIONS OF APRIL 13, 1907 (SMITH AND BROWN).

The following varieties were used, each receiving 30 pricks in groups of 5: Windsor pear, Sheldon pear, Bartlett pear, Worden Seckle pear, Wickson plum, Abundance plum, Montmorency cherry, Black Tartarian cherry, Harris apricot, J. L. Budd apricot, soft-shell almond, and American chestnut.

The trees were for the most part overgrown and in bad condition when received from the nursery, but bore no root knots or crown-galls. They were brought to the laboratory, the roots scrubbed, and then inoculated with young agar streak cultures; 2 of each sort were inoculated and 2 were held as checks.

Result.—June 10, 1907: The almond trees died without leafing out. When pulled up and examined to-day no tumors were found on the roots. Most of the chestnut trees were also dead and dying. They leafed out a little bit, but not to any great extent. No tumors were found on the roots. The two trees of Black Tartarian cherry were also pulled up and examined. There were no tumors on the roots. They had leafed out a very little and then died.

July 13, 1907: The remainder of the trees were dug and examined for galls. Condition of the roots as follows:

Worden Seckle pear—No. 363, small root tumor and several badly overgrown calluses, which are like tumors; No. 364, overgrown calluses.

Sheldon pear—No. 397, small tumors scattered along root; No. 398, badly overgrown calluses.

Bartlett pear—No. 379, small tumors along root and tumefied calluses badly overgrown; No. 380 (which is smaller than the others) bears on its roots 3 well-developed, typical root tumors. The largest one is connected with the root by a small pedicel and is over an inch in diameter. The Bartlett pear seems to be quite susceptible.

No tumors resulted from the inoculations on any other of the trees used in this experiment, but the evidence is of little negative value owing to the character of the trees at the time of inoculation. We did not at this time understand the necessity of inoculating into growing tissues.

DAISY ON CABBAGE.

INOCULATIONS OF MARCH 29, 1907 (BROWN).

Young cabbage plants were inoculated on the leaf blades with 4-day-old agar cultures isolated from the daisy.

Result.—April 15, 1907: Knobbed growths developed at all the places of inoculation.

Inoculations of April 18, 1907 (Smith and Brown).

Two cabbage plants were inoculated on the midribs of a dozen outer leaves; checks were held on another plant.

Result.—April 26, 1907: Each one of the inoculated midribs had split, and on these splits were knobbed outgrowths. The checks remained free from splits and knobs.

June 28, 1907: A cabbage stalk brought from the hothouse showed numerous tumors growing out of the leaf scars, the lower (inoculated) leaves having fallen. These appear to be secondary infections. Young sprouts are growing out of the tumors. Several other plants (none of which were inoculated on the stems, but all on the leaves) at this time showed similar tumors growing out of the leaf scars.[a]

Inoculations of March 7, 1908 (Smith).

Three leaf scars on each of three cabbage plants were inoculated with 48-hour agar slants of the daisy organism.

Result.—Nothing definite.

DAISY ON CARNATION.

Inoculations of March 2, 1907 (Townsend).

Twelve inoculations were made into stems of carnation (*Dianthus caryophyllus*), using agar streak cultures of February 27. Ten of these inoculations were made near the growing tips and two about midway of the older stems. Six controls were made in the same relative positions on other plants.

Result.—Knots or galls formed in all cases at the points of inoculation in times varying from two to six weeks. No galls formed on the controls. One of the galls was photographed September 18 (Pl. VII, fig. 1).

DAISY ON SUGAR BEET.

Inoculations of April 17, 1907 (Brown).

A row of young sugar beets about 4 inches high growing in the greenhouse was used for these inoculations. The soil was turned back from the root, the root washed with sterile water and inoculated with agar streak cultures 2 days old; the punctured places were covered with moist cotton, and the soil replaced. Twelve plants were treated in this way, and three were punctured with a sterile needle for checks.

[a] The senior writer secured infections in the laboratory on the freshly cut surface of raw turnip roots kept in deep sterile Petri dishes. The bacteria were rubbed on the surface with a platinum loop after the root had been scrubbed, soaked for an hour in mercuric chloride water (1:1,000) and cut with a sterile knife (Pl. IX, fig. 2). A yellow turnip bearing galls not due to nematodes was received from Texas.

Result.—April 29: Some of the inoculated plants bear galls, and one was photographed.

May 9, 1907: Galls half an inch in diameter were found on all of the inoculated beets; the checks had no galls.

May 29, 1907: The galls had increased in size so they were now 2 to 3 inches across.

INOCULATIONS OF NOVEMBER 15, 1907 (SMITH).

Twenty-four sugar beets (Nos. 500–519 and 523–526) were inoculated with the daisy organism from slant agar cultures of November 11, 1907. The portion of the beet projecting above ground was washed carefully with a clean cotton plug wet with filtered water, after which a large amount of milky fluid from the slant agar cultures (into which about one-half c. c. filtered sterile river water had been poured) was put on the cleaned surface with a sterile platinum loop. Ten needle pricks, about one-fourth inch deep, were made through this (close together), and more of the milky fluid was added. Three daisy plants were inoculated as checks on the virulence of the cultures, one from each tube. The daisies were Nos. 520 to 522. The sugar beets were from seeds planted in July, and were growing in pots in the greenhouse. They had made a very fair growth and were in good condition. They had good foliage, and the portions inoculated were soft and not woody. The daisies were vigorous young plants about 10 inches high, with very tender stems, and were growing rapidly. They were grown from cuttings and were in excellent condition for inoculation.

Result.—December 4, 1907: Some of the sugar beets inoculated November 15 were examined carefully and tumors as large as small peas were found on each one. A number of the sugar beets show as many tumors as there were needle pricks, i. e., 5 or 6. Some days previous very young knots were cut out of these plants, to fix for chromosome sections. The knots were at that time 10 days old and about one-tenth part as large as an ordinary pea, or perhaps even smaller than that. The daisy plants inoculated as checks showed tumors.

January 29, 1908: All of the sugar beets inoculated on November 15 produced well-developed tumors, except Nos. 509 and 524, i. e., 92 per cent. The tumors on 3 of the beets were rotting.

INOCULATIONS OF NOVEMBER 18, 1907 (SMITH).

Thirty-six sugar beets (Nos. 527 to 562) were inoculated with slant agar cultures of November 15, 1907 (from slant agar cultures of November 11, 1907, used for the preceding inoculation). The beets were from the same lot as those inoculated November 15, and were

growing well and all were free from natural tumors. The soil was removed from one side of the plant, leaving about 1 inch of the root exposed. This was rubbed with clean cotton wet with filtered water and then inoculated. A large quantity of the milky fluid from the slant agar cultures to which sterile water had been added was put on the clean surface, 10 needle pricks about one-fourth inch deep made through it, and more of the bacterial fluid put on. Six rapidly growing daisies (from cuttings) were inoculated near the tip of the stem, as checks on the virulence of the cultures.

Result.—December 4, 1907: Some of the sugar beets were examined carefully, and tumors found on all examined. The tumors were as large as small peas (16 days). Quite a number showed as many tumors as there were needle pricks, i. e., 5 or 6. All of the daisy plants inoculated as checks showed tumors.

January 29, 1908: All of the 36 beets developed tumors in the pricked area. One plant attacked by nematodes (none of the others were) also developed a second bacterial tumor about half an inch in diameter near the basal part of the root. The other 35 plants were attacked only where inoculated.

Miss M. L. Shorey isolated oxidases and peroxidases (black substances) from these tumors. These were copiously inoculated into the roots of numerous sound sugar beets, but no growths appeared.

Inoculations of June 11, 1908 (Smith).

Five sugar beets were inoculated with the daisy organism after passing it through oleander (with production of tumors) and plating it out again.

Result.—August 10, 1908: Every one of the 5 plants has a tumor at the place inoculated and not elsewhere.

Inoculations of December 4, 1909 (Brown).

Eight young sugar beets were inoculated with 2-day-old agar cultures of the daisy organism just plated from a gall; the cultures used were the first subcultures made.

Result.—December 21, 1909: No galls had formed. The soil was very cold and the temperature constantly low in the greenhouse.

April 4, 1910: Galls had formed on each one of the 8 inoculated sugar beets (Pl. VIII). Some of these galls were 5 inches across.

Remarks.—Out of a total of 85 sugar beets inoculated (5 experiments) 83 contracted the disease and 82 of them only at the point of inoculation. In 4 of the 5 experiments 100 per cent of the inoculated plants contracted the disease, pure cultures being used. Numerous uninoculated beets in the same houses remained entirely free from the disease.

DAISY ON HOP.

INOCULATIONS OF APRIL 8, 1907 (SMITH AND BROWN).

Two varieties of hop were used: The English Cluster and the Custis Late. The plants were knocked out of the pots, brought to the laboratory, and washed carefully. All were free from tumors. They came from New York. Eight of the English Cluster and 10 of the Custis Late were inoculated by means of needle pricks (20 to 40) on each fleshy root in groups of 5, with viscid agar streak cultures 4 days old. Four checks of each variety were held, each receiving the same number of punctures as the inoculated plants. The vines were then taken back to the hothouse and set in 10-inch pots.

No checks were made into daisy plants (1) because none were convenient, and (2) because the 4 tubes used in this experiment are part of the same lot used for inoculating peaches on April 6, and checks were kept on 12 daisy plants at that time (p. 39).

Result.—May 6, 1907: Knots were on the crowns of each of the 18 inoculated plants where punctured. The 8 checks were free.

July 11, 1907: The vines have grown slowly. The results are as follows:

ENGLISH CLUSTER.

No. 163: Four tumors on the roots, each one-half inch or more in diameter.

No. 164: Two small tumors on the upper part of the root.

No. 165: Two tumors on the upper part of root, each about as large as a wax bean; one on one side of the root and the other on the opposite side, evidently corresponding to the pricked places; midway between the two a small tumor is breaking through the root.

No. 166: One small tumor about as big as a pea.

No. 167: A decaying tumor at the top of the root and a smaller sound one a few inches down.

No. 168: A tumor about 1¼ inches long on the upper part of the root; really a multiple tumor, two having fused.

No. 169: Two tumors on the base of the stem; the larger is half an inch or more in diameter and the other about half as large; none on the roots.

No. 170: Three tumors on the upper part of the roots; 1 about an inch in diameter, 1 about half that size, and 1 quite small. The smallest is on a side root about 1½ inches below the largest one.

CUSTIS LATE.

No. 177: A tumor about an inch in diameter (longest way) at the top of the root; 4 smaller tumors scattered along the roots. The lowest one is at least a foot below the crown, and the root that bears this bears two others.

No. 178: This plant has made scarcely any growth, the two crown shoots being not more than a few inches long with diminutive leaves. Two small root tumors, each about as big as sweet-pea seeds.

No. 179: Seven root tumors, the largest over a half inch in diameter, the others about as big as small peas. Some are on the main root, some on the small side roots.

No. 180: Four tumors; 3 about as large as beans and 1 small; 3 of the 4 are on the stems near the roots; the smallest is on the root.

No. 181: A tumor on the root about 6 inches from the top; not larger than a small pea.

No. 182: Two tumors on the upper part of the root, each about a half inch in diameter; also a small tumor on a side root not larger than a sweet-pea seed.

No. 183: Plant missing.

No. 184: Two small tumors on the roots underground; one is not larger than a small pea and the other a little smaller.

No. 185: Four tumors on the upper part of the roots; 1 is three-fourths inch in diameter; another, a small tumor, is on the base of a young shoot which is tumefied over a distance of 2 inches toward the base, and it is probable that the organism had to do with this tumefaction. Saved separately in alcohol for sections.

No. 186: This is a defective, slow-growing root; it has a tumor about as large as a pea on the main root 4 inches from the top.

No. 187: Two tumors on the upper part of the root where stems come out and 1 just above on another stem; each half an inch or more in diameter.

Remarks.—Eighteen plants, all diseased; jar of material saved in alcohol; photographs made; 8 check plants subsequently examined—all free. These check plants received more than 200 punctures.

Inoculations of April 10, 1907 (Brown).

Two more varieties of hops were inoculated, the Red Canada and the Humphrey. The plants were left in the pots but the soil was turned back and the crown and young shoots were washed with sterile water. Eighteen of the Red Canada variety were inoculated, 9 with agar streak cultures 4 days old, and 9 with cultures 6 days old. Seven of the Humphrey were inoculated with a 6-day-old culture. Twenty to 30 needle pricks were made in the base of young shoots and in the crown. Two checks of each variety were held, the same number of pricks being made with a sterile needle.

Result.—April 27, 1907: All the 25 inoculated plants had galls except one, which was dead. Most of the galls were on the crown; only a few occurred at the base of the shoots. The 4 checks had none.

July 25, 1907: Removed the last of the inoculated hops (Pl. IX, fig. 1). The galls were three-fourths inch to 1½ inches in diameter. The checks remained free.

Remarks.—Of the 43 inoculated hops, 42 contracted the disease, or 100 per cent, if we exclude the 1 feeble plant which died soon after inoculation. Of the 12 checks, all remained free from the disease.

DAISY ON FIG.

Inoculations of August 9, 1907 (Brown).

Eleven young trees of *Ficus carica* were inoculated by needle pricks with agar streak cultures 3 days old. The plants were grown from cuttings and were about a foot high. The youngest and softest parts of the stems were inoculated. Three checks were held.

Result.—August 27, 1907: No galls had formed. (None formed later.)

Inoculations of March 7, 1908 (Smith).

Three fig plants were inoculated in the young tender tissues of the growing part of the stem from 48-hour agar slants.

Result.—June 1, 1908: No tumors have developed.

September, 1909: No galls appeared.

Inoculations of April 6, 1910 (Brown).

Twelve young rapidly growing shoots of an edible fig were selected and the inoculations made by needle pricks from a young agar streak culture of the newest isolation from daisy.

Result.—June 25, 1910: All negative.

DAISY ON CHESTNUT.

Inoculations of March 7, 1908 (Smith).

Three Paragon chestnut plants and 1 American chestnut were inoculated with 48-hour agar slants of the daisy organism. The needle pricks were made on growing shoots.

Result.—September, 1909: No galls appeared.

Inoculations of April 7, 1910 (Brown).

The newest isolation from daisy was employed, 4 of the chestnut plants being inoculated on the crown and 6 on young shoots. All were needle-prick inoculations from young agar streaks.

Result.—June 25, 1910: All negative. The shoots grew rather slowly.

DAISY ON OAK.

Inoculations of April 7, 1910 (Brown).

Eight small seedling red oaks (species ?) were inoculated by needle pricks into terminal slow-growing green shoots.

Result.—June 24, 1910: All negative. The shoots made only an inch of growth beyond the pricks.

DAISY ON PERSIAN WALNUT.

Inoculations of April 17, 1907 (Smith and Brown).

The roots of 6 trees of Persian walnut (*Juglans regia*) were inoculated with agar slants 5 days old, each receiving 30 pricks in groups of 5. Four trees were held as checks. The roots of all the trees were washed thoroughly in the laboratory before inoculating. The trees were then planted in the greenhouse.

Result.—July 13, 1907: The trees were an inch or two in diameter at the base when inoculated and have made a slow growth. No tumors resulted from these inoculations.

Inoculations of May 26, 1908 (Smith).

Three vigorous young shoots of *Juglans regia pendula* were inoculated near the growing end with 4-day-old agar streak cultures of the daisy bacterium.

Result.—July 15, 1908: The 3 shoots developed small tumors in the pricked area within a week or 10 days, and these have continued to grow slowly ever since. Two shoots were put into alcohol July 15.

DAISY ON WINGED HICKORY.

Inoculations of May 26, 1908 (Smith).

Eight young, actively growing shoots of *Pterocarya fraxinifolia* were inoculated by needle pricks near the growing point with 4-day-old agar streak cultures of the daisy bacterium. The tree stood on the grounds of the Department of Agriculture.

Result.—July 15, 1908: The Pterocarya proved much more resistant than the Persian walnut. There was nothing on any of the shoots for a month. More recently a few small tumors have developed on 3 of the 8 shoots. The others show nothing. Two were put in alcohol.

DAISY ON GRAY POPLAR.

Inoculations of April 17, 1907 (Smith).

Five gray poplar trees (*Populus canescens*) were inoculated with agar slants 5 days old, each receiving 30 pricks in groups of 5. Four trees were held as checks. There was a natural tumor at the earth's surface on No. 401, which was cut off. One also on No. 403 was left to be photographed. The checks received 60 pricks each in groups of 5. The roots of all the trees were washed thoroughly in the laboratory before inoculating. The trees were then planted in the greenhouse.

Result.—July 13, 1907: The trees were in a poor condition when received from the nursery. No tumors resulted from these inoculations, probably because the trees made too slow a growth.

Inoculations of May 25 and 26, 1908 (Smith).

Some young sprouts (cuttings rooted some weeks previous) were inoculated May 25 with 4-day-old agar streak cultures of the daisy bacterium.

One shoot on a tree was inoculated May 26 from the same cultures used on May 25.

One daisy plant was inoculated in 4 places from each of the 4 tubes used as a check on their virulence.

Result.—June 1, 1908: Evidence of tumors on 1 shoot (Z) at the place where the punctures were made. The daisy is developing tumors.

Inoculations of June 9, 1908 (Smith).

The inoculations were made on branches of 2 potted plants of *Populus canescens* several feet from the ground with 4-day-old agar cultures of the daisy organism.

Result.—November 16, 1908: Five small tumors have resulted; the largest is about 1 centimeter in diameter. This indicates clearly that gray poplar is also susceptible to the disease.

December 24, 1908: Branches were photographed (Pl. V, fig. 2).

DAISY ON LOMBARDY POPLAR.

Inoculations of April 17, 1907 (Smith).

Six trees of Lombardy poplar (*Populus fastigiata*) were inoculated with 5-day-old cultures, each receiving 30 pricks in groups of 5. Four trees were held as checks. The roots of all the trees were washed thoroughly in the laboratory before inoculating, and the trees were then planted in the greenhouse.

Result.—July 13, 1907: No tumors resulted from these inoculations. The areas punctured by the needle were still plainly visible on the yellow bark of the roots as little black patches.

Inoculations of May 26, 1908 (Smith).

Three shoots of *Populus fastigiata* were inoculated (soft wood near the growing tip) with daisy organism from agar streak 4 days old.

Result.—June 1, 1908: Nothing definite.

November 16, 1908: No tumors have resulted. It is difficult to find where the needle entered. So far as these experiments go they tend to show that the Lombardy poplar is not susceptible to this disease, but not enough tests have been made.

DAISY ON COTTONWOOD.

Inoculations of April 17, 1907 (Smith).

Six trees of *Populus deltoides* were inoculated with 5-day-old cultures, each receiving 30 pricks in groups of 5. Four trees were held as checks. The roots of all the trees were washed thoroughly in the laboratory before inoculating, and the trees were then planted in the greenhouse.

Result.—July 13, 1907: No tumors have resulted from these inoculations, probably because the trees were too old to stand transplanting

well, i. e., the careless breaking of large roots. They were an inch or two in diameter at the base when inoculated and have made a slow growth. The areas punctured by the needle are still plainly visible on the yellow bark of the roots as little black patches.

DAISY ON ONION.

Inoculations of January 6, 1907 (Brown).

The bulbs (part above ground) and also the leaves of *Allium cepa* were inoculated by needle pricks with agar streak cultures 4 days old. Three checks were held.

Result.—February 1, 1907: No knots formed.

Inoculations of April 4, 1910 (Brown).

Eleven onion plants, growing slowly, were pricked in the bottom of the plateau from an agar streak culture of newest isolation.

Result.—June 24, 1910: All negative.

EXPERIMENTS WITH SCHIZOMYCETES FROM GALLS ON OTHER PLANTS.

In connection with our studies of the organism derived from the cultivated daisy, isolations from galls on other plants, together with pure-culture inoculations and cross-inoculations, were undertaken as follows:

HONEYSUCKLE ON DAISY.

Inoculations of April 14, 1908 (Smith).

Eight daisy plants were inoculated by needle pricks with agar streak cultures 4 days old of an organism plated out of an old knot on Japanese honeysuckle, found near Washington by Mr. W. A. Orton. The honeysuckle knots were about one-fourth inch in diameter. The knot from which these cultures were made was somewhat cracked open, and the plates came up with a variety of bacterial colonies, white and yellowish. A half dozen of these colonies approaching the daisy organism in appearance were selected, from which transfers were made.

Result.—June 1, 1908: No tumors. This means perhaps that the two colonies selected for these inoculations were not the right thing.

ARBUTUS ON DAISY.

Inoculations of November 2, 1909 (Brown).

Five young daisy plants were inoculated with agar cultures 5 days old plated from gall on *Arbutus unedo* sent from France. The daisy plants were of the lot obtained from a grower in Boston and were in splendid condition. Several checks were held.

Result.—March 26, 1910: No galls formed on any of the plants.

Inoculations of May 7, 1910 (Brown).

Six terminal young flower shoots of slow-growing old daisy plants already bearing daisy galls on the main stem were selected for inoculation. Into these the Arbutus organism was pricked from young agar streaks.

Result.—June 25, 1910: All negative.

July 7: Same.

ARBUTUS ON SUGAR BEET.

Inoculations of November 8, 1909 (Brown).

Sixteen young sugar beets growing in the open bed were inoculated with 2-day-old agar cultures of the Arbutus gall organism. Six checks were held.

Result.—December 20, 1909: Examined one row and found a gall three-fourths of an inch in diameter on one beet only. It was left to grow.

March 26, 1910: Reexamined plant with gall and found that it had rotted off.

June 10, 1910: Pulled up the beets and found one other with a gall 2 inches in diameter. Much of it had rotted off, but new parts were forming. This was photographed (Pl. XXIV, *A*).

COTTON ON DAISY.

Inoculations of November 16, 1909 (Brown).

A dozen inoculations were made on young stems of plants in fine condition by needle pricks from agar cultures 5 days old. Three checks were held.

Result.—January 4, 1910: No galls formed.

Inoculations of April 29, 1910 (Brown).

Seven young shoots of the Queen Alexandra daisy were inoculated with 2-day-old agar cultures of the cotton-gall organism, using needle pricks. These plants were old but were putting out young flower shoots. They already bore large galls on the lower part of the stem as the result of earlier inoculations with daisy.

Result.—July 7, 1910: No galls resulted.

COTTON ON COTTON.

Inoculations of December 1, 1909 (Brown).

Nine seedling cotton plants about 6 inches tall were inoculated with 2-day-old cultures of the cotton-gall organism.[a] The soil was laid

[a] When this organism was originally isolated there were pure cultures on 5 of the 8 plates poured.

back from the root and the crown inoculated by needle pricks. Three checks were held.

Result.—January 4, 1910: Four of the 9 plants had tiny galls. The checks were free. The plants had grown scarcely any since the time of inoculation.

COTTON ON SUGAR BEET.

Inoculations of November 11, 1909 (Brown).

Nine young sugar beets were inoculated at the crown with 5-day-old agar cultures of the cotton-gall organism, using needle pricks. The beets were young and growing in the open bed.

Result.—January 4, 1910: No galls.

March 7: Plants were pulled up—no galls.

Inoculations of July 5, 1910 (Brown).

Six young sugar beets were inoculated with 4-day-old agar cultures of the cotton-gall organism, using needle pricks.

Result.—July 18, 1910: No galls. The beets grew very slowly, owing to the excessive heat.

GRAPE ON DAISY.

Inoculations of March 28, 1908 (Smith).

Sixteen daisy plants were inoculated from slant agar cultures of March 25, the organism being derived from a tumor on grape occurring in this country. The plants had not yet branched and were inoculated in young and tender shoots about 6 to 8 inches from the ground.

Result.—June 1, 1908: They have given no distinct tumors, but a much more corky development than would have resulted from the needle punctures alone. The plants are now in blossom. These plants may have been cuttings taken from somewhat resistant tumor-bearing plants, or it may not have been the right organism, or, finally, colonies derived from an organism of weak virulence may have been used.

Inoculations of August 31, 1909 (Brown).

Six young daisy plants of a strain that had never been inoculated with any gall organism were inoculated with grape-gall organism 4 days old (used also for inoculating sugar beet and grapevines). The plants were very small, still in 3-inch pots, and had very little soft tissue. Eight checks were held. The plants were repotted after inoculating.

Result.—October 13, 1909: Three of the 6 daisy plants had galls. These galls resembled the regular daisy gall and looked unlike those of grape, owing, perhaps, to different tissue reaction of the plants.

January 19, 1910: Photographed natural size (Pl. X, fig. 2).

April 6, 1910: Galls are 2½ inches in diameter and still growing (Pl. X, fig. 3).

GRAPE ON OPUNTIA.

Inoculations of June 30, 1910 (Brown).

Needle pricks from an agar streak 6 days old on one plant in one group of punctures.

Result.—October 21, 1910: Negative.

GRAPE ON GRAPE.

Inoculations of August 31, 1909 (Brown).

Galled grape stems of the Champion variety were sent in from Lawton, Mich., and agar plates were poured on August 23. A few colonies appeared in 3 days, transfers were made, and from these grapes and daisy plants were inoculated.

Four grapevines (variety not known) in poor condition (no young shoots being present) were inoculated, 3 to 5 stems on each, with 4-day-old agar cultures.

Result.—October 13, 1909: Two of the 4 plants inoculated were covered with the small warty protuberances characteristic of grape gall (Pl. X, fig. 1).

Inoculations of September 7, 1909 (Brown).

Inoculated 6 young grapevines, several stems each (Seedless Sultana variety), with the grape-gall organism, cultures 3 days old. The vines were in pots and in fairly good condition and were inoculated in the youngest parts. Two plants were held as checks.

Result.—September 25, 1909: All of the inoculated plants had galls of the typical grape-gall kind. The checks had no galls.

GRAPE ON ALMOND.

Inoculations of March 27, 1908 (Smith).

Three young shoots of almond were inoculated copiously by means of needle pricks from 48-hour slant agar cultures (same as used on daisy of this date which failed), the organism being derived from tumor on grape occurring in this country.

Result.—June 1, 1908: The shoots have grown much since the date of inoculation, but as yet have developed no tumors.

December, 1908: None appeared. Possibly wrong organism selected. Certainly there are sometimes noninfectious white schizomycetes in the galls which on agar are very difficult to distinguish from the right organism.

Inoculations of June 28, 1910 (Smith and Brown).

Eleven small seedling almonds and 1 grafted almond were inoculated on the crown by needle pricks from a young slant agar culture, not of the same origin as the preceding.

Result.—July 18, 1910: Galls are forming on the crowns of several of these plants.

July 29, 1910: Plants dug; 100 per cent infected. Galls occur only where inoculated and are from one-eighth to three-fourths inch in diameter (Pl. IX, fig. 3). The check plants, 2 pricked and 4 unpricked, are free from galls. The grafted plant is S. P. I. 24809, N. E. H. 257. The seedlings were grown from hard-shell California almonds cracked and germinated in sand, after removal of the shell, this spring in one of our houses, and all were free from natural infection.

GRAPE ON SUGAR BEET.

Inoculations of August 31, 1909 (Brown).

Three sugar beets were inoculated with agar cultures 4 days old (same cultures used this date also on grape and daisy).

Result.—October 13, 1909: No galls formed on the beets.

Inoculations of May 7, 1910 (Brown).

Twelve well-grown sugar beets standing in one row in a bed in the hothouse were inoculated on the upper part of the smooth, white root by means of needle pricks from an agar culture 1 day old.

Result.—June 23, 1910: Eleven plants contracted the disease at the place of inoculation. One failed; this was a small, slow-growing plant much like those inoculated in 1909. Two other plants in the row, being very small at the time of inoculation, were omitted, and these are now free from galls. The inoculated plants are also free except in the vicinity of the spot where they were inoculated. Other rows of beets in the same bed remained free except as inoculated. The largest tumors are 2 inches across. They resemble the grape gall in having numerous smaller nodules on the swollen surface. (Pl. XXIV, *B*.)

Remarks.—That these galls were produced by the visible bacteria inserted and not by some invisible hypothetical *x* transferred along with the bacteria from the original gall and unable to grow in our media but capable of inducing galls when inadvertently put back into the plant along with the bacteria, is indicated by the fact that the following eliminating transfers were made:

(1) Organism plated from grape gall, August, 1909.

(2) Subcultures to slant agar from single typical colonies.

(3) Transfers once a month to agar and beef bouillon for about 7 months to keep the bacteria alive.

(4) Poured plates from last bouillon transfer made a few days before the beets were inoculated.
(5) Agar subcultures from colonies on 4.
(6) Beets inoculated from one of 5.

ALFALFA ON DAISY.

Inoculations of June 14, 1909 (Brown).

Four young daisy shoots were inoculated with the alfalfa gall organism, using pure cultures 4 days old.

Result.—August 20, 1909: One of the shoots inoculated had a small gall about three-fourths of an inch in diameter. No good daisy plants were available at this time, and the experiment was never repeated.

ALFALFA ON ALFALFA.

Inoculations of June 14, 1909 (Brown).

Four young alfalfa cuttings were inoculated with 4-day-old agar streak cultures, the colonies for which were isolated June 1, 1909, from an alfalfa gall occurring in a field in Alabama. The fine roots were inoculated and then tied with a piece of cord, which was later replaced by a wire. This was to locate the galls should they form, and also so as not to mistake nitrogen-fixing nodules for them.

Result.—July 6, 1909: The plants were knocked out of the pots and examined. No galls were found.

August 20, 1909: Examined plants again and found the inoculations had taken on two-thirds of the roots marked by the wires. There were no distinct galls like the daisy gall, but hairlike projections on which a nodule different from the nitrogen-fixing nodule was formed and from which fine roots projected.

Inoculations of June 16, 1909 (Brown).

Inoculated 4 roots on each of 2 alfalfa plants with colonies on a plate poured June 10 (second isolation from southern alfalfa plants). These plants were old and pot-bound, but were taken from pots and repotted after inoculation. Each root was tied with a knot of strong cord.

Result.—July 6, 1909: Examined roots and found no galls.

August 20, 1909: Concluded plants were too old and had made too little growth for development of galls.

Inoculations of July 16, 1909 (Brown).

Forty-eight seedling alfalfa plants were inoculated in the crown, in the roots, and also in the nitrogen-fixing nodules with 4-day-old agar cultures from second southern isolation, making 10 to 20 pricks in each plant. Twelve checks were held.

Result.—August 20, 1909: No indications of galls forming or of hairy-root outgrowths.

December 2, 1909: Twelve plants had formed crown-galls. The checks were free.

As it was always necessary to knock the plants from the pots, wash and examine the roots, they were more or less injured and set back in their development by this procedure. Possibly the seedlings were too young for best results.

INOCULATIONS OF SEPTEMBER 7, 1909 (BROWN).

Inoculated 3 pots of 1-year-old alfalfa plants with pure cultures 3 days old and 6 pots of young plants having taproots 3 mm. in diameter. The roots in both sets were inoculated at the crown. There were 4 plants in each pot, making 36 plants in all. Eight plants (2 pots) were held as checks.

Result.—December 2, 1909: Examined the plants and found one pot (4 plants) with very distinct and numerous galls on the roots; and two other pots (8 plants) containing plants with less numerous galls on the roots, making in all 12 plants with galls. Tiny, fine roots projected from these galls. The checks were free. Plates were poured from one of these galls to reisolate the organism. Some of these galls were photographed (Pl. XVI, fig. 2*a*), some preserved in alcohol, and some given to Mr. Kellerman to preserve.

FIRST INOCULATIONS OF DECEMBER 8, 1909 (BROWN).

Gall colonies grew on the plates poured December 2 (see preceding) and inoculations were made into young seedling alfalfa plants to check the cultures.

Result.—May 11, 1910: All of the inoculated plants now show galls.

SECOND INOCULATIONS OF DECEMBER 8, 1909 (BROWN).

The plants used were several years old and in poor condition. The culture used was a subculture from a colony on a poured plate of December 2, 1909.

Result.—May 11, 1910: No galls.

ALFALFA ON PEACH.

INOCULATIONS OF JANUARY 27 AND FEBRUARY 1, 1910 (BROWN).

On January 27 six peach trees, which had just started to send out leaves, were inoculated at the crown by needle pricks with 1-day-old cultures of the alfalfa gall organism (first isolation). The trees were in pots in the greenhouse.

On February 1 eight young peach trees of the same lot as those inoculated January 27 were inoculated with 1-day-old cultures of the

alfalfa gall organism isolated from galls on alfalfa, produced in the greenhouse by inoculation.

Result.—June 25, 1910: No galls on either set. The trees were also examined before June, but no record was made of the date.

ALFALFA ON SUGAR BEET.

Inoculations of June 14, 1909 (Brown).

The same set of cultures used for inoculating alfalfa this date were used to inoculate 3 sugar beets in the open bed. The part of the root just below the surface of the soil was inoculated, using pure agar streak cultures 4 days old.

Result.—August 20, 1909: Two of the beets had large galls; diameter nearly 2 inches. One of the beets could not be found.

Inoculations of July 16, 1909 (Brown).

Five young sugar beets in pots were inoculated at the crown with agar cultures 4 days old.

Result.—August 20, 1909: Galls had formed on 4 of the sugar beets. They were small, however, because the plants had become pot-bound and had grown very little.

On August 23 a photograph was made (Pl. VII, fig. 3). On the lower roots of this plant were also some small nematode galls.

PEACH ON DAISY.

Inoculations of December 2, 1907 (Brown).

Ten daisy plants were inoculated in the stem by needle pricks from agar colonies obtained by the poured-plate method from a peach gall November 26, 1907. The plants were in pots in the greenhouse and were in a good growing condition. They were inoculated near the tip.

The variety of daisy used was Queen Alexandra, 4 plants being new ones from a firm in Philadelphia, where the gall disease of daisy was unknown.

Result.—January 9, 1908: Each of the 10 inoculations gave tumors; 4 check plants remained free from infection, as did also the uninoculated parts of the infected plants.

Inoculations of December 4, 1907 (Smith).

Thirty-six plants of the white Paris daisy were inoculated with bacteria plated November 23 from the interior of a peach gall received from a nursery in Maryland. All the inoculations were made by needle punctures, making 5 or 6 pricks. The plants were 12 to 14 inches high and growing rapidly, so there was an abundance of soft

tissues for puncturing. The slime was rubbed over an internode, pricks were made through it, and the wounds were rubbed again with the platinum loop. Check punctures were made on the opposite side of each one of these shoots, and a little higher up, or else upon twin branches. The first 12 plants were inoculated from as many colonies. The remainder were inoculated from 4 slant agar cultures made December 2 from as many colonies on the same poured plate, the tubes having remained in the thermostat at 30° C. for two days, and the surface being covered with a copious growth. There was also an abundance of cloudy fluid in the bottom of the tubes and this fluid was pricked in very thoroughly. There were always a greater number of check pricks than of punctures with the infected needle. Usually 20 punctures were made with a sterile needle on each plant. All of the punctures were near the tops of the shoots in tender tissues, i. e., the ones most certain to give results.

Result.—January 14, 1908: All but 3 of these 36 plants (92 per cent) yielded distinct tumors in the inoculated part. None of the more than 600 check punctures on the same plants showed any tendency to form tumors.

May 15, 1908: Photographs were made (Pl. XIII, fig. 1).

November 16, 1908: About one-third of the daisies inoculated December 4 of last year are still living; the rest have died this summer. The largest tumors are 2 inches in diameter.[a]

In this connection the following notes on the origin and appearance of the bacteria used for these inoculations will be of interest. The crown-gall of the peach was scraped, washed, and the denuded surface further deadened by plunging into alcohol and then for five minutes in 1:1,000 mercuric chloride water. The interior of the knot was then entered by means of a sterile scalpel and scraped into sterile bouillon, from which the poured plates were then made. The scrapings from this tumor were thrown in considerable quantity into three different tubes of bouillon. Plates were poured from each one of these and also from bouillon dilutions of the same. The tubes which received the scrapings were marked A_1, B_1, C_1, and the dilutions were marked A_2, B_2, C_2. Apparently, not a great many living organisms were in the knot, and the dilutions did not yield satisfactory plates. The number of bacteria in the plates appeared to bear a constant relation to the amount of infectious material put in, i. e., those plates which were sown thickest gave the most colonies. The plates were incubated at room temperatures varying from 20° to 23° C. The plates were poured by Miss Florence Hedges, using +15 peptonized beef bouillon containing 1 per cent of agar. The

[a] The largest natural tumor observed on the daisy was on a root and measured 4¾ by 6 by 3 inches. Toumey figures much larger ones from the almond. (See also poplar, Pl. XXIII.)

knot was scraped, washed, sterilized, etc., by Dr. Smith. After 10 days at room temperature the plates gave the following results:

(A_1) *Two-millimeter loop:* This has been given about 75 colonies. These colonies are nearly all on the surface, i. e., they have been buried and have broken through. The largest surface colonies are now 4 mm. in diameter, the smallest ones are 1.5 mm. in diameter. They are circular in outline and uniform in appearance by transmitted light, except that the margins are a little clearer. The granulations in them are too fine to be visible with a Zeiss aplanatic lens magnifying six times. Nearly all of them have been buried colonies and show a darker, elliptical, triangular, or ragged buried central portion corresponding to the original buried colony. The margins are very sharply defined. The colonies are wet-shining on the surface, smooth. They do not seem, with the hand lens, to have any structure. They are not pink, nor greenish, nor yellow, but white by reflected light, and by transmitted light very slightly brownish. So far as can be determined with the hand lens, all the colonies in this plate are one thing. The buried ones are much smaller.

(A_1) *Needle inoculation:* This plate contains 8 colonies. They are of the same general appearance as the colonies in the other plates except that 1 marginal colony seems to be different, i. e., has a yellowish tinge.

(A_2) *Inoculated with a 2-mm. loop:* This plate contains nothing.

(B_1) *Two-millimeter loop:* This plate contains 56 colonies, of which 2 are mold spores, 1 is a thin-growing buried organism of uncertain nature, and the remainder are like those already described; circular, smooth, wet-shining, rather rounded-up surface colonies, slightly darker in the greater portion of their mass than at the extreme margin, which is sharp. These colonies have a darker center, corresponding to the buried growth from which they have arisen. There is a fine granulation in the colonies, but nothing distinct under the hand lens. The largest of them measures 6 mm. in diameter. The smallest surface ones measure about 2 mm. The colonies, like those in plate A_1, are distinctly rounded up from the margin to the center. This can be seen very well by looking sidewise through the plate across the top of the agar. The buried colonies are elliptical, pointed, or triangular.

(B_2) Inoculated with two 3-mm. loops: This plate contains 4 white colonies, 3 of which are typical; 1 has crenate margins and is an intruder.

(B_2) This inoculation, made with one 2-mm. loop, contains nothing.

(C_1) Two-mm. loop: This plate contains 44 colonies, of which 1 is a small intruding mold spore, and the others appear to be the same

thing and just like those colonies in plates made from A_1 and B_1. The surface colonies are round, smooth, wet-shining, white, rounded up from the margins, uniform in structure, except a little paler toward the edge, which is sharp. They have darker centers corresponding to the buried colonies from which they arose. So far as can be seen under the hand lens they have a uniform very fine granular structure. The largest of these colonies is 5 mm. in diameter and the smallest is 1.5 mm. The buried ones are elliptical, pointed, like those already described.

(C_1) Needle inoculation: This contains 10 colonies, all of which are alike and evidently the same organism as in A_1 and B_1. The largest surface colony is 6 mm. in diameter and the smallest one is about 1.3 mm. In their internal structure and their general elevation above the surface the colonies are precisely like those already described.

(C_2) Contains about a dozen colonies (thin, wide expansions), none of which appear to be like those already described. Most of them have lobed margins and are clearly some other organism. Probably infected in pouring.

(C_2) Needle inoculation: Contains one colony, which appears to be of the right sort.

The structure of the colonies in these plates under the miscroscope (Zeiss 16 mm. and No. 12 ocular) is precisely that of the daisy organism which I have just examined.

August 10: Photographs were made of Nos. 10 and 17 (Pl. XXV, fig. *C*.)

January 27, 1908: Alcoholic material was preserved and photos were made. Plates were also then poured from two of the knots.

February 3: The results obtained from the plates (seventh day) were as follows: (1) The plates from one knot were discarded because they contained yellow and pink colonies, i. e., saprophytes; (2) the set of plates from the other knot contained yellow colonies and white ones. The latter were most numerous and looked like what was inserted. In a very thinly sown plate the largest surface colonies were 8 mm. in diameter. They were perfectly circular, smooth, wet-shining, with sharp margins. The colonies were rather dense and nearly homogeneous to the naked eye, but under the hand lens they were seen to consist of a dense buried central spindle ringed by a clearer space, which was surrounded by a denser zone followed by a marginal clear zone. None of the zones were very sharply defined. The colonies were pure white, i. e., there was no yellow, pink, or greenish in them. In plates containing about 150 colonies the surface ones were circular, white, wet-shining, smooth, and rather dense, being 2 to 4 mm. in diameter. The buried colonies were spindle

shaped, sharply pointed, and most of them had broken through to the surface, while others were doing so. The only crystals in the plate were inside some of the yellow colonies. There were about 30 of these yellow colonies. The surface of the knot was probably not bathed in 1:1,000 mercuric chloride water long enough, i. e., only 10 seconds. Subcultures were made from 10 colonies on 2 of the thinnest sown plates, and 10 other similar white colonies on the plates were used to make the successful inoculations of February 3.

Inoculations of February 3, 1908 (Smith).

Ten daisy plants, Nos. 40 to 49, inclusive, were inoculated with the peach organism (originally plated from crown-gall of the peach, inoculated December 4 into daisy with production of tumors; then on January 27, plated from one of these tumors and now reinoculated into this group of daisies). The plants were each inoculated from a separate poured-plate colony.

Result.—June 1, 1908: Each of the 10 daisy plants finally developed a tumor (from one-fourth inch to over an inch in diameter) in the inoculated spot; but they were very slow to appear. They showed no tumors in any other place except No. 48, which developed a small tumor at the surface of the ground about a foot below the inoculated part. Photographs made (Pl. XIII, fig. 2).

Inoculations of March 11, 1908 (Smith).

Two daisy plants were inoculated with peach organism from colonies on poured plates of March 4. Two plants were held as checks.

Result.—June 1, 1908: No tumors; none on checks. These daisy plants were inoculated as checks on Wealthy apple, inoculated with the peach organism from the same poured plates, and their failure to produce tumors was due probably to small amount of inoculating material left after inoculating the apples (since the latter contracted the disease), or to the fact that they were inoculated directly from the plate, as would seem to be the case, from similar looking but really different colonies, or finally to the possibility of the daisy cuttings having been made from somewhat resistant (previously inoculated) stocks (p. 177).

PEACH ON OLIVE.

Inoculations of March 11, 1908 (Smith).

The tops of 2 tender shoots were inoculated with the peach organism from poured-plate colonies of March 4.

Result.—June 1, 1908: One of the inoculated shoots had grown 15 inches since the date of inoculation and the other one about 10 inches. No tumors.

This is in the same set of inoculations as that of the Wealthy apples, which took the disease (Pl. XII, fig. 2), and the 2 daisies which did not take it.

November 16, 1908: No tumors.

PEACH ON PHLOX.

Inoculations of May 18, 1909 (Brown).

Young annual phlox plants in pots just starting to bloom were inoculated near the tips of the stems by needle pricks with agar cultures 4 days old of the peach-gall organism (isolated February 29, 1908). Ten plants were inoculated and 4 were held as checks.

Result.—June 8, 1909: No indications of galls could be seen.

July 14: Examined again—no galls. The cultures may have lost their virulence.

PEACH ON VERBENA.

Inoculations of May 18, 1909 (Brown).

Young verbena plants growing in pots were inoculated with 4-day-old agar cultures of the peach-gall organism (isolated February 29, 1908), the stems being pricked at the tips, at the base, and midway between. Ten plants were inoculated and 4 were held as checks.

Result.—July 14: No indication of galls.

PEACH ON GRAPE.

Inoculations of June 24, 1910 (Smith and Brown).

The terminal part of 2 green shoots of *Vitis vinifera* was inoculated by needle pricks from a 2-day-old agar streak culture of the crown-gall organism from the peach (isolated February 29, 1908).

Result.—July 18, 1910: Doubtful; only slight prominences.

October 31, 1910: Nothing on one; very tiny elevations in needle pricks on the other; no true galls. Organism had probably lost virulence.

PEACH ON IMPATIENS.

Inoculations of June 24, 1910 (Smith and Brown).

One pink-flowered plant on 3 shoots and 1 white-flowered plant on 2 shoots were inoculated by needle pricks from 48-hour-old agar streaks of the crown-gall of peach organism (isolated February 29, 1908). The stems were soft.

Result.—October 21, 1910: All negative.

PEACH ON PELARGONIUM.

INOCULATIONS OF OCTOBER 13, 1908 (SMITH).

Two vigorous-growing shoots on each of 2 plants of a common red-flowered *Pelargonium zonale* were inoculated by needle pricks from 3-day-old agar streak cultures of the peach organism after it had been passed through red raspberry.

Result.—November 16, 1908: Each of the 4 shoots bore a small whitish corky-looking tumor where the needle entered, i. e., about 1 sq. cm. was raised above the surface of the stem 3 mm. or more.

December 9, 1908: Two of the shoots were photographed and the material then fixed in Carnoy's solution for sections.

January 18, 1909: The other 2 shoots were brought in and photographed (Pl. XIV). These shoots were still leafy and vigorous. The tumors were more than an inch in diameter, but did not seem to have done the plants any injury, i. e., the foliage above the gall was not yellow nor dwarfed.

PEACH ON PEACH.

INOCULATIONS OF DECEMBER 5, 1907 (BROWN).

Six young peach trees were inoculated with the peach-gall organism, 25 needle punctures being made in groups of 5 along the root, beginning at the crown. Agar streak cultures 3 days old made directly from the plate colonies were used. The inoculations were made in the laboratory and the plants set out in pots in the greenhouse. Two controls were held, the needle pricks being the same in number and position.

For comparison 6 peach trees were also inoculated with the daisy organism, using agar cultures of the same age, and making the punctures in the same way, in groups of 5 on the root, beginning at the crown.

Result.—January 8, 1908: Four of the 6 trees inoculated with the peach knot organism had decided galls one-third to one-half inch in diameter; and 4 of the 6 trees inoculated with the daisy organism had galls about the same size.

January 15: A photograph was made of the peach (Pl. XI, fig. 1).

February 14, 1908: All the trees inoculated with the peach gall organism had galls, while galls had formed on only 4 of the 6 inoculated with the daisy organism. The galls were 1 to 2 inches in diameter and alike on each tree. All the galls occurred at the crown or just below it, in no case on the deeper inoculated roots, nor were there any galls at other than the inoculated places. The 2 check plants remained free from galls.

INOCULATIONS OF JANUARY 13, 1908 (SMITH).

One hundred and thirteen seedling peach trees 1 year old were received through Mr. Corbett from the Arlington Experimental Farm. All of them were in good condition except 5, which were rejected because of borers. All were free from nematode galls and also from crown galls. Twelve were given to Doctor Townsend. (See *Rose on Peach*, p. 76.) The remaining 96 were divided into 2 lots. The dirt was first thoroughly washed from the roots; then the roots were shortened a little and the tops pruned back, so that they could be planted in 10-inch pots in the hothouse. The 2 groups were then treated as follows:

(1) Thirty-six trees were held as absolute checks, 15 pricks in groups of 5 being made with a sterile needle on the roots of each tree. The check pricks on all the plants were made before any inoculations were undertaken.

(2) This group of 60 trees is like group 1, except that opposite the 15 check needle pricks (3 groups of 5 each) 15 infected pricks (introducing a pure culture of the organism from crown gall of the peach) were made on the crown and roots in groups of 5 each. The side on which the check pricks were made was marked by cutting a sliver out of the bark of the stem, above the crown, with a sharp knife. A good deal of the white slime was put on the roots in making these inoculations (in most cases on the main root, rarely on side roots), and as they were planted within an hour or two of inoculation, there was a possibility of the slime infecting some of the check pricks by finding its way to the other side of the root; with a view to lessen this possibility, instructions were given the gardener to withhold water until the following day, if this could be done without injury to the trees. They were planted in good greenhouse soil.

Result.—March 4, 1908: The peach trees planted January 13 were pulled up and examined:

(1) Group of 36 check plants: 33 absolutely sound, 3 with slightly enlarged callus about as many knife wounds, none with galls. The trees received 540 stabs, all of which healed normally.

(2) Group of 60 inoculated plants: 55 with galls; 5 free. The 55 plants bore 127 galls—with very few exceptions, only where inoculated. The few exceptions are minute galls in the vicinity of the pricks on smaller accidentally injured roots. The 900 check pricks remained free from galls. The best galls were photographed (Pl. XI, fig. 3) and put into alcohol. None were very large, but sufficient for the time concerned, i. e., less than 2 months—the largest one-half to five-eighths inch in diameter.

Had the trees been ready to leaf out when planted it is probable that the percentage of infections would have been 100 instead of 92.

A greater number of the upper sets of pricks failed than of those farther down on the root. (By "upper" is meant near the crown.)

The surface of selected washed galls was sterilized three minutes in mercuric chlorid water (1:1,000), portions from their interior removed with sterile knives, mashed in beef bouillon, and plates poured—one set by Miss Florence Hedges and another by Miss Lucia McCulloch.

March 7, 1908: Each set of plates yielded many colonies of the right organism.

Inoculations of March 24, 1908 (Brown).

On February 29 an organism was plated out of the interior of one of the galls (peach strain) obtained by the inoculations of December 5, 1907, and on March 24 inoculations were made in the greenhouse on 10 healthy peach trees to determine whether or not this was the crown-gall organism, i. e., the same schizomycete as that inserted. Four-day-old agar streak cultures were used—the first subculture from the poured-plate colonies. The inoculations were made at the crown by means of needle pricks. The trees had been moved in recently from a cold frame. Five of the trees had developed foliage; 5 others were just beginning to show foliage. Four checks were held, punctures being made in the same way as on the inoculated plants.

Result.—June 2, 1908: Galls 1 inch to $2\frac{1}{2}$ inches in diameter were found on 5 of the inoculated trees. On one other tree a gall had formed and then rotted off. Three trees showed no indication of galls, but the roots were abnormal, i. e., there were many fibrous roots. The tenth inoculated tree was missing. The 4 check plants remained free from galls. On the inoculated plants the galls were restricted to the inoculated parts.

August 10: Photograph made.

All further inoculations with the peach gall organism so far as made by Miss Brown were with this strain plated from one of the galls produced by inoculation.

PEACH ON APPLE.

Inoculations of January 16, 1908 (Brown).

Six young apple trees from the Arlington Experimental Farm were inoculated with agar streak cultures 1 day old, the fourth subculture. Fifteen needle pricks were made on the main root of each plant. Four check plants were held. The inoculations were made in the laboratory and the trees set out in pots in the greenhouse. The trees (variety Wealthy) were not in good condition and were dormant. It is important to keep these facts in mind.

Result.—April 7, 1908: No trace of a knot on any tree.

INOCULATIONS OF JANUARY 23, 1908 (BROWN).

Five young Ben Davis apple trees, and 5 young Wealthy apple trees were inoculated. Two Ben Davis and 4 Wealthy were held as controls.

A number of trees were discarded at the time of inoculation because of the fibrous condition of the roots, and all were more or less suspicious because of the soil in which they had grown. The cultures used for these inoculations were 2-day-old agar streaks, the fifth subculture from plate colonies. From 15 to 25 needle pricks were made on the main root of each plant on one side only, and the side inoculated was indicated by a notch on the stem. The trees were planted in pots in the greenhouse.

Result.—June 2, 1908: Two of the Wealthy apple trees had well-developed knots on the inoculated side. Three were without knots. Only 3 of the 5 inoculated Ben Davis trees could be found, and all of these had knots. These knots were on the punctured side of 2 of the trees; but no positive conclusion can be drawn because of the behavior of the controls. Of the 4 Wealthy used for checks 3 bore knots and 1 was doubtful. Of the 2 Ben Davis 1 had a knot and 1 was free.

As the controls bore knots, and in places where there were no needle pricks, the conclusion was drawn that some of the trees at least were infected in the field.

INOCULATIONS OF MARCH 11, 1908 (SMITH).

Three Wealthy apple trees were inoculated (1 in two places on a top shoot and in one place on the base of a shoot at a considerable distance above ground; the other 2 in the top shoots only) with the peach-gall organism from poured-plate colonies of March 4—i. e., derived from the interior of a crown gall on peach produced by a pure-culture inoculation.

Result.—June 1, 1908: The top shoot of plant 1 has given 2 well-developed small tumors, the larger one round and about five-eighths inch in diameter. The inoculation on the base of the shoot has resulted in 4 distinct small tumors, each about 4 mm. high and 2 to 5 mm. broad.

Plant 2 was inoculated in the top shoot in two places. In one place it has given a tumor about 2 mm. high and about the same diameter, and in the other place it has given 3 separate tumors, each about 2 mm. high and 2 mm. in diameter.

Plant 3 was inoculated at the base of two top shoots, each one of which has given a group of small tumors 2 mm. in diameter and 2 mm. high. One of them has 4 of these tumors and the other has 3. The development of these galls has been very slow. There are no other tumors on the plant.

August 14, 1908: All the larger galls were removed. They are now 2 inches in diameter.

November 16, 1908: The remaining tumors have grown a great deal and have partly decayed.

April 2, 1910: The hard galls on tree No. 2 were photographed (Pl. XII, fig. 2), the tree repotted, and the galls wrapped in sphagnum to see if roots would develop from them. The buds on the tree are just opening.

June 25, 1910: No roots have yet formed on peach gall growing on apple under wet sphagnum, but the gall has begun to make new growth in places.

October 13, 1910: The sphagnum was removed and the galled portion of the tree brought in and examined. A considerable area of finely warted new tissue had formed and some parts of the gall had given rise to small roots, but they were not of the hairy-root type.

The peach organism was recovered from this gall by means of poured plates.

INOCULATIONS OF MAY 10, 1908 (BROWN).

Thirteen seedling apple trees, ranging from 6 to 12 inches in height and growing in pots in the greenhouse, were inoculated with the peach gall organism as follows: Seven at the crown and also on the stem; 6 at the crown only. Agar streak cultures 4 days old were used for the inoculations. Four check plants were held. These were punctured with a sterile needle on crown and stem.

Result.—September 2, 1908: A gall 2 inches in diameter on the stem of one tree; a gall one-half inch in diameter on the stem of another tree. Galls had also formed at the crown of 3 other trees. One of these was 1½ inches in diameter. Two of the trees inoculated on stem and crown did not make any growth. Of the trees which were inoculated at the crown only, 2 died and 4 did not make any growth, consequently no tumors formed. In this experiment we may claim 100 per cent of infections if we exclude the 8 trees which did not grow.

There were no galls on the check plants.

INOCULATIONS OF MAY 22, 1908 (TOWNSEND).

Thirty-five apple seedlings (variety, Kansas) were inoculated by needle puncture on the crown just above ground, using agar streak cultures of peach organism 3 days old.

Thirty-five trees of the same kind were punctured in the same way with a sterile needle for control.

Eighteen apple seedlings (variety, Virginia) were inoculated in the same way.

Nine trees of the same kind were punctured for control.

Result.—November 24, 1908: Of the inoculated Kansas, 10 trees made no growth, 12 were missing, and 1 out of the remaining 12 bore a knot. Of the supposed controls, 5 made no growth, 14 were missing, 6 bore knots, and 10 were free.

Of the inoculated Virginia only 4 bore knots; 5 only of the controls were found. On these there were no knots.

All of these trees were grown on the Arlington Experimental Farm. They were dug, washed, inoculated, and replanted at Arlington. Doctor Townsend superintended the washing and inoculation, but not the replanting. The soil in which the trees had grown, and in which they were replanted, was believed to be free from the gall organism. The appearance of galls on the trees marked as checks was attributed to the workman's having mixed checks and inoculated trees at the time of replanting. The failure of this experiment emphasizes the necessity of safeguarding every step of an experiment.

PEACH ON RED RASPBERRY.

Inoculations of May 20, 1908 (Brown).

Nine young red raspberry bushes in a good growing condition in pots in the greenhouse were inoculated with agar streak cultures 2 days old. Seven plants were inoculated on the crown and on the stem. Two plants were inoculated on the crown only. Four checks were pricked on crown and stem.

Result.—June 12, 1908: One hundred per cent of infections. Four of the 7 plants inoculated on crown and stem bore knots on both crown and stem, while the other 3 had knots at the crown only; of the 2 plants inoculated only on the crown, both bore knots. Those plants inoculated on the stem received 2 to 3 groups of punctures, each one yielding a gall.

No galls formed on the 4 checks.

PEACH ON BLACK RASPBERRY.

Inoculations of May 19, 21, 22, 1908 (Brown).

Thirty-four plants were inoculated, as follows:

May 19. Ten young black raspberry bushes in a good growing condition in pots in the greenhouse were inoculated with agar streak cultures 8 days old; 6 plants, both on the crown and on the stem; 4 plants, on the crown only. Four checks, punctured on both crown and stem, were held.

May 21. Twelve similar plants were inoculated at the crown and on the stem with agar streak cultures 3 days old.

May 22. Twelve similar plants were inoculated on the crown only with agar streak cultures 2 days old.

Results.—June 12, 1908: Plants inoculated May 19: All the inoculated places had galls except 1. The exception was 1 of the 4 plants inoculated only on the crown.

All plants inoculated May 21 had knots on both root and stem, except 1 plant which had a knot on the stem only.

All plants inoculated May 22 had knots.

Total number of inoculated plants 34, of which 33 bore galls. In most cases there were several groups of punctures on the stem, each of which yielded a gall.

The 4 check plants remained free from galls.

April, 1909: The above galls rotted away and in 8 or 10 instances new galls developed from their margins.

PEACH ON ROSE.

Inoculations of January 15, 1908 (Brown).

Six rose bushes (variety Killarney) were inoculated at the crown by needle pricks from 1-day-old agar streak cultures, second subcultures from the poured-plate colonies. The plants used were in pots in the greenhouse. The soil was laid back carefully, and, after puncturing, the inoculated places were covered with moist cotton.

Result.—April 17, 1908: A gall one-third inch in diameter on one plant. (Pl. II, fig. 1.) No trace of an enlargement on any other. The plants were not growing rapidly.

Inoculations of June 27, 1910 (Smith and Brown).

Twelve shoots of rooted cuttings of as many rose plants (variety, Killarney) were inoculated by needle pricks from agar streak cultures 3 days old, puncturing into the softest wood.

Result.—October 21, 1910: All negative. The plants grew slowly. The organism had probably lost virulence.

PEACH ON MAGNOLIA.

Inoculations of June 24, 1910 (Smith).

The terminal part of 4 young rapidly growing shoots of a broad-leaved magnolia (*M. acuminata?*) were inoculated by needle pricks from a 2-day-old agar streak culture of the crown-gall organism isolated (February 29, 1908) from the peach.

Result.—July 30, 1910: Negative; suspect that organism has lost its virulence.

PEACH ON PEONIA.

INOCULATIONS OF MAY 6, 1909 (SMITH AND BROWN).

Four roots of *Peonia officinalis* were inoculated with peach gall organism—culture 2 days old (isolation of 1908). Leaf buds were just starting out of the root stocks, and inoculations were made at the base of these, also on the roots themselves. Three checks were held.

Result.—September 2, 1909: The plants were knocked out of the pots and examined carefully. No galls were found in those inoculated. We were led to make these inoculations because Dr. Whetzel reported finding root-galls on peonia in New York.

PEACH ON SUGAR BEET.

INOCULATIONS OF MARCH 11, 1908 (SMITH).

Five sugar beets were inoculated by needle pricks on the crown with an agar subculture of an organism isolated from a crown-gall on peach and previously passed twice though the peach with the production of tumors.

Result.—May 4, 1908: Each one of the 5 plants contracted the disease at the point of inoculation and not elsewhere (Pl. VI, fig. 2).

PEACH ON HOP.

INOCULATIONS OF JUNE 10, 1908 (BROWN).

Six young hop plants from seed grown in sterile soil in pots in the greenhouse were inoculated by needle pricks at the crown with agar streak cultures 4 days old. Two plants kept as checks were punctured in the same way at the crown.

Result.—June 30, 1908: 100 per cent of infections. Knots three-fourths inch to an inch in diameter had developed on each one of the 6 inoculated plants. These knots were white and grew more quickly than those on the peach. The checks remained free.

PEACH ON RED OAK.

INOCULATIONS OF MAY 20, 1908 (BROWN).

Young seedling red oak trees about 6 inches tall were inoculated on the crown and on the stem with agar streak cultures 2 days old. Eleven trees were inoculated; 4 were held as checks.

Result.—September 2, 1908: The trees had not made any noteworthy growth, but a small knot or knobby outgrowth was present on one of the inoculated stems. The checks remained free.

PEACH ON PERSIAN WALNUT.

Inoculations of October 13, 1908 (Smith).

Two shoots of *Juglans regia* were inoculated by needle pricks with agar streak cultures of *Bacterium tumefaciens*, plated by Miss Brown from knots on red raspberry, which were produced by inoculating with pure cultures plated out of peach crown-gall.

These agar cultures were streaked on the 10th of October. The two streaks are copious, somewhat raised up from the surface, smooth, and wet-shining. Under the hand lens the lower part of the streak has in certain lights very fine irregularities on its surface, not noticeable to the naked eye. The color of the slime is gray-white. The spread away from the needle track is considerable. At the top it extends to either side a distance of 2 mm.; toward the base it extends to either side of the needle track a distance of 5 mm. There is a slight amount of water in the V, and this also is filled with the gray slime. The edge of the track is slightly undulatory.

The walnut shoots were hard when inoculated, so no results were anticipated. The plants were in a hothouse.

Result.—No tumors developed. This negative result was attributed to the fact above mentioned, i. e., that the shoots had ceased to elongate and were hard when inoculated, so that the bacteria were inserted into slow-growing tissues. This is the more likely because inoculations made the same day on Pelargonium gave positive results.

Inoculations of June 24, 1910 (Smith).

Three green shoots of *Juglans regia* var. *pendula* were inoculated in the softer terminal portion by needle pricks from a 2-day-old agar streak culture of the crown-gall organism derived from the peach (isolated in 1908). The shoots had reached nearly their definite length and were, therefore, less satisfactory than they would have been at the beginning of the month.

Result.—August 15, 1910: No result. Possibly the organism is losing virulence.

PEACH ON TRADESCANTIA.

Inoculations of May 7, 1909 (Brown).

Six growing stems of Tradescantia were inoculated by needle pricks with 3-day-old agar cultures of the peach-gall organism (isolation of 1908). Each stem was punctured 15 to 20 times. Two stems were punctured with a sterile needle for checks.

Result.—July 14, 1909: No trace of gall formation.

ROSE ON DAISY.

Isolation of Organisms.

On December 10, 1907, 2 rose bushes were found in the propagating greenhouse of the Department of Agriculture with galls 2 inches in diameter on the root below the graft. Plates were poured from the soundest part of one of these galls after proper scrubbing and surface sterilization, i. e., the scrubbed gall was pared with a sterile knife and small selected pieces designed for cultures plunged for about 2 seconds into mercuric-chlorid water (1:1,000), rinsed in sterile water, and crushed in sterile bouillon for the plates. In three days the typical gall colonies appeared.

Inoculations of March 18, 1908 (Brown).

Eight inoculations were made with rose A on daisy plants, 4 on a large yellow-flowered variety and 4 on the Queen Alexandra. One check.

Result.—March 31, 1908: Elevations at places inoculated indicate the beginning of knots.

April 25, 1908: Knots had formed but were quite small. They had not developed as rapidly as those due to the regular daisy-knot organism. On the rambler rose these same cultures produced no galls.

Inoculations of March 21, 1909 (Brown).

Three daisy plants of the Queen Alexandra variety were inoculated by needle pricks with 5-day-old slant agar cultures from the rose gall. Each plant was inoculated on from 3 to 5 shoots. Two plants were held as checks. These plants were growing better than those previously mentioned.

Result.—April 3, 1909: Small galls had formed at half of the inoculated places. The checks remained free.

September 13, 1909: The galls grew slowly, as shown in the photograph made on this date (Pl. VII, fig. 2).

ROSE ON ROSE.

Inoculations of December 17, 1907.

Twelve Killarney rosebushes were inoculated by needle pricks at the crown with agar slant cultures 4 days old, the first subculture from poured-plate colonies made December 10. Each inoculated place was covered with a small piece of moist cotton. The crowns of 3 other rosebushes were punctured with a sterile needle for checks.

Result.—January 8, 1908: Small, white, knobbed prominences projected from the dark-colored root about a quarter of an inch on 2 of

the 12 inoculated plants. These knots were under the pieces of cotton, placed over the punctures, so there was no mistaking the infection. The checks had no knots. These roses were not making a rapid growth.

Remarks.—The rose is rather difficult to infect. Probably if one knew just the right age and stage of growth infection might not be difficult, since some varieties of rose are very liable to contract this disease in hothouse culture, the Killarney in our experience being one of them.

Inoculations of January 15, 1908.

Twelve Killarney rosebushes were inoculated with agar streak cultures 1 day old. The soil was laid back from the crown of the root, and the root washed carefully with sterile water before inoculating. Fifteen needle pricks were made on each root. Each inoculated spot was covered with a piece of moist cotton.

Result.—February 3, 1908: Only 1 root had a gall.

April 17, 1908: The entire lot was again examined carefully, and but 1 other gall found. This one was taken to the laboratory and the organism was obtained from it by poured agar plates.

Inoculations of March 18, 1908.

Six young, healthy Rambler rosebushes were inoculated by needle pricks with agar streak cultures 2 days old. The plants were taken from the pots, the soil removed, but not washed, so that none of the fine rootlets were broken off.

Result.—April 6, 1908: The plants were taken from the pots and examined but no knots were found. The growth had been slow. (For checks, see *Rose on Daisy*, p. 75.)

Remarks.—Of 30 rose plants inoculated only 4 contracted the disease.

ROSE ON PEACH.

Inoculations of January 14, 1908 (Townsend and Brown).

Eight young peach trees were inoculated at the crown with slant agar cultures 1 day old. Twenty-five punctures were made in each tree in groups of 5. Four trees were held as checks. The work was done in the laboratory and the trees planted immediately afterward in pots in the greenhouse. The trees were dormant at the time of inoculation and probably remained so long enough to interfere with the infection.

Result.—April 6, 1908: The trees were taken from the pots, washed, and carefully examined. No galls were found on any of the trees.

Inoculations of May 9, 1909 (Smith and Brown).

Twenty-five peach trees dug up and brought over from the Arlington Experimental Farm May 8 were inoculated and planted out. The trees were rather leafy, and through an oversight the young foliage was not removed from them until 10 a. m. May 9, so they suffered considerably from transpiration. Ten of these trees were pricked with a sterile needle for checks, 15 or 20 pricks each in groups of 5, and the 15 remaining were inoculated with 1-day-old and 3-day-old agar streak cultures of the rose organism, which had been on culture media since the fall of 1908. The inoculations were made by means of needle pricks in groups on both sides of the main root in the yellow bark. After the punctures were made the plants were set out in 10-inch pots. Ordinarily the plants would not have been watered immediately after inoculation, but they had suffered so much from loss of water overnight that directions were given the gardener to water them carefully as soon as he had finished potting them.

Result.—September 3: Shook trees from pots, washed and examined roots; no galls on inoculated or check plants; 5 inoculated trees and 5 check trees were dead.

These trees were set back in their development by stripping the leaves in May, but this is scarcely sufficient to account for the non-infection. The rose-gall organism does not cross-inoculate readily.

ROSE ON APPLE.

Inoculations of January 23, 1908 (Brown).

Six apple trees were inoculated with agar streak cultures 2 days old. Punctures were made at 3 different places on each tree—at the crown, above the crown on the stem, and near the end of the shoot. Four controls were held. The variety of apple used was the Wealthy. The trees were dormant.

Result.—June 2, 1908: The trees were taken out of the pots, washed, and examined carefully. No knots were found. Here again possibly the dormant condition interfered with the infection.

ROSE ON SUGAR BEET.

Inoculations of December 3, 1908 (Brown).

Seven small sugar beets were inoculated by needle pricks just below the surface of the soil with agar streak cultures 7 days old. Three checks were made. The house was cold, and the plants were making a slow growth. When pulled up they were scarcely larger than when inoculated.

Result.—December 22, 1908: The 7 inoculated plants were examined carefully and but 1 gall found (Pl. II, fig. 6). It was three-fourths inch in diameter and regular in shape. The checks were examined and no galls found on them.

RASPBERRY ON DAISY.

Inoculations of July 9, 1907 (Smith).

Four daisy plants were inoculated from the white colonies on Petri-dish poured plates of July 2. These colonies were plated out of a small growing tumor on the root of a red raspberry plant taken from one of our houses, the same being in all probability a natural infection. Most of the daisy plants inoculated had several branches, and each branch was inoculated toward the top in as soft tissue as could be found. The plants had been neglected, and the wood was rather hard for the purposes of inoculation.

Result.—Negative.

QUINCE ON DAISY.

Isolation of Organisms.

The galls on quince trees are very unlike those of apple, peach, chestnut, etc.—i. e., they are warty outgrowths massed together, rather than galls of the ordinary type.

Isolations were undertaken December 23, 1908.

The material for this work was sent from California by Mr. Ballard, and when it arrived it was very much dried out. The galled stems were soaked overnight and the plates poured from the softest part of the material after proper scrubbing and surface sterilization. Six days afterward gall colonies appeared on the plates.

Inoculations of January 27, 1909 (Brown).

Inoculations were made into the stems of 8 daisy plants of both yellow and white varieties (4 of each) with agar streak cultures 2 days old, the first subculture from poured-plate colonies. Three checks of the white variety were held. All were old plants growing slowly.

Result.—February 11, 1909: Small knobs were present on 2 of the white daisies at the point of inoculation, but no indication of any on the yellow variety. There were no outgrowths on the check plants.

Inoculations of March 21, 1909 (Brown).

Several daisy plants of the Queen Alexandra variety were inoculated by needle pricks with agar streak cultures 5 days old. Nine different shoots were punctured. Two plants were also held as checks, several shoots being punctured.

Result.—April 3, 1909: Gall formation had begun to show on two-thirds of the inoculated places. The appearance was rather warty and not like the beginning of an apple or peach gall. The checks remained free.

QUINCE ON QUINCE.

Inoculations of May 14, 1909 (Brown).

Rooted cuttings of quince, from which the leaves had been pulled off and the stems trimmed back, were scrubbed well and then inoculated. The stem and the part of the stem from which roots were growing (it could scarcely be called crown on these cuttings) were inoculated. Both nodes and internodes on the upper stem were inoculated. From 30 to 50 punctures were made on each cutting. The side inoculated was indicated by a notch in the bark. Eight cuttings were inoculated and 6 checks were held.

Result.—June 15, 1909: No indication of galls.

July 13: Still no galls.

November 28, 1910: All negative.

Inoculations of May 21, 1909 (Brown).

Some of the lot of cuttings received May 14, 1909, had been planted without inoculating and were now starting to send out buds. Three trees were inoculated on the stems by needle pricks with 2-day-old agar cultures. Nodes where leaf buds were starting and also internodes were inoculated. Each stem received at least 30 punctures. The same number of trees were inoculated with the hairy-root organism in the same manner. (See *Hairy root on quince trees*, p. 103.)

Result.—September 3, 1909: Examined the trees and found no trace of galls. The quince trees in the greenhouse seemed to grow very little, for no change had taken place in the size of the young stems inoculated.

November 28, 1910: Still no galls. Trees grew more than last year.

Inoculations of March 9, 1910 (Brown).

Reinoculated 7 of the quince trees which were inoculated last May. They seemed to be in a good growing condition. Two-day-old agar cultures of the quince-gall organism were used and only young shoots were punctured—6 to 8 shoots on each plant.

Result.—June 24, 1910: No galls formed.

November 28, 1910: Nothing.

QUINCE ON SUGAR BEET.

Inoculations of February 26, 1909 (Brown).

Seven small sugar beets were inoculated just below the surface of the ground with 3-day-old agar streak cultures, the second subculture from the poured-plate colonies. Two checks were made.

Result.—March 31, 1909: No growth of the beets had taken place since the time of inoculation and no outgrowths were found, either on the inoculated plants or on the checks. The absence of galls should be ascribed probably either to a defective culture or to slow development of the beets rather than to any special resistance.

Inoculations of July 2, 1910 (Brown).

Four inoculations were made on middle-sized plants, and 12 on younger plants growing in a bed. All were made on the upper part of the root by needle pricks, using young agar cultures.

Result.—July 18, 1910: All negative. Here again the plants were making a very slow growth, owing to the excessive heat. Whether this second failure should be ascribed to the bad condition of the host plants or to the character of the culture must be left an unsettled point. Its cultural characters were unlike those of the cultures of proved pathogenic power (daisy, hop, peach, grape, poplar).

BEET ON DAISY.

Inoculations of April 26, 1910 (Brown).

Four terminal shoots were inoculated on old slow-growing daisy plants already bearing daisy galls on the main stem. A 4-day-old culture of an organism from the sugar beet on agar was used, and this was inserted by needle pricks.

Result.—June 25, 1910: Three shoots negative. The fourth bears in the pricked part one small gall about as large as a sweet-pea seed.

BEET ON ALMOND.

Inoculations of July 30, 1910 (Brown).

These almonds were part of the lot used for the grape inoculations of 1910, i. e., young grafted stocks. Four plants were inoculated by needle pricks and 2 were held as checks.

Result.—October 22, 1910: All negative.

BEET ON BEET.

Inoculations of June 27, 1910 (Brown).

Ten half-grown plants were inoculated on the upper part of the root by needle pricks from a young agar culture.

Result.—July 18, 1910: All negative. Weather hot and beets making a very slow growth.

Remarks.—The foregoing 3 experiments were made in all probability with the wrong organism, as shown by subsequent tests on culture media. The organism used had a slightly pinkish growth on agar. (See *Cultural characters*, p. 108.)

ADDITIONAL EXPERIMENTS WITH SUGAR BEETS.

Isolation of Organisms.

In November, 1910—i. e., since the foregoing paragraphs were written—additional galled sugar beets were obtained (1) from Colorado, (2) from Kansas, (3) from Michigan, (4) from Arlington Experimental Farm in Virginia, and (5) from the State of Washington.

The material from Colorado and Kansas was not satisfactory for reasons stated on page 194. From the Michigan material what was supposed to be the gall organism was obtained twice (two sets of plates from one gall). From the Washington material a gall-like organism was obtained once. From the Arlington material it was obtained 5 times (4 different beets). Two of the above tests (Arlington) were quantitative tests. The first one was lost so far as quantitative results are concerned, either because the sand used in grinding was not sterile or because the surface of the gall was simply scraped and then washed repeatedly in sterile waters without subjecting the surface to heat or germicidal solutions. The second test, made with greater care as to sand and surface sterilization, yielded the results hereafter detailed; but before these are given it will be well to put before the reader the technique employed.

On November 15, 1910, a sound, medium-sized sugar beet was selected. It bears one tumor free from decay or cracks. It is rounded oblong, attached by a rather broad base, and free from surface irregularities, being covered by a thin secondary cork layer (wound cork). The beet was washed clean in tap water, plunged into alcohol until free from air bubbles, then into 1:1,000 mercuric chloride water for 20 minutes. During this period the tumor was scraped gently with a knife to remove all the cork without injury to the deeper tissues. With the point of the knife a few tiny black specks extending into the white surface of the gall a very little deeper

than the average cork layer were also removed. Once or twice the denuded surface was also rubbed gently under the disinfectant with the finger tip. Great care was taken not to wound the deeper tissues. At the end of the 20 minutes the beet was rinsed quickly in a large volume of sterile water, then wrapped in sterile paper and put away over night. Only that mercuric chloride lying on the surface was washed away, not that absorbed into the superficial layers of the tumor.

On November 16 washed pure white sand (for grinding the tissue) was dry heated in the oven one hour at 240° to 250° C. The Wedgwood mortar and glass pestle and the distilled water used were autoclaved for one-half hour at 110° C. As checks on the sterility of the water, the surface of the gall, and the sand and mortar used in grinding the tumor, three plates were poured.

Toward noon (about 20 hours after treatment) the beet was uncovered, the thin dead surface now covering the tumor was removed with sterile scalpels and thrown into 10 c. c. bouillon. The volume of the gall was now measured in sterile water. It displaced 8 c. c. of water. A cube of the white flesh, approximately 4 by 4 by 4 mm., was cut out and thrown into 10 c. c. bouillon to duplicate Reinelt's experiment as nearly as possible (see later). The remainder was thrown into the mortar, cut into small fragments with sterile cold scalpels, several grams of the sand added, together with 40 c. c. of sterile water, and the material then ground vigorously for 15 minutes, i. e., until the beet was pulped and the fluid began to darken from oxidation. The mortar was tilted and the mass allowed to settle for 10 minutes, after which 20 c. c. of the fluid was recovered by means of sterile pipettes and put into sterile test tubes. All of this fluid was then distributed into 10 c. c. volumes of nutrient +15 agar, using a sterile pipette, and 68 Petri-dish plates were poured during the next three hours as follows: 5 received 1 drop each; 10 received 2 drops each; 5 received 3 drops each; 5 received 5 drops each; 5 received 10 drops each; and the remaining 38 received 0.5 c. c. each. The work was done in a clean culture chamber. The agar was inoculated and poured at 39° to 40° C. The three check plates gave the following results at the end of the seventh day:

No. 1, inoculated with 1 c. c. from a tube of 10 c. c. bouillon in which all the scrapings of the sterilized surface of the gall were allowed to soak for an hour—nothing.

No. 2, inoculated with 1 c. c. of washings from the dry heated white sand—nothing.

No. 3, inoculated with 1 c. c. of washings from the interior of the autoclaved mortar before using it—1 mold spore.

The 68 plates poured from the 20 c. c. fluid (contents of the tumor plus possible contaminations from the air) gave the following results:

(1) After 24 hours at 23° C.: Sixty-four plates sterile; 4 plates contain a total of 10 colonies.

(2) After 48 hours at 23° C.: Fifty-seven plates sterile; 11 plates contain a total of 17 colonies—2 of them a widespreading white organism. All the other colonies are tiny, white, and buried.

(3) After 72 hours at 23° C.: There are now 298 additional colonies all small, slow-growing, white, and buried. Twenty-seven plates are still sterile so far as the hand lens indicates, and a number of these received 0.5 c. c. volumes of the fluid. Plate 67 was rejected from the count because overrun and spoiled by a white colony.

(4) After five days at 23° C.: There are now 792 additional colonies not counting those on two plates (53 and 59) which are now overrun and spoiled by a white rapidly growing organism. All of these colonies are small, white, slow-growing, and most of them buried. Twenty-two plates are still free from colonies. These received the smaller inoculations, but much more fluid than one ordinarily expects to use, viz, most of them 2 to 10 drops.

(5) After seven days at 23° C.: (*a*) Thirty-seven plates show no additional colonies; (*b*) 2 more plates (50 and 57) rejected because contaminated; (*c*) on the remaining 29 plates there are 386 additional colonies, all small and mostly scattered, rarely small clusters on a tiny fragment of tissue.

Two plates of lot *c* contain each 1 Penicillium spore and 2 yellow ntruding colonies. The colonies which came up at the end of 24 and 48 hours may be regarded as contaminations from the air. Those on the 5 rejected plates may also be neglected. Of the remainder very few can be regarded as air borne.

(6) On the ninth day nearly all the white colonies are still buried and small and it is too early to say what proportion of these are the parasite. A few of the most hopeful-looking ones were transferred to bouillon.

(7) On the fifteenth day those previously transferred to bouillon as hopeful were rejected and 13 other colonies were marked for transfer, most of these having appeared after the ninth day—in other words, of the 1,500 colonies which developed only 1 per cent had the appearance on the plates of the right organism. Of these colonies only 2 proved pathogenic, i. e., produced tumors when inoculated into sugar beets, and these yielded very small slow-growing galls as if feebly virulent (Pl. XXXVI, fig. 1). It should be stated, however, that the beets were not growing much.

REINELT'S EXPERIMENT.

The experiment performed in Reinelt's manner gave the following result at the end of 5 days:

Second dilution from tube containing the cube—plate 4, nothing; plate 5, nothing; plate 6, 2 small white colonies, nature doubtful; plate 7, nothing.

The dilutions were made as follows: After the 4-millimeter cube, which was cut with a cold knife, had remained in the tube of bouillon about 10 minutes, one 3-millimeter loop of this fluid was transferred to tube 2, which was shaken; then two 3-millimeter loops from this tube were transferred to tube 3, which was shaken. The plates were then poured from tube 3, the following amounts of fluid being put into each: Plate 4 received three 3-millimeter loops; plate 5 received two 3-millimeter loops; plate 6 received one 3-millimeter loop; plate 7 received one 3-millimeter loop.

After the cube had stood in tube 1 for 2 hours it was mashed in the bouillon with a sterile scalpel as well as it could be (but not nearly as effectually as the remainder of the tumor which was ground with sand), and after standing an hour longer (to diffuse) 4 additional plates were poured, inoculating as follows directly from the tube containing the crushed beet: Plate 68 received three 3-millimeter loops; plate 69 received two 3-millimeter loops; plate 70 received one 3-millimeter loop; plate 71 received one 1-millimeter loop.

At the end of the fifth day all were free from gall colonies. The same was true at the end of 15 days.

OTHER ATTEMPTS AT ISOLATION.

At the same time as the above, Miss Brown made attempts to cultivate out the organism believed to be the cause of the sugar-beet tumor from galled sugar beets from Arlington, Va.; Blissfield, Mich.; and Fairfield, Wash. On her numerous poured plates she obtained a small sprinkling of colonies which appeared to be the right thing, and with subcultures from 30 of these made inoculations upon sugar beets in one of our houses, and also with the more hopeful a few inoculations upon daisies, tomatoes, etc. Of the whole lot inoculated, greatly to our surprise, not a single one has produced galls on sugar beet. The only results obtained up to the time this bulletin goes to the press are tiny beginnings of overgrowth in needle pricks on a few daisies and oleanders, and 2 somewhat larger, but small hyperplasias, on 2 tomato stems. There can be no doubt about the growths being tumor growths and due to what was inserted, but 5 colonies only of the 30 have yielded these results. The remainder have failed.

Remarks.—The explanation of the failures and of the very slow growth of the successful inoculations is a matter which must be left to the future. Two or three possible explanations may be offered. No great amount of energy was devoted to attempting to isolate the organism from sugar beets until after we had read Professor Jensen's paper late in the autumn of 1910. The gall on sugar beet then assumed a new importance in our eyes, and we made, as we have stated, diligent attempts to get out an organism with which the tumor on sugar beets could be reproduced. We began, however, not until the end of the growing season, namely, in November, when the galls were old, and although we plated from those which had no decayed spots on them, it is quite certain that the galls had nearly or quite approached the end of their growth for the current season, and may be assumed to have been several months old. We think, therefore, either that the right organism was dead for the most part in the tissues at this time, or so weakened by its own by-products or by reactions of the plant that it had lost its virulence. There seems to be no good reason, if one thinks about it, why an organism which loses its virulence in culture tubes might not also lose it in the interior of the host plant, if it had ceased or nearly ceased to stimulate growth, and had been subject for some weeks or months to harmful reactions resulting from its own products and those of the host itself. The fact that after 3 months of hard work, out of 42 colonies selected from several thousand as the most hopeful we have found only 7 able to produce tumors, shows how difficult it is sometimes to isolate a pathogenic organism from material known or believed to contain it.

HOP ON DAISY.

Inoculations of February 8, 1908 (Brown).

Five plants of the Queen Alexandra variety of daisy were inoculated by needle pricks from a slant agar culture 5 days old. (For origin, see *Hop on Hop*, p. 90.) Four and 5 shoots were inoculated on each plant. The plants were old and growing slowly.

Result.—February 18, 1908: There were protuberances at all places of inoculation.

April 22, 1908: No large knots like those due to the daisy organism were produced, although there was definite evidence of infection in each plant.

Inoculations of February 10, 1908 (Smith).

Twelve plants of the Paris daisy were inoculated with the hop organism from cultures of February 3. They were Nos. 1 to 12, inclusive, each made from a separate colony.

Result.—June 1, 1908: Nos. 1–7, 9, 11, and 12 gave no tumors. Plant 8, inoculated with colony 8 in 2 places, yielded a tumor about one-fourth inch in diameter. Plant 10, inoculated with colony 10 in 3 places, yielded a very slight tumor—a little round nodule about 2 mm. in diameter and 2 mm. in height.

Remarks.—This experiment may be interpreted in at least four ways: (1) That the plants were old and hard when inoculated and thus resistant; (2) that the organism had lost virulence in the gall; (3) that 10 of the 12 colonies were the wrong organism; (4) that the daisy had become somewhat resistant as the result of previous inoculations. Colony 8 is the only one that has given a tumor of any considerable size.

Inoculations of April 17, 1908 (Smith).

Four daisy plants, Nos. 1 to 4, inclusive, were inoculated with the hop organism from agar cultures 48 hours old.

Result.—June 1, 1908: No tumors.

Inoculations of April 25, 1908 (Brown).

Five more plants of the ordinary Paris daisy were inoculated with slant agar cultures of the hop organism 2 days old.

Result.—May 12, 1908: The same protuberances were formed as in the first set of inoculated daisies, but no well-developed galls.

Inoculations of May 9, 1910 (Brown).

Eight terminal shoots on 3 large plants ready to blossom and already inoculated on the lower part of the main stems with daisy gall and bearing galls received punctures introducing the hop organism from an agar culture several days old.

Result.—June 23, 1910: Seven floral shoots failed to take (the main stems below now bear large daisy galls). One vegetative shoot now bears at the punctured spot 2 small smooth galls each about as large as a pea and on directly opposite sides of the small branch. Six inches below the main stem bears a large daisy gall.

The reason for selecting daisy plants already bearing tumors produced by the daisy organism was that all the numerous uninoculated daisies of the same age were so much further advanced in flowering than these inoculated ones that no soft tissues were available.

Inoculations of November 12, 1910 (Smith).

The preceding inoculations of hop on daisy having given such slight results in comparison with hop on some other plants, daisies propagated from stock never before used were inoculated with subcultures 3 days old from agar colonies (fresh isolation, California, 1910).

Checks were held on sugar beet. Both daisies and beets, especially the former, were young plants in excellent condition for inoculation.

Result.—December 12, 1910: The subcultures from 2 of the colonies proved to be noninfectious. Colony 1 produced galls on each one of the 3 inoculated beets at the place of puncture. These tumors were, respectively, 1, 3, and 5 cm. in diameter at the end of 4 weeks, when the experiment was interrupted. The largest gall was on the most vigorous plant; the smallest was on a feeble plant. At this time 2 of the 4 daisies bore each a small gall on the pricked part. These galls were 2 to 3 mm. only in diameter. The other plants were free.

February 8, 1911: Three of the 4 daisy plants now bear tumors where inoculated and not elsewhere. The smallest one is 1 cm. in diameter, the largest one is 3 cm.

Inoculations of November 30, 1910 (Smith).

The preceding experiment was repeated on 12 young growing daisy plants, using subcultures of the hop organism (colony 1) and inoculating from peptone bouillon cultures 16 days old.

Result.—February 8, 1911: Eight plants remained free, 4 developed tumors at the place of inoculation and not elsewhere. These are now one-eighth to one-half inch in diameter.

Inoculations of December 2, 1910 (Smith).

A second repetition of the new hop (colony 1) on 20 daisy plants of the same character, using agar streak cultures 2 days old, gave the following:

Result.—February 8, 1911: Seventeen plants free from tumors. On 3 plants there are 4 tumors, the largest three-fourths inch in diameter, the others one-fourth inch.

HOP ON TOMATO.

Inoculations of November 21, 1908 (Brown).

Three tomato plants of a small, red, hothouse variety, 5 feet tall and in fruit, were inoculated with the hop organism. The stems about half way down the plant showed bulgings where roots might possibly protrude and adventitious roots also projected a distance of one-eighth to one-fourth inch. The bulging places on the stem and the smallest adventitious roots were both inoculated with agar streak cultures 3 days old. More than a dozen places on each plant were punctured. Two check plants were held, the punctures being made in the same way as those of the inoculations.

Result.—December 10, 1908: Galls formed at the bulged places on the plants inoculated, but no hairy-roots.

December 22, 1908: The hop-galls had increased so that they were now one-half to three-fourths inch in diamater. No galls formed on the adventitious tomato roots inoculated with the hop organism. The checks remained healthy.

February 15, 1909: Photographs were made (Pl. II, fig. 3).

HOP ON OLIVE.

Inoculations of May 9, 1910 (Smith and Brown).

Ten rapidly growing olive shoots were inoculated near the tip by needle pricks from a young agar culture. For want of other material these inoculations were made on plants standing close together in a bed. Many of these bore olive tubercles as the result of recent inoculations, and other inoculations with the same organism (*Bacterium savastanoi*) were in progress at that time.

Result.—July 18, 1910: All negative except No. 4, which bears 25 needle pricks, from 3 of which small galls have developed. Possibly these are olive galls, as branches of the same plant were by accident inoculated two days later with the olive-tubercle organism and the gardener sprayed the plants every day.[a] Otherwise it is difficult to account for the 9 failures, as the shoots have grown very rapidly since inoculation, i. e., 16 inches to 2 feet, and were all inoculated with equal care and from the same culture.

December 7, 1910: Plates were poured in gelatin from one of these galls and only the olive-tubercle organism was isolated, thus confirming the previous supposition.

HOP ON COTTON.

Inoculations of July 20, 1910 (Brown).

Inoculated 6 young growing cotton plants (Willet's Red Leaf) at the crown with 5-day-old cultures of the hop-gall organism. Two checks were held.

Result.—October 21, 1910: All negative; growing conditions good.

HOP ON GRAPE.

Inoculations of April 16, 1909 (Brown).

Eight young shoots of European grape on 3 plants, variety not known, were inoculated with agar streak cultures 2 days old. The crowns of the 3 plants were inoculated also. Two plants were held as checks, the stems and crowns being punctured with a sterile needle.

Result.—May 6, 1909: The stems of the inoculated vines had small knobbed prominences like the regular grape gall. No galls showed on the crown.

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[a] The strain of the olive-tubercle organism used proved very infectious, every one of the 208 inoculations yielding a gall with many subsequent metastases, although all of the 105 inoculated plants had previously borne galls, and two former strains (one from California, one from Italy) had ceased to be infectious to them. The recent strain was isolated from an olive knot sent by Miss Florence Hedges from Portofino, Italy, in April, 1910.—E. F. S.

HOP ON ALMOND.

Inoculations of April 16, 1909 (Brown).

Three almond trees grown from the seed, and which were over a year old, were inoculated on the stems and the crown with agar streak cultures 2 days old. No checks were held, as there were but 3 trees.

Result.—May 6, 1909: No indication of galls forming.

August 21, 1909: Galls formed on both crown and stems of 2 trees. The inoculations did not take on the third tree. This tree was smaller, gnarled, and not in a good growing condition.

November 19, 1909: A photograph was made (Pl. XV, fig. 2).

HOP ON PEONIA.

Inoculations of May 6, 1909 (Smith and Brown).

Three roots of *Peonia officinalis* were inoculated with the hop gall organism. Checks were held.

Result.—September 2, 1909: The plants were knocked out of the pots and examined. No galls were found on those inoculated. We were led to make these inoculations because Dr. Whetzel reported root galls on peonia in New York.

HOP ON SUGAR BEET.

Inoculations of April 17, 1908 (Smith).

Six sugar beets were inoculated from agar subculture 48 hours old.

Result.—May 18, 1908: Tumors have appeared and are three-fourths inch or more in diameter (Pl. XII, fig. 1).

June 1, 1908: All of the inoculated sugar beets bear tumors. The largest tumors are now 2 inches in diameter.

Inoculations of March 7, 1910 (Brown).

Five sugar beets were inoculated on the crowns by needle pricks from 3-day-old agar cultures.

Result.—April 20, 1910: Galls are forming in the pricked parts.

May 11, 1910: All contracted the disease and developed tumors several inches in diameter (Pl. XXI). Other beets in the same and adjoining rows remained free from the disease.

Remarks.—The hop organism used for the last inoculations on sugar beet had been in the laboratory a long time, having been transferred (without passage through plants) to fresh slant agar and bouillon once a month for over 2 years, i. e., about 26 times, to keep it alive, and yet with all of these transfers it remained actively pathogenic.

(See also checks, Hop on Daisy, Nov. 12, p. 86.)

HOP ON HOP.

On October 23, 1907, a hop root from California with a good-sized gall was brought into the laboratory by Dr. W. W. Stockberger for examination. Plates were poured from this gall and the gall organism obtained.

Inoculations of November 21, 1907 (Brown).

Only 4 hop plants could be obtained, and these were inoculated by needle pricks at the crown with cultures 2 days old.

Result.—January 15, 1908: The plants had made almost no growth, but 3 of them had small galls at the inoculated places.

Isolation of organisms.—On January 28, 1908, some more hop roots with irregular galls 4 to 6 inches in diameter were received from California through Doctor Stockberger. Parts of these galls were blackened and decayed; some swarmed with nematodes and some had small white nodules of new gall tissue on the margin of the old blackened gall tissue. These young portions were used for pouring agar plates, and 7 days later the gall colonies appeared on the plates.

Inoculations of June 10, 1908 (Brown).

Eight seedling hop plants grown in the greenhouse were inoculated at the crown with slant agar cultures 4 days old. Four other seedlings were punctured with a sterile needle for checks.

Result.—July 20, 1908: Galls 1 to 2 inches in diameter had formed on all the inoculated plants. The checks bore no galls.

CHESTNUT ON DAISY.

Inoculations of November 13, 1908 (Brown).

Four shoots of a daisy plant were punctured and a little of a pure culture introduced on the point of the needle (same cultures used on sugar beets).

Result.—December 2, 1908: The inoculations showed only as a slight swelling.

December 19: Perceptible galls are now visible on the daisy at the points of inoculation.

March 13, 1909: The galls are now 1 to 1½ inches in diameter (Pl. XVI, fig. 1). They grew more slowly than any gall heretofore observed except perhaps peach on daisy (inoculations of February 3, 1908) and some of peach on apple (inoculations of March 11, 1908).

May 3, 1909: The galls are still growing, nearly 2 inches in diameter, and quite tough.

CHESTNUT ON GRAPE.

Inoculations of June 10, 1910 (Brown).

In 1910 a fresh strain of the chestnut gall organism was plated from an old gall growing on the crown of a young chestnut (material from Mr. David Fairchild). A single shoot of *Vitis vinifera* was inoculated by needle pricks from a subculture on agar.

Result.—June 24, 1910: Distinct small knobs are visible on some of the needle pricks. Altogether about a dozen are now visible.

October 31, 1910: Slight elevations in the needle pricks, but no true galls.

CHESTNUT ON SUGAR BEET.

On November 7, 1908, among a lot of chestnut trees received by the Department of Agriculture there was one with a gall 3 inches in diameter on the stem. This gall was rather soft and of a texture much like that of a nut meat. Plates were poured from it and the gall colonies appeared 4 days afterwards.

Inoculations of November 13, 1908 (Brown).

Five young sugar beets were inoculated with colonies from the plates poured November 7, which colonies were 2 days old (visible 2 days). The beets were grown in an open bed in the greenhouse.

Result.—December 26, 1908: All the inoculated sugar beets had knots three-fourths to 1 inch in diameter (Pl. II, fig. 4). They were white and not so hard as the ordinary galls of daisy and peach.

January, 1909: The galls on the sugar beets rotted off before the end of the second month.

Inoculations of May 24, 1910 (Brown).

Five medium-sized sugar beets were inoculated from an agar streak by needle pricks on the root.

Result.—July 18: All negative.

Inoculations of June 10, 1910 (Brown).

A fresh isolation called "new chestnut" was made out of a rather old gall procured from the estate of Mr. David Fairchild, in Maryland. Five beets were inoculated on the roots by needle pricks.

Result.—July 18, 1910: Negative.

POPLAR ON OLEANDER.

Inoculations of July 20, 1910 (Smith).

Six young shoots on vigorous plants were inoculated by needle pricks from a peptone water culture 5 days old.

Result.—October 22, 1910: One shoot missing; the other 5 diseased, but only in the pricked area. The results in detail are as follows: (1) Four rounded knots (one-eighth to one-half inch in diameter); 16

pricks failed. (2) Four rounded tumors, largest one-half inch; several pricks failed. (3) Missing. (4) Three very small knots (one-sixteenth to one-eighth inch); rest of pricks failed. (5) Six small rough galls, each about one-eighth inch in diameter, 5 on stem, 1 on petiole; 10 pricks failed. (6) Six small rounded tumors (one-eighth to one-quarter inch).

POPLAR ON OPUNTIA.

Inoculations of July 5, 1910 (Smith and Brown).

Growing branches were inoculated by needle pricks from an agar streak culture 4 days old. Four varieties were used, having the following appearance, viz: (*a*) Elongated, dark-green joints, which are uniformly fine-hairy, and nearly free from prickles, i. e., short, weak spines; (*b*) smooth, light-green, elliptical, or pear-shaped, thin joints, with inch-long, single spines; (*c*) like *b* but with clusters of spines; (*d*) somewhat like *b*, but with round flat branches, very long spines, and clusters of brownish short prickles.

Result.—October 21, 1910: (*a*) Negative, one shoot inoculated; (*b*) one of the two pricked joints has, where inoculated, a smooth round tumor half an inch in diameter; (*c*) two young shoots, negative; (*d*) two shoots, negative.

December 19, 1910: The tumor on *b* is smaller than it was, and nipple-like projections have appeared on it.

February 8, 1911: The tumor has not increased in size.

POPLAR ON COTTON.

Inoculations of July 20, 1910 (Brown).

Inoculated the crowns of 6 young growing cotton plants (Willet's Red Leaf), with a 5-day-old 2 per cent peptone water culture of Flats poplar gall organism. (For origin see "*Poplar on sugar beet*," p. 93.) Held 2 checks.

Result.—October 21, 1910: All negative. The plants grew well.

POPLAR ON GRAPE.

Inoculations of June 4, 1910 (Smith and Brown).

Two small shoots of *Vitis vinifera* were inoculated in the green terminal growing parts by needle pricks with an agar subculture from a colony derived by poured plate from a gall found on the trunk of a large tree of *Populus deltoides* (*P. monilifera*) in Washington (Flats below the Washington Monument).

Result.—July 18, 1910: One of the inoculated shoots bears a dozen small tumors, and the other 26 (Pl. XXIV, *C*). There are no galls on the plants except where they were pricked.

POPLAR ON APPLE.

INOCULATIONS OF JULY 20, 1910 (SMITH AND BROWN).

Six shoots were inoculated by needle pricks from an agar streak culture 5 days old.

Result.—All grew well. One yielded in the pricked spot a distinct, rounded, corky tumor one-fourth inch in diameter. The other 5 failed.

POPLAR ON BRASSICA.

INOCULATIONS OF JULY 20, 1910 (SMITH).

The organism derived from the Flats poplar was inoculated by needle pricks into growing stems, using a peptone water culture 5 days old: (1) Early Summer cabbage; (2) Early Wakefield cabbage; (3) collard; (4) Tall Green Scotch kale; (5) kohl-rabi.

Result.—October 21, 1910: (1) The four inoculated plants bear galls in the pricked area and not elsewhere. On one they are small; on the others they are 1 to 2 inches in diameter (Pl. XXXIII, fig. *B*). Eleven uninoculated plants in the same pots are free. (2) Two plants bear large galls (1 to 2 inches in diameter), but only in the pricked areas; 6 checks are free. (3) One large collard bears a dozen small knots (one-fourth to one-half inch in diameter) in pricked area and not elsewhere. One-half of one knot shows hairy-root (Pl. XXXIII, fig. *A*). No other collards. Cabbage checks free. (4) One plant has 2 well-defined small galls (one-fourth inch) in pricked area and none elsewhere. Eight checks free. (5) Top of inoculated plant missing, i. e., broken off by someone (this was the pricked part); 10 checks free. These stood close to the Wakefield cabbage and the kale.

POPLAR ON SUGAR BEET.

INOCULATIONS OF JUNE 4, 1910 (SMITH AND BROWN).

In May, 1910, an organism resembling *Bacterium tumefaciens* was plated from a gall on *Populus deltoides* (called "Flats poplar" because the tree stands on the Flats near the river below the Washington Monument in the District of Columbia). These galls grew in clusters on the extreme base of the trunk of a large tree, but were not large; i. e., only 1 to 3 inches in diameter.

The material used for inoculation was an agar streak subculture (May 31) from a poured-plate colony. Ten sugar beets in a row were inoculated on the root toward the crown by needle pricks.

Result.—July 5, 1910: 100 per cent of infections. Galls began to develop at once in the pricked parts, but the plants were allowed to

remain in the bed until this date that they might get larger. Each of the 10 plants now bears a good-sized tumor, and at least half are larger than the root which bears them (Pl. XXII, *B*, *C*). The surface is white or pinkish, and they have not yet begun to decay. Material for sections was fixed in Carnoy. No checks were held, but the adjoining row inoculated at about the same date with an isolation from a chestnut gall might be considered as a check row, since the numerous needle pricks have yielded nothing.

Remarks.—Cultures were also made from the large old poplar gall shown on Plate XXIII, and subcultures from 2 of the most hopeful looking colonies (called Newport No. 1 and Newport No. 2) were inoculated into sugar beets (upper part of root) by needle pricks on June 30, 1910, but without results. Nine plants were inoculated, but the weather was hot and they did not grow much. The examinations were made on July 18.

POPLAR ON CALLA.

Inoculations of July 5, 1910 (Brown).

The corms of 8 growing callas were inoculated with 4-day-old agar cultures of the Flats poplar organism. Both leaves and corms were in good condition.

Result.—October 25, 1910: Examined the callas and found a pebble-like outgrowth on 1 of those inoculated; also knobbed portions on 2 others. Plates were poured from 2 corms, but no gall colonies appeared.

WILLOW ON DAISY.

Inoculations of May 9, 1910 (Brown).

In the spring of 1910 a small willow gall was received from South Africa. Plates were made from it, and colonies obtained which resembled *Bacterium tumefaciens*. A subculture from one of these colonies was pricked into 3 terminal shoots of old, slow-growing daisies already bearing large daisy galls near the ground.

Result.—June 25, 1910: Two of the shoots bear smooth brown galls one-half inch or more in diameter at the place inoculated. The third shoot, which is yellow and sickly, has not developed any.

WILLOW ON WILLOW.

Inoculations of December 12, 1910 (Smith).

Six recently rooted small cuttings of *Salix babylonica* were inoculated on rather slow-growing young shoots by needle pricks, using a 4-day agar streak culture which was a subculture (possibly the twelfth) from a colony plated from the South African willow gall in December, 1909.

Result.— January 5, 1911: Typical small galls (Pl. XXXV, fig. 1) have appeared in a portion of the needle pricks on 4 of the 6 plants, 5 shoots, 14 galls in all.

RELATION OF SO-CALLED HARD-GALL OF APPLE TO SOFT-GALL.

BOTH KINDS OF CROWN-GALL DUE TO BACTERIA.

Doctor Hedgcock has distinguished between hard and soft gall of the apple. He has not pointed out any good means of separating the two, but has stated the more common hard-gall to be noninfectious. As a matter of fact, the two kinds of tumors under consideration intergrade and both are due to bacteria, the differences being referable probably either to variation in the rate of growth of the host plant or else to varying degrees of virulence on the part of the bacteria, perhaps to both of these factors.

APPLE-GALL (HARD AND SOFT) ON VARIOUS PLANTS.

PRELIMINARY ISOLATIONS AND INOCULATIONS OF 1908 (BROWN).

On October 15, 1908, an apple seedling with a gall $1\frac{3}{4}$ inches in diameter was found among trees purchased by the Department of Agriculture to be used for the congressional distribution. An organism very much like the daisy gall organism in appearance and manner of growth was plated from this gall. Inoculations into apple trees, peach trees, daisy plants, and sugar beets produced galls in each species, although the per cent of infections was low.

On November 25, 1908, apple-galls were received from a nursery in Maryland. Plates were poured from the softest of these knots and the same organism obtained as before.

On November 27, 1908, Dr. G. G. Hedgcock brought in some of his so-called hard-galls of apple and challenged us to plate out the gall organism from them. These, as well as those used for the previous work, were galls without the accompanying tufts of roots (hairy-root). Plates were poured. Colonies resembling the gall organism appeared on the plates, and inoculations into sugar beets proved that it was the gall-forming organism, for in 18 days galls were produced at the inoculated places. The plants were from the vicinity of Washington (second D. C. test).

On November 4, 1908, Doctor Hedgcock also sent hard-galls of apple from Iowa to Doctor Smith, asking that tests be made. Miss Brown plated out what seemed to be the gall organism from one of these plants, but no inoculations were made by her. For result of independent isolations and inoculations into daisy by Doctor Smith, see inoculations of November 9, 1908.

HARD GALL OF APPLE ON DAISY.

Inoculations of February 24, 1908 (Smith).

Six Paris daisy plants were inoculated with 4-day-old slant agar cultures (from beef-bouillon culture of February 18, from stock agar stab of January 6) of Doctor Hedgcock's first (D. C.) apple gall. Each plant was inoculated in two places in the top.

Result.—June 1, 1908: No tumors. Plants discarded. Same cultures were negative on apple of January 23, 1908.

Inoculations of October 22, 1908 (Brown).

Four daisy plants were inoculated on the upper part of the stem from colonies on plates poured October 15. These colonies, however, had not appeared until October 20, so that the greater part of the cultures were in reality only 2 days old.

Result.—November 6, 1908: Small knotty growths had formed on all the daisies; not like the regular daisy gall in shape, but more like that of the hard gall of apple.

August 21, 1909: The galls have increased in size very materially, as shown by the photograph (Pl. XV, fig. 1).

Inoculations of November 9, 1908 (Smith).

Eight young Paris daisy plants were inoculated from cultures plated November 4 out of a very hard gall of the apple, forwarded by Doctor Hedgcock from Iowa. A 1-millimeter loop was usually scraped across a half dozen of the small colonies in order to get enough material, and then this was rubbed on a small area on the surface of the stem near the top of the plants and pricked in with a sterile needle. What remained on the platinum loop was rubbed over the wounds afterwards. As checks, the daisy plants were punctured in another place with a sterile needle. For this purpose plants were selected which had twin branches, one branch being inoculated and the other check pricked. The gardener was directed to withhold water for a few days until the check wounds should have healed over, so that the organism might not be scattered from the surface of the inoculated part into the pricks on the other branch. The plate used for these inoculations was photographed (Pl. XXV, fig. *D*).

Isolation of organisms.—The details of making these poured plates, their later appearance, etc., are as follows:

Two plants only of the hard gall were sent. The galls were found to be very hard indeed and not much raised above the surface of the apple stem. The surface of one of the galls was washed and then soaked for 3 minutes in 1:1,000 mercuric-chloride water. It was then

dug into and plates poured (November 4). After 2 days they yielded scattering saprophytic colonies of two types: (1) Whitish, circular, rather dense, of moderately rapid growth; and (2) larger, dendritic white ones, consisting of a large nonmotile schizomycete. At this time there appeared to be nothing else on the plates, but 2 days later the plates came up thickly with small, round, white colonies, and on the fifth day these had grown sufficiently so that there appeared to be very little doubt of their being the same sort of organism that we had plated from the crown-gall of the peach. These surface colonies were mostly less than 1 mm. in diameter, wet-shining, very translucent, white, circular; the buried ones were elliptical.

On November 9 transfers were made to agar streaks from 8 of these small colonies. One of the characteristic plates was then selected for the above inoculations. Plates were now made from the second hard gall of the same lot, and these yielded similar colonies, with which galls were also produced.

Result.—November 16, 1908: As yet no indications of tumors on any of these plants.

July 22, 1909: Galls have appeared. The best growths are about one-half inch in diameter and raised above the surface of the stem one-fourth inch or less. They are typical hard galls. Only about half the plants contracted the disease. This appeared at the inoculated spots, and not elsewhere. The growths resembled the original hard gall from which they were taken, rather than the ordinary daisy gall (Pl. III, bottom, stem 21).

Inoculations of November 18, 1908 (Smith).

Three daisy plants were inoculated from colonies on poured plates made November 9 from Hedgcock's second Iowa gall. At the time of inoculation the small colonies were white, dense, fleshy, circular, wet-shining.

Result.—July 22, 1909: Galls on each one of the three plants (Pl. III, bottom, stems 64, 65, 66). The growths are about one-half inch in diameter, and raised above the surface of the stem one-fourth inch or less. They are typical hard galls, i. e., not like the soft, rapid-growing daisy galls.

HARD GALL OF APPLE ON TOMATO.

Inoculations of December 4, 1908 (Brown).

The protruding adventitious roots of some nearly full-grown tomato plants were inoculated with the apple gall organism obtained from plates poured November 27. The projections were well out from the stem as though the roots were going to take hold of the soil. The cultures used were agar slants 2 days old, the first subculture

from the poured-plate colonies. The stems of the tomatoes were not inoculated.

Result.—February 2, 1909: No appearance of galls or hairy roots.

HARD GALL OF APPLE ON PELARGONIUM.

Inoculations of November 9, 1908 (Smith).

Two growing shoots of Pelargonium were inoculated from poured-plate colonies made November 4 from the hard gall of apple received from Iowa. These inoculations were from the same plate as the daisies inoculated on this date and were made in the same manner (p. 96).

Result.—November 16, 1908: As yet no indications of tumors on any of the plants.

April, 1909: No tumors appeared.

Inoculations of June 24, 1910 (Smith).

Ten growing shoots of *Pelargonium zonale* (pink and red flowered varieties) were inoculated in the soft terminal portion by needle pricks from an agar streak culture 2 days old. This was descended from a colony isolated in 1908. Four similar shoots were pricked with a sterile needle as checks.

Result.—August 10, 1910: Negative.

October 21, 1910: Negative.

HARD GALL OF APPLE ON APPLE.

Inoculations of January 23, 1908 (Smith).

Inoculated 10 growing Wealthy apple trees, part of them on the stock, with a bacterium plated from a hard knot on an apple tree furnished by Doctor Hedgcock (first D. C.). The roots were washed thoroughly and 50 or 60 pricks on each tree were made in some peculiar form, so as to recognize the place of inoculation. The gray-white slime from 44-hour-old slant agar cultures grown at 30° C. (5 subcultures, all descended from one small, white colony) was used for these inoculations. These cultures came from three 10-day-old slants and those from stock cultures made from the colony. After inoculation the trees were planted in 10-inch pots in the greenhouse.

Result.—No infections. Trees continued to grow, but very slowly. The colony used was, perhaps, a wrong one. Many small round white colonies appeared on the set of plates from which this colony was selected.

INOCULATIONS OF FEBRUARY 24, 1908 (SMITH).

Three Wealthy apple trees were inoculated with 4-day-old slant agar cultures (from beef-bouillon culture of February 18, from stock agar stab of January 6) of Hedgcock's first (D. C.) apple gall. Four inoculations were made on each plant, 2 into young shoots and 2 into old stems.

Result.—June 1, 1908: No tumors. Same set of plates as the preceding.

INOCULATIONS OF OCTOBER 22, 1908 (BROWN).

Three apple trees were inoculated on the crown and on the stem from hard-gall colonies on plates poured October 15. The colonies, however, had not appeared until October 20, so that for the most part the cultures were in reality only 2 days old. The apple trees were small and in a poor condition.

Result.—November 24, 1908: Two of the 3 apple trees had small galls at the crown. One of these trees bore 2 galls, one at the crown and the other a little below the crown.

December 22, 1908 (see Pl. II, fig. 2).

INOCULATIONS OF NOVEMBER 12, 1908 (SMITH).

Four apple trees were inoculated from colonies on poured plates made November 4 from Doctor Hedgcock's first Iowa apple gall. When used for inoculation the colonies were white, dense, fleshy, circular, wet-shining. The trees were leafy but growing very slowly. They are of the same lot that failed to take daisy inoculation (p. 43), and earlier root inoculations (January 23, 1908) with organism from first (D. C.) hard gall of apple. Each of the 4 trees was inoculated on the root just underground; 2 of them on 2 roots each, and 2 also on parts aboveground—1 in 1 place and the other in 3 places.

Result.—Nothing. Trees growing very slowly. The same organism inoculated into daisies gave slow-growing hard galls.

HARD GALL OF APPLE ON SUGAR BEET.

INOCULATIONS OF NOVEMBER 12, 1908 (SMITH).

Nine sugar beets were inoculated from poured-plate colonies (each from a separate colony) made November 4 from first hard gall of apple received from Iowa. At the time of inoculation the colonies were white, dense, fleshy, circular, wet-shining.

Result.—Negative. Plants small and making scarcely any growth.

INOCULATIONS OF DECEMBER 4, 1908 (BROWN).

Eight young sugar beets were inoculated with subcultures 2 days old from apple-gall colonies. These were descended from the same poured-plate colonies (second D. C.) used for the tomato inoculations of this date (p. 97). The beets were in a cool house making a slow growth.

Result.—December 18, 1908: Galls formed on only 2 of the beets and these were not more than half an inch in diameter. The beets had not grown much since the time of inoculation.

INOCULATIONS OF JUNE 24, 1910 (BROWN).

This is the culture recorded under "Morphology" as "old apple" and now believed to be something other than the crown-gall organism. Ten beets were inoculated on the roots by needle pricks from a young culture.

Result.—July 18, 1910: All negative.

HARD GALL OF APPLE ON MONSTERA.

INOCULATIONS OF NOVEMBER 12, 1908 (SMITH).

Six root tips (aerial roots) of *Monstera deliciosa* were inoculated from colonies on plates poured November 4 from Hedgcock's hard gall of the apple (first Iowa). When used for inoculation these colonies were white, dense, fleshy, circular, wet-shining.

Result.—No galls. Some of the roots bifurcated owing to injury of the growing point.

RELATION OF CROWN-GALL TO HAIRY-ROOT.

HAIRY-ROOT OF APPLE DUE TO BACTERIA.

Originally we had no intention to touch the subject of hairy-root, but Doctor Hedgcock having expressed a belief that it was not due to any organism and having sent on material with the request that we examine it, plates were poured and inoculations were made with the following results:

On November 7, 1908, an apple tree with small roots in clusters on the main root was sent in from Iowa by Doctor Hedgcock to Doctor Smith to experiment with for isolation of the hypothetical organism which we believed to exist therein and he did not. There was no typical gall on either roots or stem, but there were small enlargements at the base of the little clusters of hairy roots. A few of the rootlets of the bunched mass were rather fleshy. The root-

lets themselves were not used, but the thickened bases were cut out and used for pouring agar plates. In 4 days the characteristic gall colonies appeared. In 5 days they were of good size and looked very much like the colonies obtained from apple galls. This was the first time any such organism had been isolated from hairy-root.

EXPERIMENTS TO DETERMINE WHERE THE ORGANISM IS LOCATED.

November 9, 1908.—In order to find out whether the organism believed by us to be the cause of the hairy-root of apple was located in the main root under the point of origin of the hairy roots, or in the fleshy small roots themselves, plates were poured from material cut from these two locations. Colonies came up on those plates which has been made from that portion of the main root lying under the base of the hairy-root tuft, but none at all on the other plates; i. e., the organism was not found in the hairy roots themselves.

November 27, 1908.—An apple tree affected with hairy-root was brought in from his Washington, D. C., plantation by Doctor Hedgcock, who challenged us to prove the presence of an organism. The clustered roots were not dry and wiry, but fleshy and tender. Where they joined the main root there was a broad, flat enlargement. Plates were poured from the fleshy roots and also from the enlargement at the base of these roots. As in the previous experiment, the gall colonies appeared only on the plates poured from the thickened base.

February 16, 1909.—Some apple trees affected with hairy-root were sent to Doctor Smith by Doctor Whetzel, plant pathologist in Cornell University, for us to prove the presence of a pathogenic organism. The roots were very dry and had to be soaked before they could be cut. Little knobs grew on the main root just where the clustered roots came out, and these were used as material with which to pour agar plates. In four days the typical gall colonies were up on the plates and were used to infect young apple trees.

HAIRY-ROOT ON DAISY.

INOCULATIONS OF MAY 9, 1910 (BROWN).

Six terminal shoots on old slow-growing daisy plants were inoculated with the hairy-root organism.

Result.—June 25, 1910: Four shoots are negative; 2 show several tiny galls growing out of the inoculation pricks. These do not bear any roots. The stems lower down bear large daisy galls.

HAIRY-ROOT ON TOMATO.

Inoculations of November 21, 1908 (Brown).

In connection with the inoculations of the apple-gall organism into the adventitious roots of tomato, the hairy-root organism was also tried on tomato. The tomato plants were of the same sort as those used for the apple-gall inoculations, viz, a small, red, hothouse variety, 5 feet tall and in fruit. The stems about halfway down the plant showed bulgings where roots might possibly protrude, and adventitious roots projected a distance of one-eighth to one-fourth inch. Both the bulging places on the stem and the smallest adventitious roots were inoculated from agar streak cultures 3 days old. Three plants were inoculated, more than a dozen places on each being punctured.

Two check plants were held, the punctures being made in the same way as those of the inoculations.

Result.—December 10, 1908: No trace of hairy roots or galls on the plants inoculated with the hairy-root organism.

December 22, 1908: No galls or hairy roots formed on the adventitious tomato roots inoculated with the apple hairy-root organism.

The checks remained healthy.

Inoculations of December 10, 1908 (Brown).

A second test of the apple hairy-root organism on tomato was made, much younger plants in a better growing condition being inoculated with 3-day-old cultures of the hairy-root organism. The nascent roots on the stem were treated in the same way as the first set.

Result.—January 2, 1909: No effect produced on the stem of the plant or on the nascent roots by the inoculation.

Inoculations of April 1, 1909 (Brown).

Ten young tomato plants about 6 inches tall were inoculated with agar streak cultures of the apple hairy-root organism 2 days old. The crown of the root, the middle of the stem, and the growing point of the stem were inoculated. Four checks were held.

Result.—May 2, 1909: Examined the plants and found that 6 had roots projecting in a cluster, but whether these were adventitious roots put out to hold the plant in position or due to the presence of the organism could not be determined. The checks had not these decided rootlets, but the inoculated plants did not always have the rootlets in the immediate area of the puncture. The plants were replaced in larger pots and left to grow.

September 3, 1909: Decided that no hairy roots had formed.

HAIRY-ROOT ON YOUNG APPLE TREES.

INOCULATIONS OF APRIL 5, 1909 (BROWN).

Twelve young apple trees entirely free from gall or hairy-root were washed carefully and 8 of them inoculated with 2-day-old agar cultures of the apple hairy-root organism (Whetzel tree). Twenty-five pricks in groups of 5 were given each root, beginning at the crown and going down. The stem was notched to indicate the side inoculated. Four checks were held, the punctures being made in the same way.

Result.—May 3, 1909: Turned back the soil, examined, and found that hairs were forming in the pricked places.

September 3, 1909: Dug the trees, washed, and examined them. Five of the 8 showed very good cases of hairy-root (Pl. XVIII, figs. 1 and 2). Two failed and one bore rather small hard galls without hairy-root. One of the checks also had several small galls. This tree must have become infected during the planting. Plates were made from one of the hard galls obtained from the inoculated tree which did not bear hairy roots and an organism isolated. This was successfully inoculated into sugar beets on November 11, 1909, *both galls and hairy roots* developing. This indicates that the apple gall was actually due to the hairy-root organism, as suspected.

HAIRY-ROOT ON QUINCE TREES.

INOCULATIONS OF MAY 21, 1909 (BROWN).

Three quince trees were inoculated with a 2-day-old agar culture of the apple hairy-root organism. The trees were in the greenhouse and just starting to bud out. The wood was very tough, but the stems were inoculated at the nodes and internodes; at least 30 punctures were made on each stem.

Result.—September 3, 1909: Negative.

November 28, 1910: (*A*) Three galls bearing hairy-root (Pl. XXXIII, fig. *D*) on stem well aboveground; (*B*) one stem gall, no roots from it; (*C*) several small galls on stem, one bearing hairy roots.

Five checks on the same bench remained free; also 10 plants of same lot inoculated with the organism marked "Quince."

HAIRY-ROOT ON SUGAR BEET.

INOCULATIONS OF NOVEMBER 13, 1908 (BROWN).

Six young sugar beets growing in an open bed in the greenhouse were inoculated just below the surface of the ground, care being taken not to puncture along the line of the root hairs. The inoculations were made with agar-plate colonies of the apple hairy-root organism 2 days old. One daisy plant was also inoculated.

Result.—December 2, 1908: Examined the beets and found that fine roots were growing out at the punctured places, and little warty growths were at the bases of these roots. One and sometimes 2 roots protruded from a punctured place. This was observed on 4 out of the 6 beets.

December 22, 1908: Hairy roots were found on 1 more of the inoculated beets, making 5 out of the 6. No galls like the distinct galls of the daisy, peach, or apple were produced.

The daisy did not develop either hairy root or galls, although it was under observation until April 17, 1909.

INOCULATIONS OF DECEMBER 22, 1908 (BROWN).

Eleven young sugar beets were inoculated with slant agar cultures of the hairy-root organism 3 days old, the second subculture from agar poured-plate colonies. The organism was obtained from the apple tree brought in by Doctor Hedgcock. The inoculations were made just below the surface of the soil.

Result.—January 9, 1909: Four of the beets were pulled up; clustered roots of rather a fleshy texture were found on all 4 at the place of inoculation. There was no possibility of confusing these roots with those that occur regularly on either side of the beet, for they were too near the crown of the beet and besides were growing from small nodules.

April 10, 1909: Three more beets were removed and examined. Each one bore typical hairy-root (clustered roots) at the point of inoculation, which was midway between the two lines of lateral roots (Pl. XVII, figs. 1 and 2).

April 29, 1909: The remaining four beets were removed and examined. Three of these showed undoubted hairy-root at the point of inoculation. The fourth one probably developed hairy-root, but was rejected from the count because the needle entered on the line of the lateral roots rather than on the smooth surface between them, the inoculation having been made when the plant was very small.

INOCULATIONS OF FEBRUARY 24, 1909 (BROWN).

Seven small sugar beets were inoculated just below the surface of the ground with 1-day-old agar streak cultures of the apple hairy-root organism, the first subculture from poured-plate colonies obtained from one of the apple trees sent from New York by Doctor Whetzel.

Result.—March 10, 1909: Two beets were pulled up and examined; clustered roots were found on one of them at the inoculated places, which were on the smooth part of the beet.

March 31, 1909: Three more of the 7 beets had clusters of little roots at the inoculated places. The checks showed the punctured places healed; no hairs had formed.

INOCULATIONS OF NOVEMBER 11, 1909 (BROWN).

Eight sugar beets were inoculated with 6-day-old agar streak cultures made from colonies plated from gall on apple root produced by inoculation (p. 103) with culture of apple hairy-root organism derived from the Whetzel tree.

Result.—March 10, 1910: One beet was pulled and both galls and hairy-root found present.

April 7, 1910: The remaining 7 beets were pulled and typical hairy-root found on all of them. Three had both hairy-root and galls, i. e. clusters of roots (hairy-root) growing out of galls which were not large (Pl. XVII, fig. 3; Pl. XIX, figs. 1 and 2).

MISCELLANEOUS.

In 1910 organisms were isolated from salsify gall, turnip gall, and parsnip gall, and inoculated respectively into salsify, turnip, and parsnips, and each also into sugar beet, but all of the inoculations were negative.

DESCRIPTION OF BACTERIUM TUMEFACIENS[a] FROM DAISY.

MORPHOLOGICAL CHARACTERS.

Vegetative cells.—The daisy knot organism is a small schizomycete of variable length, but usually short and generally not over 0.6 to 1μ in diameter, unless treated with severe flagella stains. None as slender as 0.2 or 0.3 μ have been observed.

Taken directly from a gall and stained with gentian violet, the following measurements were obtained in 1909: Single rods, 0.6 to 1.0μ by 1.2 to 1.5μ; paired rods, 0.6 to 1.0μ by 2.4 to 2.8μ. These were obtained in the following way: The surface of a young gall was scraped, washed, and sterilized; thin slices were then placed in distilled sterile water on sterile slides and the bacteria allowed to diffuse out of the sections for an hour. The sections were then lifted with sterile forceps, the fluid dried and stained. Only scattering rods were visible.

When grown on agar for two days and stained with Loeffler's flagella stain (in 1907) its length was 2.5 to 3μ and its breadth 0.7 to 0.8μ, or occasionally a little wider. Some recorded as 6μ long were probably paired rods.

[a] Name first used in Science, n. s., vol. 25, April 26, 1907, p. 672.

Other slides made at various times and measured February 25, 1910, gave the following results:

(1) Van Ermengem: Average diameter 1.2μ; some less, a few more. Result on a second slide: Average diameter 1 to 1.2μ.

(2) Pitfield's (Smith), agar 24 hour: Average diameter 1.2μ, widest 1.75μ, narrowest 1μ. Occasionally Y-shaped rods. Pitfield's (Brown): Widest diameter 1.1μ; many less wide, i. e., 0.8 to 0.9μ.

(3) Carbol fuchsin, without a mordant (30 minutes) flagella visible: Average diameter 1.2μ.

(4) Löwit's stain: Average diameter 1.56μ.

(5) Loeffler's flagella stain (1909): Diameter 0.8 to 1μ; none seen wider than 1μ.

In making these measurements a Zeiss photomicrographic stand, 3-mm. apochromatic oil-immersion objective, No. 12 compensating ocular, and Schraubenmikrometer were used (Smith).

The rods are straight, have rounded ends, thin walls, and a uniform diameter.

When taken from young agar cultures, the limits of size are 1 to 3 by 0.4 to 1.8μ. Size of the majority 1.2 to 2.5 by 0.5 to 0.8μ. Occasionally one finds more than two rods attached, end to end, forming short chains (seldom more than 3 or 4 elements). Long chains have never been observed in the daisy organism except under abnormal conditions, e. g., in old 3.5 per cent salt bouillon, where sinous or curved rods 20 times the ordinary length were seen.

Endospores (?).—*No endospores have been observed, and probably none occur.* Certainly they are not formed under most culture conditions, as shown by the short life of cultures and by their sensitiveness to heat. The following additional experiments were made in 1910:

Bouillon cultures some weeks old were boiled for 3 minutes with the result that all were killed. The experiment was repeated after some months with the same result. Bouillon cultures were then heated for 20 minutes at 80° C., after which some grew. It was thought that owing to the lumpy character of the slime the heat might not have penetrated to the center of all the pseudozoogloeæ. The experiment was repeated, therefore, exposing the tube for 60 minutes at 80° C. After this none of the transfers grew, not even when large quantities of the fluid were used (1 drop, 2 drops, etc.).

Flagella.—The organism is motile by means of a polar flagellum. Sometimes 2 or 3 terminal flagella are present, but more often in the slides examined there was only 1. The rods of young cultures show a distinct movement when examined in hanging drops. The flagella were first stained by the senior writer from 24-hour agar cultures, using Pitfield's flagella stain (fig. 1, *a*). Afterwards they were

stained by Miss Brown, using Loeffler's flagella stain (fig. 1, *c*), and subsequently Van Ermengem's stain (fig. 1, *b*). They were also stained by Miss Lucia McCulloch without a special mordant, by simply exposing the flamed covers to carbol fuchsin for from 30 to 60 minutes and then washing in alcohol (fig. 1, *d*).

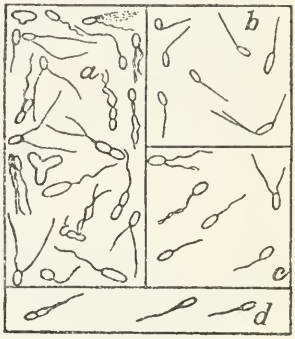

FIG. 1.—Flagella of *Bacterium tumefaciens* from daisy: *a*, Pitfield's flagella stain; *b*, Van Ermengem's stain; *c*, Loeffler's flagella stain; *d*, from a slide stained 30 minutes in carbol fuchsin without mordant.

Capsules (?).—The organism is viscid after some days on agar, etc., but capsules have not been demonstrated. Welch's stain was tried.

Zooglœæ (?).—Pseudozooglœæ occur, and perhaps the stringy masses in peptonized beef bouillon should be regarded as transitions toward zooglœæ. Under the microscope these masses consist of short rods held together by a viscid slime.

Involution forms.—Numerous involution forms (fig. 2) were observed in bouillon cultures which were making a slow growth at 0° C. The cultures were first examined under the microscope on the fourteenth day. Occasional Y-shaped rods occur in young agar cultures (fig. 1, *a*). Club-shaped and Y-shaped involution forms were also seen in salt bouillon and in bouillon and agar to which acetic acid was added. See also note on ordinary bouillon.

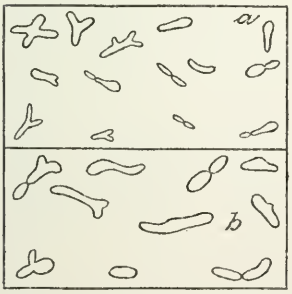

FIG. 2.—Involution forms of daisy organism after two weeks in bouillon at 0° C. Bottom growth: *a*, drawn by E. F. S.; *b*, drawn by Brown. Similar involutions forms were produced in young agar cultures and also in bouillon by exposure to acetic acid.

BEHAVIOR TOWARD STAINS.

This organism when taken from young agar cultures stains readily in all ordinary basic anilin stains so far as tried, e. g., gentian violet, fuchsin, carbol fuchsin, amyl Gram, methyl violet. It is not surrounded by any substance that interferes with staining. When stained from a 2-day agar streak in Loeffler's alkaline methylene blue, the rods were either a uniform pale blue or showed round to oval, inner portions bearing a much heavier stain. There were sometimes two of these bodies in a rod, but more often one and that usually polar. About one-fourth of the rods stained in this manner, and the part not heavily stained was of a uniform pale blue.

It does not stain by Gram. It stains readily a uniform deep blue if amyl alcohol be substituted for ethyl alcohol in the washing after exposure to the anilin gentian violet and the iodine-potassium iodid of Gram's stain.

Taken from beef bouillon 7 weeks old it did not show glycogen stain when exposed to iodine water; i. e., there was only a uniform yellow color.

It is not acid-fast.

Brizi's method was employed on daisy galls without success. By the use of methylene green (not methyl green, but that was tried also), without subsequent exposure to acid, numerous cell inclusions, consisting of bacteria-like granules, were demonstrated, but whether really bacteria remained undetermined.

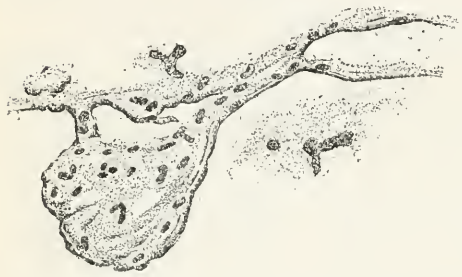

FIG. 3.—Daisy organism. Slime from pellicle on beef-bouillon culture three weeks old. Stained with carbol fuchsin, and camera-drawn by Miss Brown.

The bacteria Brizi succeeded in staining readily in poplar tumor tissues by an acid-fast method were probably not this organism. In our hands the gall-producing organism in American poplar galls stains like the daisy. It is not an acid-fast organism.

In sections it is often stained with difficulty, and we have seldom been able to differentiate it well from the surrounding tissue. It seems to us to occur, for the most part, at least, in the interior of the parenchyma cells rather than in the intercellular spaces or vessels. Repeated efforts to stain in situ have not yielded, as a rule, well-stained, sharply defined rods such as one would expect, but occasionally stained and unstained we have seen rods inside the cells which we believe to be the bacteria.

CULTURAL CHARACTERS.

NUTRIENT AGAR.[a]

Colonies.— When the organisms are obtained from a crushed knot the colonies come up in from 3 to 12 days (usually 4 to 6) at a room temperature of about 25° C. on poured agar plates. They come up very much slower when taken from knots than when taken from young cultures. The white smooth surface colonies are circular with an even margin, rounded up to the center, and have a shining semitransparent luster. The colonies increase in size slowly at 25° C.,

[a] +15 peptonized beef bouillon with 1 per cent Merck's agar flour.

attaining their maximum size of 2 to 4 mm. in thin-sown plates in from 2 to 4 days after becoming visible. They attain a larger size on culture media after many transfers than when first plated from the galls. The colonies, especially when they pile up in the center, are opaque, but never of a chalky white. They often resemble the watery colonies of some forms of the root-tubercle organism of legumes. Plates poured from a young bouillon culture show colonies sometimes in 24 hours, and nearly always in 48 hours at 25° C. Old colonies are sometimes iridescent.

Streaks.—Needle stroke not wide-spreading and very translucent at first. On slant agar (+15) the streak made with a platinum needle has a moderate filiform growth and does not branch on the surface nor penetrate the agar. The white streak widens slowly (chiefly toward the bottom), is slightly to considerably raised, sometimes nearly convex in cross section, and has a glistening luster; it is opaque or translucent and is slightly opalescent; is free from odor, somewhat slimy (especially after the first two or three days), and does not color the agar upon which it grows—at least not for some weeks, after which it may sometimes show a slight brownish color. In one strain (1909) the old slime, especially where it had run down into the V, had a trace of brownish in it lighter than Ridgway's tawny olive and somewhat resembling his buff or cream buff. This culture looked suspicious, but proved pathogenic. This has happened a number of times. The rods taken from this substratum stain readily with carbol fuchsin, gentian violet, methylene blue, or anilin methyl violet.

Two streaks from "B," made February 14, 1907, on slant 6 per cent glycerine agar by use of a loop, covered at the end of 48 hours the whole surface of the agar with a smooth, wet-shining growth, which was white by transmitted light. This growth was slightly viscid and plainly alkaline. There was no stain of the agar, nor were there any crystals. The cultures were pathogenic, as shown by inoculations of February 18 on daisy, tomato, and tobacco (Smith).

Stab.—Nontypical in stab cultures. The growth is filiform and best toward the top of the stab; the surface growth is scanty to abundant and generally restricted. Agar is not liquefied nor softened.

CORN-MEAL AGAR.

Feeble growth at end of five days. This experiment was repeated twice with the same result—growth at the end of four weeks was very slight, the media being made by the same formula, but in another laboratory.

POTATO.

On sterile potato cylinders (lower end in water) the organism makes a much more rapid growth than on agar. The growth is first visible

along the line of the streak, which is slightly elevated with entire margin. It spreads rapidly and in from one to two days covers the entire surface of the cylinder. The white growth has a smooth surface with a wet-glistening appearance. It has a slimy to viscid consistency, is free from odor, and turns the potato cylinder a grayish color, which becomes darker with age. It is never yellow on potato. The organism has but little action on the potato starch and its growth on potato is correspondingly transient.

STARCH JELLY.[a]

Growth scanty; diastasic action absent or feeble; medium unstained or only slightly stained. Some years later the experiment was repeated and continued for a longer time with the same result, except that the old daisy strain, which had become noninfectious, now stained the medium brown.

NUTRIENT GELATIN.[b]

Colonies.—Colonies dense, white, circular, small, nonliquefying. In plates poured March 26 from a 3-day-old bouillon culture carried to a second dilution the colonies were numerous, but remained very small. The surface colonies were 1 to 1.5 mm. at the end of 4 days at 20° C. and were not larger 2 days later (Brown). In thinly sown plates the growth of the surface colonies was slow, the largest being 2 mm. in diameter at the end of 24 days at 16° C.; they were round, white, dense, flat to raised, with entire edge, and no sign of liquefaction (Smith). They were never yellow with fringed margins.

Streaks.—There is a very *good growth, starting off slowly* at a temperature of 11° C. on gelatin streak cultures.

This experiment was repeated two years later, using gelatin from another laboratory with identical results. The gelatin was +12 on Fuller's scale. The temperature varied from 9° to 10° C. There was very slight growth up to the end of the third day. At the end of 12 days there was a good white growth.

At room temperatures (22° to 23° C.) on the same gelatin at the end of 2 days the streaks were as good as streaks of the same age on +15 peptonized beef agar, but this parallel growth did not continue. At the end of 4 days the agar streaks showed a copious growth, while the gelatin streaks showed only a moderate growth. At the end of 13 days the gelatin streaks were pure white, wet-shining, smooth on the surface, with bunches (tufts) of small crystals projecting from the under surface of the streaks into the unstained gelatin. Some white slime had also run down into the V. There was no liquefaction.

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[a] For composition, see "Bacteria in Relation to Plant Diseases," vol. 1.

[b] Peptonized beef bouillon with 10 per cent Nelson's photographic gelatin No. 1 and made +10 on Fuller's scale with sodium hydroxid.

Stabs.—In gelatin stabs the growth is best at the top; the line of puncture is filiform; liquefaction is absent, and the medium is not stained. It did not make an abundant growth.

LOEFFLER'S BLOOD SERUM.

Medium not liquefied. After 14 days at about 26° C. the growth along the streak was moderate, filiform, flat, glistening, and smooth; color white, tending toward cream in the old cultures. The medium was slightly grayed below the condensation water.

NUTRIENT BEEF BROTH.[a]

Clouding often absent or inconspicuous; a rim of gelatinous threads and more or less pellicle; also in young cultures very delicate suspended short filaments, best seen on shaking. In 48 hours after inoculating from bouillon there was no surface growth and no clouding; there was a slight, usually filamentous sediment, which became visible upon shaking the tube containing the culture. On thorough shaking the fluid becomes thinly clouded with numerous white suspended delicate threads (1 to 10 mm. or more in length). Under the microscope these threads are seen to be made of innumerable closely compacted small rods several times as long as broad. In 4 days a ring had formed, but the clouding of the liquid was either absent or not noticeable, although long gelatinous threads extended from the ring at the top to the bottom of the broth. These threads appeared to flatten out at the lower ends, forming a flocculent sediment. In cultures 3 weeks old the same phenomena were conspicuous. When stained with carbol fuchsin, there were many much swollen, irregularly staining, vacuolate, branched slime threads containing bacteria (fig. 3). In old cultures (7 weeks) the strings when examined under the microscope appeared in the form of irregular fine threads more or less vacuolate. At first these threads were taken for bacterial filaments undergoing disorganization, but on further study and careful staining they proved to be slime threads containing numerous involution forms and unmodified bacteria. No odor is noticeable in these cultures. There is often no true pellicle, only what might be termed an interrupted one. At other times, especially on standing for some weeks undisturbed, a true pellicle forms which fragments on shaking. It may be mentioned, however, that in a strain under cultivation for three years a continuous thick firm (nonfragmenting) pellicle finally formed, and coincident with this the virulence greatly lessened.

Sometimes in 12 hours at 25° C., when inoculated copiously from a young culture, the bouillon contains *numerous suspended delicate filaments* easily visible, especially on shaking.

[a] Containing 1 per cent Witte's peptone and sodium hydrate to read +15 on Fuller's scale.

ALKALINE BEEF BROTHS.

The organism grows better in acid than in alkaline bouillons. The optimum reaction in peptonized beef bouillon lies between +12 and +24 of Fuller's scale. In May, 1908, growth was found to be more rapid in +15 bouillon than in phenolphthalein neutral bouillon. This was true at end of the second and the eighth day. The inoculations were from an agar culture 4 days old.

This experiment was repeated in 1910 with +15 bouillon, neutral bouillon, and −15 bouillon. At the end of two days there was most growth in the +15 and least in the −15. All showed more or less clouding, especially the alkaline ones. At the end of five days at 25° C. there was a plain white rim in the +15 tubes, and a very scanty one in the others; doubtfully present in some of the −15 tubes. On shaking, the fluid was at least three times as cloudy in the +15 bouillon as in the 0 or −15. The tubes were inoculated from 10 c. c. of sterile water in which a loop from a 2-day agar culture was diffused by shaking. For tests in other grades of alkalinity see following table and the chart under Comparative Tests.

SUGARED PEPTONE WATER.

Heavy pellicle, long continued growth, and final brown stain of the fluid, in flasks of autoclaved river water containing Merck's c. p. dextrose, Witte's peptone and c. p. calcium carbonate. Frequently the pellicle settled and a second one formed. The cultures were alive at the end of 4 months when turned over to the chemist for examination.

MILK.

Coagulation delayed; extrusion of whey begins only after several days; coagulum not peptonized (?). There is usually a pellicle or interrupted pellicle. For example: Four tubes of sterile milk were inoculated with a 1-millimeter loop of beef-broth culture 3 days old and kept at 23° C. Two days after inoculation no change had taken place in the consistency of the milk. Six days after inoculation the only change noticeable was the formation of a very shallow layer of whey on the surface of the inoculated milk. This is the customary behavior in milk. Often separation of whey is long delayed.

In 6-months-old milk cultures, three-fourths dried out, but still alive, the color of the gelatinous curd was Rhamnin brown No. 2 nearly (Repert. de Couleurs, Soc. Fr. des Chrysanth.), or between drab and ocher of Standard Dictionary (spectrum). Under the microscope, the bacteria were in the form of short rods, single and end to end in pairs, mostly as a pure white precipitate, 3 mm. wide, which

did not take stain very readily (carbol fuchsin). No tyrosin crystals were found (hand lens and compound microscope), and if there is any solution of the curd it is very slow and incomplete.

At the end of 10 days, tubes of milk inoculated with the daisy organism in February, 1910, showed about 2 mm. depth of whey on top of a separating curd, which remained fluid. At the end of the twenty-fourth day there was a small amount of clear whey over a copious fluid curd, beneath which was a small amount of clear white bacterial precipitate. Color much as in checks, which were brownish from overheating.

On May 9, 1910, 6 additional inoculations were made from 3 of the 6-months-old milk cultures into sterile white milk (i. e., milk not overheated), 3 check tubes being held. At the end of 9 days the only visible change was a white pellicle on the inoculated milks. Examined July 1, the inoculated tubes contained about 1 centimeter depth of clear whey supporting a well-defined white pellicle and resting on a homogeneous-looking opaque white curd about 3 centimeters deep (Pl. XXV, figs. *g*, *h*). The curd was not browned and yet not as white as in the checks. This so-called curd was fluid, as shown by gentle shaking.

There is never any rapid separation and digestion of the curd such as Brizi describes for his *Bacillus populi*.

LITMUS MILK.

The litmus is gradually blued, then reduced. Inoculations into litmus milk, using a 1-mm. loop of a 3-day-old beef-broth culture, resulted in 8 days in a deeper blue color (indigo blue); and in 24 days the blue color disappeared with a slight formation of whey at the surface. It is apparent from this test and others which were subsequently instituted (Table XII) that the culture is alkaline from the start, the litmus becoming reduced later. The litmus is never reddened. The behavior in litmus milk indicates the presence of a lab ferment. The reduction of the litmus may be partial or complete and is always slow. There was not much, if any, peptonization of the curd, and the whey at the end of 2 months was dark by reflected light (not red), the curd being either bluish, drab, or wholly bleached.

SILICATE JELLY.

Slow white growth. (See p. 156.)

COHN'S SOLUTION.

Growth scanty or absent, medium nonfluorescent. Many tests were made.

USCHINSKY'S SOLUTION.

Growth scanty, not viscid. Tubes were inoculated from a beef-broth culture 3 days old, a 1-mm. loop being used for each tube of Uschinsky's solution. In 2 days a scanty growth, which was slightly flocculent, could be seen, together with a few white filamentous flaky particles which were in suspension in the liquid. At the end of 6 days no further change was perceptible and the fluid did not become viscid, nor fluorescent. There was no pellicle. Under the microscope, at the end of 2 months, the filamentous flakes consisted of numerous short rods staining readily in carbol fuchsin. These rods appear to lie in an unstained slime. No chains were detected.

SODIUM CHLORIDE BOUILLON.

Four per cent of salt inhibits growth, 3 per cent retards growth or inhibits it.

A 1-mm. loop of a 3-day-old +15 peptonized beef-broth culture was placed in each tube of peptonized beef broth, containing 1, 2, 3, 4, 5, and 6 per cent c. p. sodium chloride—several tubes of each sort. At the end of 6 days, growth was apparent in tubes containing 1, 2, and 3 per cent, but no growth could be detected in tubes containing 4 per cent or more of sodium chloride, indicating that 4 per cent will inhibit the growth of the organism. The growth in the 3 per cent was slight.

In another experiment the daisy organism refused to grow in 3 per cent salt bouillon (Table VI).

In a repetition test it grew in 3.5 per cent.

GROWTH IN BOUILLON OVER CHLOROFORM.

Growth is unrestrained. Chloroform to the amount of 5 c. c. was run into 5 tubes of bouillon by means of a sterile pipette. Three tubes were then inoculated with the organism from a 10-day-old bouillon culture. In 2 days there was a good growth at the top of the bouillon; 12 days after inoculating a heavy growth was present. The tubes were not shaken.

NITROGEN NUTRITION.

Nitrogen is obtained from peptone, asparagin, etc. In filtered river water containing 0.5 per cent dextrose and 0.5 per cent urea there was no growth. The experiment was repeated some months later with the same result In filtered river water containing 1 per cent asparagin the organism made a slow initial growth, first visible after 5 days. Strain B, which had been in the laboratory several years and had lost its virulence, grew better than a recent isolation. This

indicates ability of the organism to take both its nitrogen and its carbon from asparagin. A decidedly less amount, but some growth, was obtained in river water containing only dextrose. In river water alone no growth was obtained. For further data see tables under *Observed Differences in Organisms from Various Sources.*

BEST MEDIA FOR LONG-CONTINUED GROWTH.

Milk, bouillon, dextrose peptone water with calcium carbonate are the best media we have tried. In tubes of milk the organism has lived for six months.

QUICK TESTS FOR DIFFERENTIAL PURPOSES.

The following are recommended tests:

Gelatin and agar plates, especially time of appearance of colonies on +15 agar plates made from the tumors; young agar stroke cultures; behavior in milk and litmus milk; growth on potato; behavior in Cohn's solution; behavior in the thermostat at 37° C.; stringy ring and suspended filaments in peptonized beef bouillon; inoculations into young, rapidly growing daisy shoots or into growing sugar-beet roots.

FERMENTATION TUBES.

No gas is produced, and the organism is aerobic in its tendencies. A basal solution was made by adding 2 per cent of Witte's peptone to water. Six solutions were then made from this, each containing 1 per cent of the following carbon compound: Glycerin, cane sugar, mannit, dextrose, maltose, lactose. One-half dozen fermentation tubes were filled with each of these solutions and sterilized by heating 20 minutes on three days in succession. Four tubes of each set were inoculated and 2 were left for control. The inoculated tubes each received a 1 mm. loop from a 2-day-old culture growing in water containing 2 per cent Witte's peptone and 1 per cent glycerin. Four days after inoculation there was a slight cloudiness in the open end of all inoculated tubes. The clouding was most conspicuous in the tubes containing dextrose and this extended down to the elbow. Next to the dextrose in point of cloudiness stood the maltose with threadlike thickenings floating at the surface. At the end of 10 days the cloudiness in the dextrose tubes had extended slightly into the closed end. A distinct deposit had also formed and particles of solid matter were floating in the liquid in the clouded part of the tube. Maltose had clouded to the middle of the U; the mannit solutions were clouded slightly beyond the U into the closed end of the tube, and a distinct deposit had formed. The cane-sugar and milk-sugar solutions were clouded almost to the bend in the tube, and

the clouded part was distinctly separated from the clear part as if by a veil. The glycerin solution contained a fine white cloudiness different in appearance from any of the other fluids.[a] At the end of 14 days the clouding had extended up the closed end of the tube, about 1½ cm., in the solution containing dextrose and that containing cane sugar. Eighteen days after inoculation the tubes were tested with neutral litmus paper. In the tubes containing glycerin, mannit, and lactose the paper turned blue and in the dextrose and cane-sugar solutions it turned slightly red. In the tubes containing maltose there was no change in the sensitized paper.[b] No gas formed in any of the tubes, nor were any of them clouded throughout the whole of the closed end. All the control tubes remained sterile.

AMMONIA PRODUCTION.

Moderate to strong.

NITRATES.

Nitrates are not reduced. Five tubes each containing 10 c. c. peptonized beef broth to which had been added just enough nitrate of potash to make 1 per cent nitrate-bouillon solution were inoculated with the daisy organism. At the end of four days the tubes were distinctly clouded and tests were made for nitrites as follows: To 10 c. c. of the nitrate bouillon containing the growing organism 1 c. c. of boiled starch water and 1 c. c. of potassium-iodid solution (1:200) were added. A few drops of strong sulphuric-acid water (2:1) were then added, but no trace of a blue color resulted, indicating that no nitrites had formed. This experiment was repeated several times at long intervals with subcultures from various isolations, but always with the same result. (See p. 148.)

INDOL.

Indol is produced in small quantity and very slowly. In 1908 several tubes of Uschinsky's solution with 1 per cent Witte's peptone added were inoculated with fresh agar cultures of the daisy organism. The inoculated tubes showed marked growth in four days and a test was made for indol, using concentrated sulphuric acid, and dilute sodium nitrite (1:200 in water). This test showed no trace of indol, even upon heating to 80° C. after the sulphuric acid and nitrite were added. This test was repeated at the end of 10 days, but again with negative

[a] We were not able to duplicate this in subsequent cultures and now think it may have been due to precipitation of some of the peptone on standing.

[b] This experiment was repeated two years later with practically the same result—the neutral litmus paper showing only the barest trace of alkalinity after the cultures were 16 days old. After three months the fluid was clear, or nearly so, until shaken. There was enough white precipitate to make the unstained fluid floeculent filamentous turbid on shaking. It was still neutral to litmus paper.

results. This experiment was twice repeated with the same negative results.

In 1910 another trial was made inoculating into river water containing 2 per cent Witte's peptone and testing after 26 days' growth. This time the results were positive. There was a trace of pink before heating, and after 5 minutes in the water bath at 80° C. there was a decided red about half as deep as that given by those strains of *Bacillus coli* which are considered to be typical indol producers.

The indol reaction can not be obtained at the end of 24 hours, and seldom sooner than the eighth to tenth day.

TOLERATION OF ACIDS.

Slight toleration for citric, malic, and acetic acids. For the first tests 0.5, 1, and 2 per cent of the first two acids were added to tubes of neutral bouillon. A 7-day bouillon culture was used for inoculating the acid media. In six days there was some cloudiness which was least in the tubes containing 2 per cent acid. This cloudiness had not increased in any case a month after inoculating.

This test was repeated some years later with 1 per cent citric and malic acid, with negative results.

Tests were then made in 0.5 per cent citric and also in 0.5 per cent malic acid bouillon (+71) with negative results.

A final test was made in a beef bouillon containing 0.25 per cent citric acid (+34), and in another containing 0.25 per cent malic acid (+38). In the citrated bouillon of this strength both the old and the new strains of the daisy organism grew. In the malated bouillon only the old strain of the daisy organism grew (but there was only one test—1 tube). Undoubtedly the clouding observed in the first experiments (1907) should be attributed to chemical precipitates which in such acid solutions are frequently thrown out upon standing and become confusing.

One additional test was made in July, 1910, into peptonized beef bouillon acidulated to +26 with malic acid. Four tubes were inoculated. On the seventh day there was a bacterial pellicle on each one. The fluid was nearly clear—i. e., there was no fine clouding, but it contained strings, filaments, and flocks. The organism used was the newest strain of daisy.

The tests with acetic acid were made in 1911 adding it to both agar and bouillon cultures. Small amounts sterilized the cultures.

TOLERATION OF SODIUM HYDROXID.

The toleration for alkali is slight. Transfers were made to −15, −30, and −45 peptonized beef bouillon from a +15 bouillon culture 3 days old. Sixteen days after inoculating there was a slight growth

in the −15 tubes only. This experiment was several times repeated, with the same results.

Afterwards the organism was tested in −16 peptonized beef bouillon and in −34, with the following results: In the −16 the old strain (now nonvirulent) made a copious growth, and a more recently isolated virulent strain made a slight growth (about one-twentieth as much as the preceding). In the −34 the recent isolation made no growth, and the old strain (B) about one-twentieth as much growth as it did in the −16.

OPTIMUM REACTION FOR GROWTH IN BOUILLON.

The optimum reaction appears to lie between +12 and +24 on Fuller's scale. The first tests were made in May, 1908. Subsequent experiments (1910) gave the confirmatory results detailed in Table I, from which it appears that the organism is able to overcome moderate alkalinity and grow vigorously down to 0 on Fuller's scale. Judging from these results, the optimum (sodium hydroxid) alkalinity for growth in peptonized beef bouillon lies between +12 and +24 on Fuller's scale, and the limits for growth between −16 and some undetermined point between +24 and +34, the tubes being inoculated soon after the final titrations but left exposed at room temperature to the CO_2 of the air.

TABLE I.—*Relative growth* [a] *of daisy bacterium (newest strain) in peptonized bouillons of varying grades of alkalinity or acidity.*

[Inoculated from (A) 2-day-old peptone bouillon culture; also, for comparison from (B) water suspension of a 4-day agar streak, and from (C) subsequent young cultures. The +34 was acidulated with malic acid, the +36 with citric.]

Titration (grade of alkalinity or acidity).	(A) 2-day peptone bouillon culture.						(B) Water suspension of 4-day agar streak.				(C) Subsequent young cultures.			
	Three days.			Six weeks.			Three days.		Six weeks.		(1 to 2 months.)			
	Tube.[a]		Remarks.	Tube.[a]		Remarks.	Tube.[a]	Remarks.	Tube.[a]	Remarks.	Tube.[a]			Remarks.
	1	2		1	2				1	2				
−29	0	0		0	0		0		0		0	0	0	
−25	0	0		0	(b)		0		0		0	0		
−23											0	0	0	
−20	0	0		0	0		0		(b)		0	0		
−17											(?)	(?)	(?)	No growth or merest trace on bottom; no rim or pellicle; fluid clear.
−16	1	1		3	3	Suspended filaments; no pellicle.	1		3	Suspended filaments; no pellicle.				
0	2	2	No rim or pellicle; clouding with threads.	5	5	Crystals	2	No rim or pellicle	5	Crystals				
+2	2	2	do	5	5	Few large crystals	2		5	Few large crystals				
+7	2	2	Scant, fallen rim	5	5	Fluid nearly clear; irregular crystals.	2	Scant, fallen rim	5	Fluid nearly clear; crystals.				
+12	3	3	Fallen rim; better developed.	5	5	Coarse prismatic irregular crystals; fluid clear.	3	Fallen rim	5	Crystals; fluid clear				
+16	3	3	do	5	5	do	3	do	5	do				
+21	3	3	do	5	5	do	3	do	5	do				
+24	3	3	do	4	4	Wide rim	3	do	4	4 Wide rim				
+34											0			11 days only.
+36											0			11 days only.

[a] Explanation of figures indicating growth: 0=no growth; 1=trace; 2=slight; 3=moderate; 4=good; 5=heavy, including pellicle.
[b] Broken.

VITALITY ON CULTURE MEDIA.

The life of this organism on culture media is brief to moderate. Often in hot summer weather agar streak cultures were found dead after 15 to 25 days when exposed to room temperatures. In cooler weather agar stab and streak cultures have lived for 4 or 5 weeks, but many observations made in the course of the prolonged inoculation experiments indicate that the organism is rather short lived on agar. It lives somewhat longer on agar kept in the ice box. Cultures freshly made from the galls have to be transferred as often as every three weeks if one would be certain of keeping them alive. The length of life of this organism is considerably prolonged by growing it in liquid media, notably in milk, in which it will retain its vitality for more than twice the length of time that it will on agar, whether kept in the ice box or at room temperature. (See Milk, p. 112.)

Flask cultures made in February, 1910, in river water containing 1 per cent dextrose, 1 per cent Witte's peptone, and some grams of calcium carbonate, were alive at the end of 7 months.

TEMPERATURE RELATIONS.

THERMAL DEATH POINT.

The death temperature is about 51° C., exposing for 10 minutes in +15 peptonized beef-bouillon. After several preliminary tests, e. g., at 43° to 53° C., it was concluded that the thermal death point must lie between these two temperatures. The following tests were then carried through in order to determine the point more accurately. Three sets of 4 tubes each of peptonized beef broth were inoculated with the daisy organism from 3-day-old slant agar cultures, and these tubes were placed in water at constant temperatures of 50°, 51°, and 52° C. At the end of 10 minutes the tubes were removed and kept for several days at about 29° C. At the end of 4 days growth appeared in all the tubes that had been exposed to 50° C. for 10 minutes. Slight growth appeared in some of the tubes that had been kept at 51 C., but no growth appeared even after 10 days in the tubes that had been kept at 52° C. for 10 minutes. This experiment was repeated several times with the same result, indicating that under the conditions named 51° C. is near the thermal death point of this organism.

OPTIMUM TEMPERATURE.

This appears to lie between 25° and 28° C. Growth at 25° C. on standard agar and in peptonized beef bouillon was three times better than at 30° C., and decidedly better than at 12° C. At the latter temperature growth was better in the bouillon.

MAXIMUM TEMPERATURE.

The highest temperature at which growth will take place is ±37° C. Several slant agar cultures were made (some from a 3-day-old agar culture, and others from a 9-day-old culture) and placed in a constant temperature oven at 39° C. No growth occurred. At the end of 3 days at this temperature some of the cultures were removed and kept at room temperature for 3 days, but no growth appeared. The control tubes gave a good growth. This experiment was repeated at 39° C. with the same result: No growth for 5 days, and none after removal to room temperature (5 days more).

Bouillon tubes were then inoculated and placed in a thermostat at 40° C., the controls being kept at room temperature. The controls grew. The tubes in the thermostat remained clear (6 days). Plates were then poured from them with negative results.

In another experiment glycerine agar streak cultures failed to grow at 37° C., but check tubes grew readily at room temperatures. The indications from these experiments are that a temperature of 39° to 40° C. soon destroys the life of this organism—under the conditions named. The following experiments were also made:

In March, 1910, in a well-regulated thermostat, carefully controlled, some very precise results were obtained confirming and extending the earlier observations. The temperature during the first 4 days ranged from 37° to 37.2° C. During the next 4 days the temperature increased a trifle, ranging from 37.2° to 37.5° C. This thermometer was compared with a standard instrument calibrated at the Reichs Anstalt in Berlin.

The experiment was begun at 9.45 a. m. March 1, by inoculating four +14 peptonized beef agar slants and 6 tubes of +15 peptonized beef bouillon from a peptone beef bouillon culture of February 26: One 3-mm. loop of the fluid was used for each agar slant and two 3-mm. loops for each tube of bouillon. One of the inoculated tubes of bouillon was kept at room temperature as a check and the other tubes were placed in the thermostat. At the end of 24 hours the tube at room temperature showed a moderate amount of growth. At the end of 48 hours there was a good growth in the check tube, but none in any of the 9 tubes exposed in the thermostat. At noon of March 4 there was still no growth in the thermostat. The same was true on March 7. On March 4, at noon, 1 tube of beef broth and 1 of agar were removed from the thermostat and put at room temperature. On March 5 at 3 p. m. another tube of beef broth and 1 of agar were removed from the thermostat. On March 7 at 11 a. m. the remaining tubes (3 beef bouillon, 2 agar) were removed from the thermostat.

On March 7 there was no visible growth in any of the tubes which had been in the thermostat. On March 9 the tube of bouillon removed March 4 showed numerous white bacterial flocks but no clouding. On March 14 typical strings appeared in the tube of bouillon removed March 4, and later on a pellicle. No growth took place in any of the other tubes.

Conclusion.—No growth in +15 bouillon or agar at 37° to 37.5° C. Exposure for 3 days retards subsequent growth at room temperature, and exposure for 4 days kills.

This experiment was repeated in the same thermostat using litmus milk, potato, slant peptonized beef agar (+16), and peptonized beef bouillon (+15). It was begun March 7 at noon. The range of temperature during the next 7 days was 37° to 37.4° C. Ten tubes (4 milk, 4 potato, 1 agar, 1 bouillon) were held as checks at 19° to 22° C. Thirteen tubes (5 milk, 4 potato, 2 agar, and 2 bouillon) were placed in the thermostat. All the checks showed distinct growth at the end of 24 to 48 hours. At the end of 4 days there was no visible growth in any of the tubes in the thermostat. On March 12 the 4 tubes of potato showed a trace of growth out of the water, i. e., at the extreme top of each cylinder. The 5 tubes of litmus milk were also now bluer than an uninoculated tube.

On March 14 (end of 7 days), the litmus milk was bluer than on March 12. There was still no visible growth either on the agar or in the bouillon, and that on the potato cylinders was scanty and restricted to the top. All the tubes were now removed to room temperature and agar streaks were made from the litmus milk. Two days later the agars streaked from the milk bore a good typical growth. Growth in the litmus milk and on the potato increased at room temperatures during the next week but no growth developed in the bouillon or on the agar.

Conclusion.—37° C. is above the limit for growth in +15 bouillon and on agar, and close to the limit for milk and potato. Exposure on agar or in bouillon for 7 days at 37° to 37.4° C. (mostly 37.1° to 37.3° C.) destroyed the organism.

MINIMUM TEMPERATURE.

Growth occurs at 0° C. Tests were made at temperatures varying from +10° to 0° C. with the result that growth was obtained even at the lowest temperature in peptonized beef broth and on agar. The ice box was used for temperatures above 3° C., and the records, made night and morning, were continued for a period of 2 weeks, or less if growth appeared earlier.

For tests of growth at 0° C. the experiment was continued for 2 weeks in the following manner: Transfers were made from 3-day-old agar cultures to agar and bouillon (2 tubes of each) which were cooled

to 0° C. before inoculating. The tubes were thrust far down into finely pulverized ice in a wine cooler which was set into the ice box close to large cakes of ice. Ice was added to the cooler night and morning after siphoning off the accumulated water. The temperature held constantly at 0° to 0.2° C. It was never above +0.2° C. The experiment was begun March 3, 1908. On March 7 there was a decided growth in the bouillon tubes but none on the agar. On March 9 a very slight growth was detected on the agar. On March 17, when the experiment was discontinued, the growth in the bouillon, although not heavy, was sufficient to show the usual characteristics. It was all at the bottom of the tube, none on the surface. There was enough growth on the agar to be visible, but it was slight. The bacteria in the bouillon were examined microscopically and many involution forms were found and drawings were made (fig. 2). Plates were poured from the bouillon and the daisy organism obtained in pure culture.

In order to obtain a still lower temperature, salt was mixed with the ice in a quinine can and the can was placed in a galvanized-iron bucket 10 inches in diameter. The bottom of the can, as well as of the bucket, was perforated to allow the water to escape. Both can and bucket were iced twice daily, using 4 tablespoonfuls of salt at each icing. The tubes were inoculated as before, and placed in the ice-salt mixture in which the thermometer was also placed. The bucket containing the ice and the quinine can with its contents was placed in the ice compartment of the ice box. Within a few minutes after the tubes were placed in the ice-salt mixture the contents had solidified and remained solid during the 2 weeks that the experiment was continued. The temperature varied from 0° to −14° C., but was never higher than 0° C. during the 2 weeks. At the expiration of this time the cultures were removed to room temperature where the beef broth quickly melted and was found to contain a distinct characteristic stringy growth, but only very slight. No growth was visible on the slant agar tubes.

EFFECT OF DRYING.

The daisy organism is killed readily by drying. An experiment made in April, 1910 (temperature 25° C.), gave the following result: Tiny drops of a bouillon culture 5 days old were spread on 25 clean, sterile, small cover glasses and set away on a shelf in the culture room (in diffused north light) in a covered, sterile Petri dish. The covers were then taken up by means of sterile forceps and dropped into tubes of sterile bouillon, one into each tube, with the following results:

Alive: Number of days dried, 1, 3, 7, 12.
Dead: Number of days dried, 2, 5, 6, 8, 9, 10, 13, 14, 15, 16.

The remaining 11 covers were dropped into 6 tubes of bouillon at the end of 20 days but no growth ensued (28 days). The character of growth of the daisy organism in beef bouillon renders it difficult to get an even, thin distribution and proper drying on cover slips, and to this is probably attributable the fact that the organism was alive on 3 of the 25 covers after the first day.

This experiment was repeated in June, 1910 (temperature 30° C.), using a peptone bouillon culture 5 days old and thinner smears, the covers being kept in the dark, with the following result:

Test begun at end of 2 days—
(1) Two days, 2 tubes—no growth.
(2) Three days, 1 tube—no growth.
(3) Seven days, 16 tubes—no growth.
The 16 tubes were under observation for 13 days.

A second repetition in July, 1910 (temperature 30° C.), using a 6-day-old peptone bouillon culture, the covers being kept in the dark, gave the following results:

Test begun first day—
(1) One day, 2 tubes—both grew, one very slowly.
(2) Two days, 1 tube—no growth.
(3) Three days, 1 tube—no growth.
(4) Five days, 1 tube—no growth.
(5) Six days, 1 tube—no growth.
(6) Nine days, 1 tube—no growth.
(7) Ten days, 1 tube—no growth.
(8) Twelve days, 18 tubes—no growth.
The last lot was under observation for 10 days.

EFFECT OF SUNLIGHT.

Organism moderately sensitive to sunlight.—Agar tubes of the daisy organism inoculated from 3-day bouillon cultures were poured into Petri dishes and placed in the bright sunlight for 45 and 60 minutes, half of each plate being covered with black paper. After 4 days on each of the four plates there were numerous colonies under the covered parts, but none on the exposed parts. On 8 plates exposed at this time for shorter periods (30, 15, 10, and 5 minutes) colonies appeared on the exposed parts, but they were fewer than those on the shaded parts. Results similar to those just detailed were obtained by a repetition at 30 minutes. Colonies came up slower on the exposed side of the plate, but finally there were many. A second repetition gave similar results at 30 and 35 minutes. In a repetition at 35, 40, and 45 minutes, made a few weeks later (experiment begun April 3 in bright sunlight and final examination made April 11) tiny colonies appeared the third day on the shaded side of all the plates, but none at all developed on the exposed side (8 days).

ACIDS.

Acetic acid is produced in peptone water in the presence of grape sugar, and calcium carbonate (see report by Doctor Alsberg). Cane sugar is also broken up with production of an acid.

ALKALIES.

The blueing of litmus milk is due to ammonia. (See under *Crystals* [below]).

ALCOHOLS.

Ethyl alcohol is produced in peptone water in the presence of dextrose and calcium carbonate.

FERMENTS.

Invertase and lab are inferred to be produced: The former because an acid is produced and an invert sugar appears when the bacterium is grown in the presence of cane sugar; the latter because the casein is thrown down without the formation of an acid. (See under *Litmus Milk*, p. 154.) Litmus is also reduced.

CRYSTALS.

Prismatic crystals are formed in old cultures on agar partially neutralized by sodium hydrate (+15 agar), in bouillon and also in +10 nutrient gelatin. The washed crystals from bouillon cultures were determined for us by Dr. Carl L. Alsberg to be ammonium magnesium phosphate.

EFFECT OF GERMICIDES.

Copper sulphate.—This organism, as shown by poured-plate cultures, grew after 10, 15, and 20 minute exposures to 1:1,000 commercial copper sulphate in water, which had been acidulated with 19 drops of glacial acetic acid per 1,000 c. c. It did not grow after exposure for 30 and 40 minutes.

This experiment was repeated using copper sulphate 1:5,000, acidulated with 5 drops of glacial acetic acid per liter. Plates were poured after exposure of the organism to this solution for 10, 20, 30, 40, and 60 minutes. At the end of 6 days colonies appeared only on the plates made from the 10-minute exposure. This experiment was repeated a week later with the same result, namely, colonies only on the check plate and on the 10-minute exposure.

Exposures were made for 1 and 2 hours in 1:10,000 copper sulphate water with 8 drops of acetic acid per liter. At the end of 4 days there were numerous colonies on the check plate but none on the others.

Five days later this experiment was repeated, exposing 1¼ and 2 hours. At the end of 2 days there were colonies only on the control plate. Two days later colonies appeared also on the 1¼-hour plate, but none on the 2-hour exposure. Daisy plants sprayed with this strength of solution were not injured.

Formalin.—In September, 1907, tests were made in formalin diluted with water (1:500), exposing 10, 20, 30, 40, and 60 minutes. Colonies were abundant in all the plates except the 60-minute exposure, which yielded only a few.

Suspecting the strength of the formalin used, this experiment was repeated in May, 1910, as follows:

Transferred two 3-mm. loops of a 2-day-old bouillon culture to 10 c. c. of formalin in distilled water (1:500). This formalin solution was made from a freshly opened stock bottle. A check was made by transferring one loop from the bouillon tube to 10 c. c. of sterile water and pouring 2 plates.

The plates of the organism exposed to the formalin were poured at intervals of 10, 20, 30, 40, and 60 minutes.

Results: May 26, the plates are free from colonies. May 27, the plates are free from colonies. May 28, the check plates have numerous tiny colonies; the others are free. May 31, a few colonies up on the 10-minute plates; the others are free. June 4, colonies appeared only on the plates made from the 10-minute exposure.

Mercuric chloride.—Tests were made in a water solution of mercuric chloride (1:10,000), exposing 15 minutes, 30 minutes, and 5 hours, with a check plate of the organism from a suspension in distilled water. After 3 days there were numerous colonies on the check plate but none on the others and none appeared later.

PATHOGENICITY.

This organism was first isolated from galls occurring on the hothouse daisy (*Chrysanthemum frutescens*), but it causes, at least by inoculation, tumors in plants of many families, viz, Compositae, Solanaceae, Oleaceae, Umbelliferae, Vitaceae, Leguminosae, Rosaceae, Cruciferae, Caryophyllaceae, Chenopodiaceae, Urticaceae, Juglandaceae, Salicaceae.

LOSS OF VIRULENCE.

In cultures carried on for several years a slow gradual loss of virulence has been observed, but this was not detected until after the second year.

GROUP NUMBER.

The group number according to the descriptive chart, Society of American Bacteriologists, is: 212.2322023.

IMMUNITY.

Our studies are still incomplete. As far as they have gone they seem to indicate that repeated inoculations produce a heightened resistance to further inoculations with organisms of the original or of a lessened grade of virulence, but that more virulent strains will still produce galls on such plants, although the initial growth is usually slow.

OBSERVED DIFFERENCES IN CROWN-GALL ORGANISMS FROM VARIOUS SOURCES.

MORPHOLOGY AND BEHAVIOR TOWARD STAINS.

METHODS OF STUDY.

The measurements were made from 2-day agar streaks inoculated from agar streaks. The slime was diluted in sterile water and spread thinly on clean covers. These covers were then dried, flamed slightly, and stained with gentian violet. They were washed in water only, mounted in balsam, and examined at once. All the measurements were made by the same person (the senior writer) and represent the range of variation observed. All the rods were straight or nearly so, with rounded ends. With one or two exceptions all stained freely and uniformly. The measurements were made in the summer of 1910, using a Zeiss 2-mm. n. a. 1.3 oil-immersion lens and an eyepiece micrometer in a No. 6 compensating ocular, with a No. 12 compensating ocular for orientation and confirmation, using north light. One space of the Zeiss stage micrometer (1 mm. in 100 Th.) exactly equaled 12 spaces on the eyepiece micrometer, making one space on the latter equal to 0.833μ (confirmation of a determination by Miss Brown), but in making the measurements the value was for convenience reckoned at 0.8μ. The morphology did not vary greatly from culture to culture, as may be seen from the measurements given.

Similar young agar cultures were used for the demonstration of flagella (Pitfield's stain in most cases). The flagella were stained by Miss Katherine Bryan, but the slides were also examined by the senior writer.

For the acid-fast (Erlich Weigert) stain and the Gram's stain somewhat older agar cultures were used.

Old cultures were examined for spore formation, i. e., agar streak 19 days. For the examinations unstained in sterile water the top of the streak was used. It was then stained in carbol fuchsin 3 minutes and reexamined. Transfers were then made to sterile peptone water (3 mm. loop) from each tube and these were then at once

heated for 1 hour at 80° to 86° C., after which there was no growth (tubes under observation 23 days).

The name denotes the kind of gall from which the organism was isolated.

NEWEST DAISY.

A culture recently isolated and still infectious.

(*a*) Size 0.6 to 0.8 by 1.2 to 2.0μ. Most, I think, about 1.5μ long. Many short chains (4 to 8 segments) occur. Staining irregular and the protoplasm so pulled apart that it is very difficult to decide on the extreme length. Occasional club-shaped rods occur.

(*b*) Repetition two days later (unflamed): Size 0.5 to 0.7 by 1.0 to 3.0μ. Stained uniformly. The rods are single, paired, or in short chains often with indistinct constrictions, making it difficult to determine the maximum length of rods. The longest with indistinct septation are 4 to 10μ. A few are twice as broad as the multitude; a few are branched; a few are club shaped.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative. Agar streak 4 days.
Flagella.—Polar, 1 to 3. Pitfield's stain.
Spores.—Negative.

OLD DAISY (B).

A strain not now infectious: Size 0.4 to 0.6 by 1.0 to 2.4μ. Looks like the right thing. The bacteria adhere on the cover in small clumps. They are often paired and occasionally 4 are joined end to end. There is also an occasional club-shaped rod.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 3. Pitfield's stain, etc.
Spores.—Negative.

PEACH.

Size 0.5 by 1.0 to 2.0μ.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 3. Pitfield's stain.
Spores.—Negative.

HOP.

Size 0.4 to 0.8 by 1.0 to 1.6μ. Occasional club-shaped rods are present, but they are much less numerous than in the grape.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 3. Pitfield's stain.
Spores.—Negative.

NEW ROSE.

Size 0.5 to 0.6 by 1.0 to 2.5μ, rarely 3μ.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

OLD ROSE.

No measurements.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 3. Pitfield's stain.
Spores.—Negative.

OLD APPLE.

Size 0.6 to 0.8 by 0.6 to 0.8μ. Often 3 to 8 elements in a chain. Almost like a streptococcus. Does not look like the right thing. Probably an intruder which has displaced the original pathogenic organism. Recent inoculation tests on sugar beet (June, 1910) gave negative results. (See also *Cultural Characters.*)

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Positive. Agar 5 days.
Flagella.—Unable to stain.
Spores.—Negative.

APPLE HAIRY-ROOT.

Size 0.4 to 0.7 by 1.0 to 2.0μ. Occasionally one thicker.

Acid fast.—Negative. Agar 7 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, mostly 1 flagellum (Loeffler's stain). No results with Pitfield's stain.
Spores.—Negative.

NEW APPLE.

Descended from apple hairy-root, i. e., from apple gall which bore no roots but which produced both galls and hairy roots on sugar beet: Size 0.4 to 0.8 by 1.0 to 1.6μ. Occasionally one may be longer.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative. Agar 4 days.
Flagella.—Unable to stain.
Spores.—Negative.

ALFALFA.

Size 0.4 to 0.7 by 0.8 to 2.0μ. Average length about 1.2μ. Most are 0.5 to 0.6μ in diameter. In those which are 1.8 or 0.2μ long a slight equatorial constriction is usually visible. Occasional club-shaped rods are present.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

GRAPE.

Size 0.5 to 0.9 by 1.0 to 1.5μ. Swollen and club-shaped rods 1.2 to 1.3μ in diameter are frequent. Perhaps degeneration forms.[a]

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 4 days.
Flagella.—Polar, 1 to 2. Van Ermengem's stain. No result with Pitfield's stain or Loeffler's stain.
Spores.—Negative.

NEW CHESTNUT.

Size 0.5 to 0.7 by 0.8 to 1.4μ. Seems to be rather shorter than most. The average length is about 1.0 to 1.2μ long. An exceptionally long pair measures 2.8μ, another average pair 2.2μ. The rods are single, in pairs, 4's or 8's, with distinct rounded constrictions. Pathogenicity not proved. Beets and grape inoculated gave no conclusive results.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Unable to stain.
Spores.—Negative.

ARBUTUS UNEDO.

Size 0.5 to 0.9 by 1.2 to 2.2μ.

Acid fast.—Negative. Agar 5 days and bouillon 14 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Unable to stain.
Spores.—Negative.

COTTON.

Size 0.4 to 0.6 by 1.0 to 2.4μ. Rarely as long as 2.4μ and most about 0.5μ in diameter. A few are club-shaped; a few are broader, and deeper stained than the majority. Slide overwashed.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Very feeble stain—should be estimated as negative. Agar 4 days.
Flagella.—Polar, 1 to 3. Pitfield's stain.
Spores.—Negative.

QUINCE.

Size 0.5 to 0.7 by 1.2 to 2.0μ. Greater tendency to short chains than in most of the slides so far examined, i. e., like newest daisy, but constrictions plainer.

Acid fast.—Negative. Agar 7 days.
Gram's stain.—Negative, i. e., very feebly stained. Agar 1 day. Positive (same strain), agar 5 days. No satisfactory inoculations.
Flagella.—Polar, mostly 3. Stained by Loeffler's stain. No results with Pitfield's stain.
Spores.—Negative.

[a] After 2 weeks on agar slant, Y's and swollen rods were very common, i. e., much more so than in any of the other 24 examined.

SUGAR BEET.

Organism which shows slightly pinkish on agar after a few days and is not infectious: Size 0.5 to 0.9 by 1.2 to 2.5μ. Two well-developed rods not yet separated measure together 0.8 by 3.2μ. Rods 2.5μ long without any distinct constriction are frequent.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative (feeble stain).
Flagella.—Unable to stain.
Spores.—Negative.

WILLOW FROM SOUTH AFRICA.

Size 0.4 to 0.7 by 1.2 to 2.4μ. Most of the rods are 1.5 to 2.0μ long. They are single or in pairs, occasionally in 4's joined end to end. The pairs frequently are curved a little. There are occasional club-shaped rods.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 3 days.
Flagella.—Unable to stain.
Spores.—Negative.

POPLAR (FLATS).

Size 0.3 to 0.6 by 1.0 to 1.8μ. Most are 0.4 to 0.5μ in diameter. No spores are present.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative (a feeble stain).
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

POPLAR (NEWPORT, R. I.).

Two colonies, which looked alike, were transferred from the poured plate, but they are not alike in morphology.

Colony 1.—Size 0.7 to 1.0 by 1.0 to 1.2μ. A very short, plump rod, with rounded ends, almost a coccus form, mostly paired. Not yet proved up and doubtful if the right organism. It has been inoculated into sugar beet with negative results.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Unable to stain.
Spores.—Negative.

Colony 2.—Size 0.4 to 0.5 by 0.8 to 2.0μ. Mostly 1.0 to 1.4μ long. The rods are longer than those of colony 1. Sugar beet inoculated unsuccessfully; pathogenicity not yet determined.

Acid fast.—Negative. Agar 4 days.
Gram's stain.—Negative. Agar 3 days.
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

TURNIP NO. 1.

Size 0.4 to 0.5 by 1.0 to 2.2μ. A common length is 1.6μ. Pathogenicity not yet established. Tests made on sugar beets failed.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 2 days.
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

TURNIP NO. 2.

Fresh isolation in July, 1910, from another gall on same plant, which had been kept alive in the hothouse. Growth on agar stroke resembled daisy; also the colony on agar plate. Not tested on plants.

Acid fast.—
Gram's stain.—
Flagella.—
Spores.—Negative.

SALSIFY.

Size 0.4 to 0.5 by 1.2 to 2.0μ. Rather slender, single, in pairs or 4's, end to end. Many of the rods are 1.5 to 1.8μ long. Beets inoculated unsuccessfully; pathogenicity not yet established.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 3 days.
Flagella.—Unable to stain.
Spores.—Negative.

PARSNIP.

Size 0.4 to 0.8 by 1.0 to 2.4μ. Rarely as long as 2.4μ. Most about 1.2 to 1.5μ long, but frequently 1.8μ. A well-developed pair measured 3.2μ. Single, in pairs, or in 4's, rarely 8, end to end.[a] Rods frequently somewhat pointed at the ends. Beets inoculated unsuccessfully; pathogenicity not yet proved.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 3 days.
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

[a] Short filaments were found in agar streaks when 19 days old.

TABULATED RESULTS OF INOCULATIONS.

TABLE II.—*Showing positive results of the pure-culture inoculations.*

Date of the inoculation.	Origin of culture.	Number and kind of plants inoculated.	Kind of culture used and its age.	Per cent which developed tumors.	Number and behavior of controls pricked.
Nov. 27, 1906	Daisy	7 daisy	1st agar sub., 3 d	100	21 plants inoculated with other organisms; free.
Dec. 13, 1906	do	8 daisy	1st agar sub., 16 d	100	None; at first only where inoculated.
Dec. 21, 1906	do	do	2d agar sub., 8 d	100	4; free.
Jan 8, 1907	do	do	Agar sub., 6 d [a]	100	None; at first only where inoculated.
Jan. 19, 1907	do	7 daisy	Agar sub., 5 d [b]	100	Do.
Feb. 6, 1907	do	8 daisy	2d agar sub., 9 d	100	4; free.
Feb. 18, 1907	do	9 daisy (12 shoots)	Agar sub., 7 d	[c] 100	1 plant, 50 punctures; also 72 check pricks on the 9 inoculated; all free.
Do	do	3 tomato	do	100	None; only where inoculated.
Do	do	3 tobacco	do	100	Do.
Mar. 2, 1907	do	18 potato	Agar	100	Several; free.
Do	do	12 carnation	Agar sub., 3 d	100	6; free.
Mar. 11, 1907	do	18 peach	Agar sub., 5 and 6 d	[d] 88 or 100	9; free, 180 punctures.
Mar. 20, 1907	do	6 tobacco	Agar sub., 2 d	100	1; free.
Mar. 27, 1907	do	6 potato	Agar sub., 3 d	100	None; only where inoculated.
Do	do	Rose	Agar sub., 2 d	100	3; free.
Mar. 29, 1907	do	Several cabbage	Agar sub., 4 d	100	None.
Apr. 3, 1907	do	8 American grape	Agar sub., 2 d	12	4; free.
Do	do	3 European grape	do	33	None.
Apr. 6, 1907	do	48 peach	Agar sub., 2 and 5 d	73	21 (420 punctures); free.
Apr. 8, 1907	do	18 hop	Agar sub., 4 d	100	8; free; more than 200 pricks.
Apr. 10, 1907	do	25 hop	Agar sub., 4 and 6 d	[e] 96 or 100	4; free.
Apr. 15, 1907	do	4 field daisy	Agar	[f] 100	1; free.
Apr. 17, 1907	do	12 sugar beet	Agar sub., 2 d	100	3; free.
Apr. 18, 1907	do	2 cabbage	Agar sub	100	Uninoculated; free.
Date (?)	do	Several turnip slices	Young agar sub	100	See text, p. 45.
Apr. 26, 1907	do	3 red beets, 4 carrots, 4 long radishes.	Agar sub., 2 d	100 100 100	None.
May 6, 1907	do	3 Jap. chrysanthemum	do	100	2; free.
Do	do	3 oleander	do	100	2; free.
May 9, 1907	do	9 European grape	Agar sub., 4 d	100	3; free.
May 14, 1907	do	do	Agar sub., 3 d	100	3; free.
July 23, 1907	do	6 Ch. coronarium	Agar sub., 5 d	100	No. ?— All free.
Do	do	6 Shasta daisy	do	100	Do.

[a] First from plate of December 18, 1906. [b] First from plate. [c] Every prick gave a gall; total, 102. [d] See text, p. 38. [e] See text, p. 49. [f] About 25 galls.

TABLE II.—*Showing positive results of the pure-culture inoculations*—Continued.

Date of the inoculation.	Origin of culture.	Number and kind of plants inoculated.	Kind of culture used and its age.	Per cent which developed tumors.	Number and behavior of controls pricked.
Aug. 1, 1907	Daisy	6 corn marigold	Agar sub., 2 d	100	2; free.
Do	do	8 English daisy	do	63	2; free.
Aug. 9, 1907	do	8 European grape	Agar sub., 3 d	75	3; free.
Sept. 19, 1907	do	6 tobacco	do	100	4; all free.
Sept. 26, 1907	do	7 pyrethrum	Agar sub., 2 d	100	3; free.
Nov. 15, 1907	do	3 daisy	Agar sub., 4 d	100	Check on next.
Do	do	24 sugar beet	do	92	None. See below.
Nov. 18, 1907	do	36 sugar beet	Agar sub., 3 d	100	No specific checks held, but one side of the house was full of beets, and only the inoculated ones contracted the disease.
Do	do	6 daisy	do	100	Checks on the preceding.
Feb. 11, 1908	do	do	Agar sub., 2 d	100	3; free.
Mar. 5, 1908	do	4 oleander	do	100	2; free.
Mar. 7, 1908	do	6 oleander	do	100	None; only where inoculated.
Do	do	3 European grape	do	33	None.
Do	do	8 almond	do	50	All uninoculated are free.
Mar. 12, 1908	do	5 daisy	do	100	None.
Do	do	10 oleander	do	100	None; only where inoculated.
Do	do	4 clover	Agar sub., 3 d	100	2; free.
May 25, 1908 May 26, 1908	do	5 white poplar	Agar sub., 4 d	20	Only where inoculated.
Do	do	8 Pterocarya	do	37	Do.
Do	do	3 Persian walnut	do	100	No other galls on tree.
June 9, 1908	do	2 gray poplar	do	50	Only where inoculated.
June 11, 1908	do	5 sugar beets	Agar sub	100	None.
Dec. 4, 1909	do	8 sugar beets	Agar sub., 2 d	100	House full of beets; only inoculated beets became diseased.
Nov. 8, 1909	Arbutus	16 sugar beet	do	12	6; all free.
Dec. 1, 1909	Cotton	9 cotton	do	44	3; all free.
Aug. 31, 1909	Grape	6 daisy	Agar sub., 4 d	50	8; free.
Do	do	4 European grape	do	50	
Sept. 7, 1909	do	6 European grape	Agar sub., 3 d	100	2; free.
May 7, 1910	do	12 sugar beet	Agar sub., 1 d	92	Only inoculated beets took the disease.
June 28, 1910	do	12 almond	Young agar sub	100	6; free.
June 14, 1909	Alfalfa	4 daisy	Agar sub., 4 d	25	None.
Do	do	4 alfalfa	do	[a] 100	Only where inoculated.
Do	do	2 sugar beet	do	100	None.
July 16, 1909	do	48 alfalfa	do	25	12; free.
Do	do	5 sugar beet	do	80	None; galls only where inoculated.
Sept. 7, 1909	do	36 alfalfa	Agar sub., 3 d	33	8; free.
Dec. 8, 1909	Alfalfa (Sept. 7)	Several young alfalfa	Agar pl., 6 d	100	

Dec. 2,1907	Peach	10 daisy	Colonies, 6 d	100	4; free.
Dec. 4,1907	do	36 daisy	12 col. 9 d. and agar sub., 4 d	92	600 + check pricks sterile.
Dec. 5,1907	do	6 peach	Agar sub., 3 d	100	2; free.
Do	Daisy	do	do	66	See preceding.
Jan. 13,1908	Peach	60 peach	Agar sub., 2 d. (?)	92	36 (540 pricks) all free; also free, 900 check pricks on the inoculated trees.
Jan. 15,1908	do	6 rose	Agar sub., 1 d	17	None.
Feb. 3,1908	do	10 daisy	10 col., 7 d	100	None; only where inoculated.
Mar. 11,1908	do	3 apple (each several places).	Colonies, agar, 7 d	100	Galls only where pricked.
Do	do	5 sugar beet	Agar sub	100	Only where inoculated.
Mar. 24,1908	do	9 peach	Agar sub., 4 d	66	4; free.
May 19,1908	do	13 apple	do	[b] 38 or 100	Do.
May 20,1908	do	9 red raspberry	Agar sub., 2 d	100	Do.
May 19,1908 / May 22,1908	do	34 black raspberry	Agar sub., 2 3, and 8 d	97	Do.
June 10,1908	do	6 hop	Agar sub., 4 d	100	2; free.
Oct. 13,1908	do	2 geranium (4 shoots)	Agar sub., 3 d	100	Many other geraniums on same bench free.
Dec. 17,1907	Rose	12 rose	Agar sub., 4 d	17	3; free
Jan. 15,1908	do	do	Agar sub., 1 d	17	None.
Mar. 18,1908	do	8 daisy	Agar sub., 2 d	100	1; free.
Dec. 3,1908	do	7 sugar beet	Agar sub., 7 d	14	3; free.
Mar. 21,1909	do	3 daisy (each in 3 to 5 shoots)	Agar sub., 5 d	50	2; free.
Jan. 27,1909	Quince	8 daisy	Agar sub., 2 d	25	Do.
Mar. 21,1909	do	3 daisy (9 shoots)	Agar sub., 5 d	66	2; free.
Apr. 26,1910	Beet	1 daisy (4 shoots)	Agar sub., 4 d	25?	None.
Nov. 21,1907	Hop	4 hop	Agar? sub., 2 d	75	Do.
Feb. 8,1908	do	5 daisy (4 and 5 shoots on each).	Agar sub., 5 d	[c] 100	Do.
Feb. 10,1908	do	12 daisy	12 col., 7 d	17	Do.
Apr. 17,1908	do	6 sugar beet	Agar sub., 2 d	100	Only inoculated plants gave tumors.
Apr. 25,1908	do	5 daisy	do	100	None.
June 10,1908	do	8 hop	Agar sub., 4 d	100	4; free.
Nov. 21,1908	do	3 tomato (36 groups of punctures).	Agar sub., 3 d	100	2; free.
Apr. 16,1909	do	3 European grape	Agar sub., 2 d	100	Do.
Do	do	3 almond	do	66	None.
Mar. 7,1910	Hop (after 26 transfers on media).	5 sugar beet	Agar sub., 3 d	[d] 100	Other beets in same and adjoining rows remained free.
May 9,1910	Hop	3 daisy (8 terminal shoots)	do	33	None.
Nov. 12,1910	do	3 sugar beet	do	100	Many plants in same bed.
Do	do	4 daisy	do	75	Numerous daises on same bench picked at same time from col. 2 and 3 hop failed.
Nov. 30,1910	do	12 daisy	Beef-bouillon, 16 d	33	
Dec. 2,1910	do	20 daisy	Agar sub., 2 d	15	
Nov. 13,1908	Old chestnut	1 daisy (4 shoots)	Colonies, 6 d	[e] 100	None; galls only where inoculated.
Do	do	5 sugar beet	do	100	None; galls only where pricked.
June 4,1910	Flats poplar	10 sugar beet	Agar sub., 4 d	100	Only where inoculated. An adjoining row, pricked at this time with new chestnut gall, failed and would also serve as a check.
June 10,1910	do	2 European grape	Young agar sub	100	Galls only where punctured.
May 9,1910	Willow	3 daisy	do	66	None; the galls appeared in the spots punctured.

[a] Including two-thirds of roots pricked. [b] Eight trees made no growth. [c] None well developed. [d] Galls large. [e] 4 galls, finally large.

TABLE II.—*Showing positive results of the pure-culture inoculations*—Continued.

Date of the inoculation.	Origin of culture.	Number and kind of plants inoculated.	Kind of culture used and its age.	Per cent which developed tumors.	Number and behavior of controls pricked.
Dec. 12, 1910	Willow	6 willow	3 d. agar sub	66	
Oct. 23, 1908	Apple	6 peach	Agar plate, 8 d	33	
Do	do	8 sugar beet	Agar sub., 2 d	25	
Oct. 22, 1908	Apple (soft), Washington	4 daisy	Agar col., 7 d., or 1st sub., 2 d	100	
Do	do	3 apple	Agar col., 7 d	66	None.
Nov. 9, 1908	Apple (hard), Iowa	8 daisy	Agar col., 5 d	50	8; on same plants; negative.
Nov. 18, 1908	do	3 daisy	Agar col., 9 d	100	No galls except where inoculated.
Dec. —, 1908	Apple (hard), Washington	Sugar beet	Agar	100	
Dec. 4, 1908	do	8 sugar beet	Agar sub., 2 d	25	None.
Nov. 13, 1908	Apple hairy-root (Iowa)	6 sugar beet	do	83	Do.
Dec. 22, 1908	Apple hairy-root (Washington).	11 sugar beet	Agar sub., 3 d	[a] 90 or 100	Do.
Feb. 24, 1909	Apple hairy-root (New York).	7 sugar beet	Agar sub., 1 d	57	Number doubtful; all sound.
Apr. 5, 1909	do	8 apple seedlings	Agar sub., 2 d	[b] 75	4; one galled.
May 21, 1909	Apple hairy-root	3 quince (90 punctures)	Agar sub., 2 d	100	Hard tissues pricked; galls did not appear for a long time.
Nov. 11, 1909	Apple hairy-root (New York)	8 sugar beets	Agar sub., 6 d	100	None.

[a] See text, p. 104.

[b] See text, p. 103.

TABLE III.—*Showing negative or doubtful results of inoculations.*

Date of the inoculation.	Origin of culture.	Number and kind of plants inoculated.	Kind of culture used and its age.	Per cent which developed tumors.	Remarks.
Jan. 6,1907	Daisy	Several bulbs and leaves of common onion.	Agar sub., 4 d	0	Slow growth.
Jan. 18,1907	.....do	7 daisy (24 places)	Agar sub., 49 d	0	Culture probably dead.[a]
Feb. 14,1907	.....do	2 olive (several shoots each)	Agar sub., 7 d	0	Probably not susceptible.
Mar. 2,1907	.....do	12 salsify	Agar sub., 3 d	0	
Mar. 11,1907	.....do	1 olive	Agar sub	0	Same culture successful on peach.
Mar. 12,1907	.....do	5 olive	Agar sub., 2 d	0	Daisy inoculated from same culture gave knots.
Apr. 3,1907	.....do	18 rose	.....do	0	Daisy controls took the disease.
Apr. 11,1907	.....do	2 European grape	Agar sub. 5 d	0	One dead; other no growth.
Do	.....do	34 blackberry	Agar sub., 5 and 6 d	0	Many died; others made a moderate growth.
Apr. 12,1907					
Do	.....do	29 raspberry	Agar sub., 6 d	35?	Rejected because the checks also contracted the disease.
Apr. 13,1907	.....do	6 apple	Agar culture	50?	Rejected because some had hairy root when inoculated.
Apr. 17,1907	.....do	6 walnut	Agar sub., 5 d	0	Slow growth.
Do	.....do	5 gray poplar	.....do	0	Do.
Do	.....do	6 Lombardy poplar	.....do	0	Inoculated on roots.
Do	.....do	6 cottonwood	.....do	0	Slow growth.
June 9,1907	.....do	1 European grape	Agar sub., 4 d	0	Plant died.
Aug. 9,1907	.....do	11 common fig	Agar sub., 3 d	0	Probably not susceptible; produced galls on grape.
Dec. 19,1907	.....do	7 daisy	Agar sub., 2 d	0	Plants not growing.
Mar. 7,1908	.....do	3 common fig	.....do	0	
Do	.....do	3 cabbage	.....do	0	
Do	.....do	3 chestnut	.....do	0	
May 26,1908	.....do	1 Impatiens sultana (several places).	Agar sub., 4 d	0	
Do	.....do	5 scarlet clover	.....do	0	Dwarfed old plants growing very slowly.
Do	.....do	5 apple	.....do	0	
Do	.....do	3 Lombardy poplar	.....do	0	Inoculated in rapidly growing shoots.
Apr. 4,1910	.....do	11 onion	Young agar sub	0	Newest isolation.
Do	.....do	2 Impatiens sultana[b]	.....do	0	Culture actively pathogenic to daisy.
Apr. 6,1910	.....do	12 common fig	.....do	0	Recent isolation from daisy.
Apr. 7,1910	.....do	10 chestnut	.....do	0	Newest isolation.
Do	.....do	8 red oak	Agar sub	0	Shoot grew only 1 inch after inoculation.
Apr. 14,1908	Honeysuckle	8 daisy	Agar sub., 4 d	0	Possibly the wrong organism.
Nov. 2,1909	Arbutus	5 daisy	Agar sub., 5 d	0	Young growing plants.
May 7,1910	.....do	6 daisy	Young agar sub	0	
Nov. 11,1909	Cotton	9 sugar beet	Agar sub., 5 d	0	Young growing plants.
Nov. 16,1909	.....do	12 daisy	.....do	0	Plants in good condition.

[a] For additional failures see page 181.

[b] One (red flowered) 5 places; 1 (white flowered) 7 places.

TABLE III.—*Showing negative or doubtful results of inoculations*—Continued.

Date of the inoculation.	Origin of culture.	Number and kind of plants inoculated.	Kind of culture used and its age.	Per cent which developed tumors.	Remarks.
Apr 29,1910	Cotton	7 daisy	Agar sub., 2 d	0	
July 5,1910	do	6 sugar beet	Agar sub., 4 d	0	Plants grew very slowly.
Mar 27,1908	Grape	3 almond	Agar sub., 2 d	0	Suspect wrong organism.
Mar. 28,1908	do	16 daisy	Agar sub., 3 d	0	Do.
Aug. 31,1909	do	3 sugar beet	Agar sub., 4 d	0	Same cultures were partially successful on grape and daisy.
June 16,1909	Alfalfa	2 alfalfa (8 roots)	Agar col., 6 d	0	Dwarfed old plants.
Dec. 8,1909	do	Several old alfalfa	Young agar sub	0	Plants in poor condition.
Jan. 27,1910 / Feb. 1,1910	do	14 peach	Agar sub., 1 d	0	Trees were beginning to leaf.
Jan. 16,1908	Peach	6 apple	4th agar sub., 1 d	0	Trees dormant.
Jan. 23,1908	do	10 apple	5th agar sub., 2 d	50?	Rejected because checks also contracted disease.
Mar. 11,1908	do	2 daisy	Agar col., 7 d	0	Possibly wrong colonies used; apple took disease from other colonies on same plate.
Do	do	2 olive	do	0	
May 22,1908	do	35 apple	Agar sub., 3 d	(?)	Mixed with checks in planting?
Oct. 13,1908	do	2 walnut	do	0	Shoots hard when inoculated.
May 6,1909	do	4 peonia	Agar sub., 2 d	0	
May 18,1909	do	11 phlox	Agar sub., 4 d	0	
Do	do	10 verbena	do	0	
Jan. 14,1908	Rose	8 peach	Agar sub., 1 d	0	
Jan. 23,1908	do	6 apple	Agar sub., 2 d	0	Trees dormant.
Mar. 18,1908	do	6 rambler rose	do	0	
May 9,1909	do	25 peach	Agar sub., 3 d	0	Trees not in best condition.
July 9,1907	Raspberry	4 daisy	Colonies, 7 d	0	Possibly wrong organism used.
Feb. 26,1909	Quince	7 sugar beet	Agar sub., 3 d	0	Beets made slow growth.
May 14,1909	do	8 quince	Agar?	0	Material not suitable.
May 21,1909	do	3 quince	Agar sub., 2 d	0	
Mar. 9,1910	do	7 quince	do	0	
July 2,1910	do	16 sugar beet	Young agar sub	0	Beets made slow growth.
June 27,1910	Sugar beet	10 sugar beet	do	0	Probably of wrong organism; beets made slow growth.[a]
Apr. 17,1908	Hop	4 daisy	Agar sub., 2 d	0	
May 6,1909	do	3 peonia	Young agar sub	0	
May 9,1910	do	10 olive	do	0	Shoots growing very rapidly; many pricks. Infectious to beet.
May 24,1910	Old chestnut	5 sugar beets	do	0	
June 10,1910	New chestnut	5 sugar beets and 1 European grape.	do	0	
Jan. 23,1908	Apple (hard), Washington	10 apple	Agar sub., 2 d	0	All from 1 colony; probably wrong organism.
Feb. 24,1908	do	6 daisy	3d agar sub., 4 d	0	Probably wrong organism.

Do	do	3 apple	Agar sub., 4 d	0	Organism from same set of plates as preceding.
Nov. 9, 1908	Apple (hard), Iowa	2 pelargonium	Agar col., 5 d	0	Daisies contracted disease.
Nov. 12, 1908	do	4 apple	Agar col., 8 d	0	Trees growing very slowly; daisies gave galls.
Do	do	9 sugar beet	do	0	Plants nearly dormant.
Do	do	6 root tips of Monstera	do	0	
Dec. 4, 1908	Apple (hard)	Several plants of tomato	Agar sub., 2 d	0	Inoculated on well-developed stem roots.
June 24, 1910	do	10 pelargonium	do	0	
Do	Old apple	10 sugar beet	Young agar sub	0	See "Morphology;" probably an intruder.
Nov. 21, 1908	Apple, hairy-root	3 tomato (36 groups of punctures).	Agar sub., 3 d	0	Plants full grown; inoculated on stem roots.
Dec. 10, 1908	do	Tomato (several much younger plants).	do	0	
Apr. 1, 1909	do	10 tomato	Agar sub., 2 d	0	See text.

[a] For additional failures, see remarks p. 85.

CULTURAL CHARACTERS.

EXPLANATORY STATEMENT.

Such comparative studies as we have been able to make are included in the following tables and memoranda. Many of them were made or repeated in the spring and summer of 1910 during the preparation of this bulletin.

In offering these incomplete data it may be pointed out that probably some errors have slipped into these records, as the time was not sufficient for exhaustive tests of all the cultural characteristics of all these strains and for the elimination of all possible intruders through repeated poured plate separations and further inoculations. It is the more likely that some errors are included owing to the fact that in 1910 a number of our forms had ceased to be pathogenic, e. g., peach, chestnut, apple, quince; but whether this was due only to loss of a peculiar quality, or to the right organisms having been driven out of our cultures by unobserved intruders was not determined beyond all doubt, except that clearly the "old apple" appeared to be something entirely different from what we had on the start, and very probably the quince and the sugar beet.

To straighten out fully all the tangle of interrelations here touched upon would require so many additional months of work that it has appeared best to publish at once what we have, leaving the unsettled problems for further study.

GROWTH ON AGAR.

When these organisms were grown for 3 days at 23° to 25° C. upon slant +15 peptonized beef agar containing 1 per cent agar flour, and inoculated by needle stroke from 18-day-old slant agar cultures, there was in each case a well-developed shining white streak and some growth in the condensation water. The agar was not stained. Slight differences not easily definable were visible, the most pronounced of which were the following:

(1) White watery translucent streaks: Newest daisy, arbutus, new apple, apple hairy root, new chestnut, grape, alfalfa (the last showing transitions to 2). There were no crystals, or only a trace (new apple).

(2) Similar streaks but whiter, i. e., less translucent: Old daisy, peach, hop, old rose, new rose, beet, cotton. Numerous prismatic crystals, except in beet which had only a few.

(3) White shining flat growth, i. e., thinner than in the preceding and trace of crystals: Old apple. The growth of this when first isolated (2 years ago) was like No. 1, i. e., translucent watery. (See under *Morphology*, p. 129.)

(4) A thin white growth inclined to wrinkle, crystals few and large: Quince. This is the only culture showing any wrinkles.

This experiment was repeated in July, 1910, at a higher temperature (30° to 31° C.), inoculating from younger cultures (1 to 3 day agar), with less typical growth (in some cases colony wise) but nevertheless with essentially the same results. Under group 2 fewer with crystals. The old apple looks decidedly unlike the others and is probably an intruder.

GROWTH IN +15 BEEF BOUILLON WITH 1 PER CENT WITTE'S PEPTONE.

At the end of 3 days at 23° to 25° C., inoculating from slant agar cultures 18 days old, the appearances in test tubes containing 10 c. c. of the fluid were as follows:

(1) Incomplete easily fragmenting pellicle, fluid nearly clear, stringy threads on shaking: Arbutus, alfalfa, newest daisy.

(2) Cloudier and with a heavier pellicle but otherwise like 1: New rose, old rose, cotton, hop, old daisy (B), peach.

(3) Cloudy and more or less stringy but destitute of pellicle, some precipitate: New apple, old apple, apple hairy-root, beet.

(4) Cloudy with some flocks, but no pellicle or strings: Quince.[a]

This experiment was repeated in July, 1910, at 30° to 31° C., inoculating from 3-day-old bouillon cultures. The results at the end of 3 days were the same, except that now the newest daisy had no pellicle, and the old rose an easily fragmenting pellicle. The previously untried strains fell into the above-named groups as follows:

Group 1.—Newport poplar No. 1, salsify.
Group 2.—Turnip No. 1.
Group 3.—Grape, Newport poplar No. 2.
Group 4.—New chestnut, willow.

The parsnip did not resemble the others. It was heavily and uniformly clouded with a precipitate which rose in a swirl on shaking. There were no strings, rim, or pellicle.

CANE-SUGAR PEPTONE WATER.

When grown for 18 days in river water containing 2 per cent Witte's peptone and 2 per cent c. p. cane sugar the strains did not brown the fluid, but behaved as follows:

(1) Fluid clear and not much precipitate or flocculence, but a very copious thick white pellicle (0.5 to 1.5 cm. thick, mostly the latter): Alfalfa, turnip No. 1, Flats poplar, new rose (pellicle 0.5 cm.) cotton, hop, peach, old daisy, newest daisy.

[a] The old chestnut was discarded as contaminated, and the grape did not grow.

(2) Fluid feebly clouded, some strings and flocks, pellicle incomplete, thin, fragmenting, not much precipitate: Salsify, parsnip, arbutus, grape, apple hairy-root, new apple, Newport poplar No. 1.

(3) Thin cloudy, no rim, no pellicle, but a precipitate which makes the fluid cloudy on shaking: Beet.

(4) Trace of rim, no pellicle, no strings, moderate clouding, slight precipitate: Willow.

(5) Moderately cloudy, trace of rim, no pellicle, quite cloudy on shaking, but not much flocculence, and no strings: New chestnut.

(5a) Moderate white rim and very scanty pellicle, quite cloudy with much coarse flocculence on shaking, but no strings: Newport poplar No. 2.

(6) Fluid clear, no rim, no pellicle, no strings, or filaments; thinly clouded on shaking by a great number of fine pseudozoogloœæ: Old apple.

(7) Like 6, but clouds on shaking with a finer precipitate: Quince.

At the end of a month the tubes still fell into the old groups and none of the fluids were brown stained. When tested with neutral litmus paper the cultures gave the following reactions:

Group 1.—All strongly alkaline except alfalfa (slightly alkaline) and newest daisy (neutral or slightly acid).

Group 2.—Much less growth: New apple (slightly acid); apple hairy-root and arbutus (neutral); grape (alkaline); Newport poplar No. 1 (alkaline); parsnip (alkaline); salsify (neutral).

Group 3.—Beet (strongly alkaline).

Group 4.—Willow (strongly alkaline).

Group 5.—New chestnut (slightly alkaline).

Group 5a.—Newport poplar No. 2 (strongly alkaline).

Group 6.—Old apple (2 tubes, plainly acid).

Group 7.—Quince (2 tubes, alkaline).

Only groups 1 and 2 contained organisms of recently proved virulence. Subsequently the willow was proved to be pathogenic to willow.

MALTOSE PEPTONE WATER.

When grown for 3 months in river water containing 2 per cent Witte's peptone and 1 per cent maltose the strains behaved as follows:

(1) Fluid clear, unstained, slight stringy rim, moderate flocculent precipitate which clouds fluid on shaking: Newest daisy (neutral to litmus), alfalfa (strongly alkaline), arbutus (acid to litmus), apple hairy-root (strongly alkaline).

(2) Like 1, but fluid brownish and alkaline: Grape.

(3) Clear unstained fluid strongly alkaline to litmus, pellicle 0.5 cm. thick, slight precipitate: New rose.

(4) Fluid dried out one-third. Dense white slimy pellicle nearly filling the remainder of the fluid, i. e., 2 cm. deep, not much precipitate, fluid alkaline: Old daisy, hop.

(5) Exactly like the preceding except that fluid and pellicle are slightly brownish, fluid alkaline: Peach, cotton.

(6) Thinly clouded, no rim, or pellicle, fluid unstained, a moderate precipitate which shakes up in a coarse flocculence, which is most abundant in the beet: Old apple (acid to neutral litmus paper), beet (strongly alkaline), old chestnut (neutral to litmus).

(7) Fluid moderately cloudy and yellowish (alkaline to litmus), rim yellowish white, not stringy, no pellicle, moderate precipitate which shakes up in coarse flocculence: Quince.

TABLE IV.—*Showing behavior*[a] *of crown-gall organisms in peptonized beef bouillon of varying grades of alkalinity or acidity.*

[Inoculated from 3-day-old and 7-day-old peptonized bouillon cultures, except −25, which was from 19-day-old beef bouillon. Examined at end of 28 or 31 days, except −25, which was 9 days old. The +34 was acidulated with citric acid, the +38 with malic; the alkali was sodium hydroxide.]

Organism.	Titration (grade of alkalinity or acidity).		
	−34	−25	−24
Newest daisy	0, 0	0, 0, 0	3, 3, 3.
Old daisy	2	3, 3 *b*	3 *b*.
Peach	3		3 *b*
Hop	3	3 *b*	4 Ragged pellicle.
New rose	0	2 Thin pellicle	3 Good pellicle.
Old apple	0	0	0.
Apple hairy-root	0	0	0.
Alfalfa	0	1	0.
Grape	0	1 or 2	0.
Chestnut	0	3 Uniformly cloudy. Cont.?	3 Uniformly cloudy Cont.?
Arbutus	0	0	0.
Cotton	3	3 Thin pellicle	3.
Quince	2	2?	0.
Beet	0	2	2 or 3.

Organism.	Titration (grade of alkalinity or acidity).		
	−16	+34	+38
Newest daisy	2	3 Thin firm pellicle; clear fluid.	0.
Old daisy	3 *b*	5 *b*	4 *b* Clear fluid.
Peach	4 *b*	5 *b*	5 *b* Fluid cloudy.
Hop	4 Ragged pellicle	5 *b*	5 *b*.
New rose	3 Ragged pellicle	0	0.
Old apple	0	3 Uniformly cloudy. Cont.?	4 Rim and cloudy fluid.
Apple hairy-root	0	0	0.
Alfalfa	0	3 White rim with precip.	0.
Grape	0	3	2 Strings and feeble clouding.
Chestnut	3 Uniformly cloudy. Cont.?	0	0.
Arbutus	0	2 Thin white rim	0.
Cotton	4 Fragmenting pellicle	5 Fragmenting pellicle	5 Heavy pellicle, breaking on hard shaking.
Quince	0	0	0.
Beet	3 Strings, precipitate pale salmon.	0	0.

[a] Explanation of figures indicating growth: 0=no growth; 1=trace; 2=slight; 3=moderate; 4=good; 5=copious.

[b] Thick firm pellicle, not broken by shaking.

Some additional tests were made in 1910 in acid and alkaline peptonized beef bouillon with the results shown in Table V.

TABLE V.—*Showing behavior* [a] *of crown-gall organisms in bouillons of varying reaction, the records being taken some weeks after inoculation.*

[The alkali was sodium hydrate.]

Organism. (The first four strains are of most recently established virulence.)	Titration (grade of alkalinity or acidity).				
	−29	−25	−23	+36 (citric acid).	+34 (malic acid).
Flats poplar	0	4	2	4	4
Hop	4	4	.....	.....	.....
Grape	0	0	2	.....	.....
Peach	4	4	.....	.....	.....
Apple hairy-root	.....	.....	0	0	0
Old apple	.....	.....	4	.....	.....
Chestnut	.....	.....	0	4	4
Alfalfa	.....	.....	4	4	4
Quince	.....	.....	2 or 0	0	0
Beet	.....	.....	4	0	0
Arbutus	.....	.....	2 or 0	0	0
New rose	.....	.....	.....	0	0

[a] Explanation of figures indicating growth: 0=no growth; 2=slight; 4=good, with pellicle.

TABLE VI.—*Showing behavior* [a] *of crown-gall organisms in +15 bouillon containing sodium chloride at room temperatures.*

[Transfers from 5-day-old bouillon cultures.]

Organism.	Time (days).		
	4.	15.	31.
Daisy: [b]			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Peach:			
3.0 per cent	2	3	3
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Hop:			
3.0 per cent	2	5	5
3.5 per cent	2	2	2
4.0 per cent	0	0	0
Rose:			
3.0 per cent	2	5	5
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Apple:			
Old strain—			
3.0 per cent	3	5	5
3.5 per cent	4	5	5
4.0 per cent	2	5	5
New strain—			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Apple hairy-root:			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Alfalfa:			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Grape:			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
New chesnut:			
3.0 per cent	.....	0	.....
3.5 per cent	.....	0	.....
4.0 per cent	.....	0	.....
Old chestnut:			
3.0 per cent	0	0	2
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Arbutus:			
3.0 per cent	0	.....	0
3.5 per cent	0	.....	0
4.0 per cent	0	.....	0
Cotton: [c]			
3.0 per cent	2	5	5
3.5 per cent	1	1	1
4.0 per cent	0	0	0
Beet:			
3.0 per cent	3	.....	5
3.5 per cent	3	.....	4
4.0 per cent	2	.....	4
Quince:			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Flats, poplar:			
3.0 per cent	.....	0	.....
3.5 per cent	.....	0	.....
4.0 per cent	.....	.....	.....

[a] Explanation of figures indicating growth: 0=no growth; 1=very slight; 2=slight; 3=fairly good. 4=good; 5=heavy.

[b] In a repetition of daisy there was moderate growth at end of six weeks in 3 and 3.5 per cent.

[c] In a repetition of cotton there was no growth at end of three days and at end of fifteen days; at end of forty days the growth was 3, 3, and 0, respectively, for 3, 3.5, and 4 per cent.

TABLE VII.—*Showing behavior[a] of crown-gall organisms in Cohn's solution at room temperature (20° to 30° C.).*

[Transfers from 4-day-old cultures.]

Organism.	Date of examination.						Remarks.
	3 days.	1 week.	13 days.	2 weeks.	24 days.	6 months.	
	Growth.	Growth.	Growth.	Growth.	Growth.	Growth.	
Daisy	0	0	.....	0	.....	.....	
Peach	0	2	.....	2	.....	4	Cloudy; numerous flocks.
Hop	0	2	.....	[b]3	.....	4	Faint clouding; stringy precipitate.
Rose	0	2	.....	2	.....	4	Many flaky strings.
Apple	0	0	.....	0	.....	0?	No clouding; some small fine flocks.
Apple hairy-root	0	1	.....	3	.....	4	Milky cloudy; membranous precipitate visible on shaking.
Alfalfa	0	1	.....	1	.....	3	Cloudy; some flocks.
Grape	0	1	.....	1	.....	4	Cloudy flocculent; clumps of branched crystals.
New chestnut	.....	.....	(c)	.....	.....	.....	
Old chestnut	0	2	.....	2	.....	3	Milky cloudy; no flocks or precipitate.
Arbutus	0	0	.....	0	0	.....	
Beet	0	0	.....	0	.....	.....	
Cotton	0	0	.....	0	0	.....	
Quince	0	2	.....	2	.....	5	Yellowish stain.
Flats poplar	.....	.....	[d]0	.....	.....	.....	

[a] Explanation of figures indicating growth: 0=no growth; 1=very slight; 2=slight; 3=fairly good; 4=good; 5=heavy.
[b] Stringy growth.
[c] One tube milky cloudy; one tube clear.
[d] Two tubes, no growth; the same stock grew in nitrate bouillon.

TABLE VIII.—*Showing behavior* [a] *of crown-gall organisms on starch jelly at room temperature.*

[Transfers from 5-day-old agar cultures.]

Organism.	First test.			Repetition.			
	3 days.	11 days.		8 days.		34 days.	
	Growth.	Growth.	Remarks.	Growth.	Remarks.	Growth.	Remarks.
Daisy:							
Old strain	5	5	Color unchanged	3	(*b*)	3	(*c*)
Newest strain				1	(*d*)	1	(*e*)
Peach:							
Old strain	5	5	Light brown	3	(*b*)	3	(*c*)
Another strain	5	5	Color unchanged				
Hop	5	5	Medium gray	3	(*f*)	3	No diastasic action; no brown stain.
Rose:							
Old strain	5	5	Medium light brown.	3	(*b*)	3	(*c*)
New strain				3	(*f*)	3	No diastasic action; no brown stain.
Apple:							
Old strain	1	3	Color unchanged	1	(*d*)	1	(*e*)
New strain	1	3	do	1	(*d*)	1	(*e*)
Apple hairy-root	1	3	do	1	(*d*)	1	(*e*)
Alfalfa	3	3	do	1	(*d*)	1	(*e*)
Grape	3	4	do	1	(*d*)	1	(*e*)
New chestnut	1	3	do	3	Same as 34 days.	3	No diastasic action; no brown stain.
Arbutus				1	(*d*)	1	(*e*)
Beet				5	(*g*)		
Cotton	5	5	Light brown	3	(*b*)	3	(*c*)
Quince	1	5	Color unchanged; liquefaction.	3	(*f*)	3	No diastasic action; no brown stain.
Flats poplar						[b] 3	Brownest of all.[h]

[a] Explanation of figures indicating growth: 1=scant; 3=moderate; 4=good; 5=copious.

[b] Diastasic action feeble or absent. A brownish stain mixed with the white. In cotton the original streak is brownish, but the young growth pushing out to either side of the old is white. In old rose the base of streak is white, upper three-fourths is brownish, but beyond the brown the new growth is white The margin of old daisy streak is also whitish.

[c] Old daisy, old rose, peach, and cotton look alike—decided browning of the slime in each case, and a paler (brown) staining of the body of the jelly. No diastasic action and not a very copious growth. The margin of streak in cotton and base in old rose, which were white at end of 8 days are now brownish, while the older portions have become dark brown.

[d] No indication of any diastasic action. Purest white growth is that shown by alfalfa.

[e] No diastasic action; only slight increase in growth. A little increase in color toward cream, and in grape toward brownish.

[f] Diastasic action absent or scanty. More color in slime which approximates a pale cream.

[g] Abundant salmon-colored growth which has run down and filled the V. On plating out a pinkish intruder was discovered.

[h] In Flats poplar, recently tested on sugar beet and found to be virulent, there was at the end of 30 days a decided brownish stain throughout the medium (Ridgway's drab to drab gray).

TABLE IX.—*Showing indol reaction of crown-gall organisms [a] in two media at room temperature.*

[Transfers from 5-day-old bouillon cultures.]

Organism.	Uschinsky's solution + Witte's peptone.		2 per cent Witte's peptone in water.		
	3 days.	10 days (no change on heating).	24 hours.	26 days. [b]	33 days.
Daisy:					
Newest	0	0	0	Distinct	
Old strain				Feeble	
Peach:					
Strain 1	0	0	0	Distinct	
Strain 2	0				
Hop	0	0	0	Feeble	
Rose:					
Old strain	0	0	0		
New strain				Feeble	
Apple	0	0	0	0	
Apple hairy-root:					
Old strain	0	Faint pink	0	(c)	
New strain	0				
Alfalfa	0	0	0	Feeble	
Grape	0	Faint pink	0	Distinct; good as daisy.	
Chestnut:					
New					Moderate on heating.
Old	0	0	0	0	
Arbutus			0	Feeble	
Beet			0	(?)	
Cotton	0	Faint pink	0	Distinct	
Quince	0	0	0	0	
Flats poplar					Distinct.

[a] Tested by adding to each tube 1 c. c. of 1 : 200 sodium nitrite and 10 drops of sulphuric acid.
[b] Positive reaction only after heating to 80° C., except daisy and trace in peach and Flats poplar.
[c] No growth.

Owing to Brizi's statements respecting the rapid production of indol by his *Bacillus populi* additional tests for indol were made in river water containing 2 per cent Witte's peptone, using Flats poplar, the virulence of which had been recently established:

(1) Tube copiously inoculated and incubated 24 hours at 30° C., which gave an unusually heavy growth. Result: No trace of color on adding reagents, and none, or merest trace, on heating for five minutes at 80° C.

(2) On July 26, 1910, the above experiment was repeated. Several tubes were inoculated copiously and incubated at 30° C., the growth being prompt and typical. They were tested as follows, using for each tube 1 c. c. of sodium nitrite water (1 : 200) and 10 drops of sulphuric acid.

July 27 (28 hours): Considerable growth but no trace of red color at first, nor after some minutes, nor on heating at 80° C. for 4 minutes.

July 28 (50 hours): Growth has increased. No trace of red color cold, and none on heating for 5 minutes at 80° C.

July 29 (74 hours): Good growth continues. No red color on adding reagents cold. A trace (?) on heating at 80° C.

This experiment was repeated in October, 1910, with the same result: In 48 hours, no reaction either cold or on heating, growth good in form of shreds and strings; 83 hours, no reaction cold; on heating 5 minutes at 80° C., doubtful or possibly a trace of pink. The test was made in both 1 and 2 per cent peptone water.

REDUCTION OF NITRATES.

PRELIMINARY STATEMENT.

As already pointed out, the daisy organism does not reduce nitrates.

It is probable also that none of the others reduce nitrates, in the ordinary meaning of the term, but some doubt still exists.

Early tests by the junior writer having led to contradictory results (in some instances), further experiments were made in 1910, but not enough wholly to clear up the situation. The following is a summary of all our experiments:

DAISY.

(1) *Several old tests by Doctor Smith*—all negative.

Old tests by Miss Brown.—(2) 13 days, negative; (3) 5 days, both old and newest strains, negative; (4) 33 days, negative.

Tests in 1910 by Doctor Smith.—(5) 69-day-old cultures by Miss Brown in her nitrate bouillon No. 600. Old daisy, 2 tubes (strongly alkaline to litmus): Trace of blue in bottom which disappears on shaking; reagents pure, i. e., tested. Newest daisy: Trace of blue in bottom which shakes out. The checks also give a purple reaction in bottom of tube but not if the starch is withheld. The starch was then suspected and retested, but on adding KI water and H_2SO_4 to 20 c. c. of the boiled starch no blue reaction whatever was obtained.

PEACH.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, slight reduction; (3) 33 days, reduced.

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her bouillon No. 600 (culture strongly alkaline to litmus), copious reduction; does not shake out; check tubes give some blue reaction at bottom which goes on shaking; reagents pure. (5) Plates poured and portions of 8 colonies transferred to 8 tubes of bouillon; strong growth. Tested after 7 days, all negative, i. e., 4 failed, and 4 showed traces of blue at bottom in the bacterial precipitate, which color disappeared on shaking. Check tubes showed no nitrite present. The cultures were then tested for nitrate (H_2SO_4 and diphenylamine) with customary blue reaction. (6) Sec-

ond set of plates poured and portions of 11 typical-looking colonies transferred to as many tubes of the bouillon. Tubes tested on third day, when well clouded, all negative. These tubes foamed on shaking; the check tube did not. Before the reagents were added, the fluid in the check tube was neutral (or nearly so) to litmus, and that in the inoculated tubes strongly alkaline.

HOP.

Old tests by Miss Brown.—(1) 13 days, negative; (2) 5 days, reduced; (3) 33 days, trace of blue which disappeared on shaking.

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her nitrate bouillon No. 600 (fluid strongly alkaline to litmus). On adding the reagents, a trace of blue in bottom, which shakes out.[a]

ROSE.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, reduced (both new and old strains); (3) 33 days, reduced (both strains).

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her nitrate bouillon No. 600: New rose—Copious reduction; does not shake out. Old rose (strongly alkaline to litmus)—Copious blue reaction, does not shake out; reagents tested and found pure. (5) New rose—Plates poured and portions of 10 colonies transferred to as many tubes, also 3 tubes inoculated from mixed colonies; tubes tested when 5 days old and fluid well clouded; one check tube, negative; of 10 tubes, 7 gas forming, 3 non-gas forming; the latter did not reduce; the former showed trace of blue on pellicle where acid fell; of the 3 tubes from mixed colonies, 1 reduced, 2 did not.[b] (6) Old rose—Plates poured and portions of 8 colonies transferred to as many tubes of bouillon; two checks held: tests after a week; fluid heavily clouded and contamination suspected. Result—Checks negative; of the inoculated tubes, 4 show a trace of blue on the bottom, which disappears on shaking, 2 are heavily blued throughout, and 2 are inter-

[a] A fresh isolation from hop made in 1910, and pathogenic to daisy and sugar beet, was tested as follows for nitrate reduction:

Five tubes at end of 7 days' growth (colony 1), negative. Two tubes of same lot at end of 27 days gave a very strong reduction. Contamination was then suspected and poured plates were made. At the end of 3 days 12 colonies were transferred to nitrate bouillon. These cultures were tested at the end of 10 days, when the bouillon was well clouded. Ten gave no reduction, 1 gave a slight color which shook out, 1 reduced moderately, color persisting. Transfers from these 12 tubes were made to plain bouillon before testing, and from these bouillon cultures other 12 tubes of nitrate bouillon were inoculated on January 27 and tested at end of 14 days, when all were moderately clouded and bore a heavy pellicle which fragmented easily on shaking. The result on adding 10 drops of boiled starch water, 1 c. c. of 1 : 250 fresh potassium iodide water, and 5 drops of 2 : 1 sulphuric acid water were as follows: Two tubes, no reduction; 10 tubes, blue reaction in precipitate (fallen pellicle), in two of these there was also a trace of blue at the top of the fluid. The mass of the fluid was entirely free from blue color, and all of the color disappeared on shaking.

[b] Growth on agar slant August 3, 1910, looked wrong, l. c., it was pinkish white. Examined after 19 days.

mediate, i. e., one blue at bottom, shaking out, the other remaining pale blue after shaking. (7) Old rose—Second set of plates poured and portions of 11 colonies transferred to as many tubes; cultures tested on third day, when well clouded. Result—Check negative; 11 inoculated tubes all blue at bottom in bacterial precipitate; 1 tube remained pale blue after shaking; the other 10 became colorless; on shaking, the inoculated tubes became half filled with foam.

Conclusion: New rose, contaminated; old rose, doubtful.

APPLE.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, old apple, reduced; new apple, negative, possibly no growth; (3) 33 days, old apple, reduced; new apple, trace (?), gave scarcely any growth.

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her bouillon No. 600 (fluid strongly alkaline), copious blue reaction which does not shake out; the new apple gave no growth.

APPLE HAIRY-ROOT.

Old tests by Miss Brown.—(1) 13 days, negative; (2) 5 days, reduced; (3) 33 days, trace(?), scarcely any growth.

Tests in 1910 by Doctor Smith.—(4) Plates poured and portions of 12 colonies transferred to as many tubes of the bouillon. The tests were made at the end of 13 days. One tube is cloudy with a moderate rim and precipitate, but the others show scarcely any growth. Result: All negative; no reduction.

ALFALFA.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, reduced; (3) 33 days, trace of blue which disappears on shaking.

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her bouillon No. 600—blue in bottom on adding the reagents (color shakes out). (5) Plates poured and portions of 12 colonies transferred to as many tubes of the bouillon; cultures tested when 5 days old and well clouded; check, negative; 12 cultures, all negative.

GRAPE.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, negative; (3) 33 days, negative.

Tests by Doctor Smith in 1910.—(4) 69-day-old culture by Miss Brown in her nitrate bouillon No. 600, moderate growth, negative.

OLD CHESTNUT.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, negative; (3) 33 days, negative.

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her bouillon No. 600, negative; growth abundant.

ARBUTUS.

Old tests by Miss Brown.—(1) 5 days, reduced; (2) 33 days, trace of color, scarcely any growth.

Tests by Doctor Smith in 1910.—(3) Plates poured and portions of 9 colonies transferred to as many tubes of the nitrate bouillon; tested on twelfth day, when all had grown; fluid more or less stringy and not much clouded, rim and pellicle fallen; all negative. The presence of nitrate in each tube was then determined by obtaining the evanescent blue reaction with diphenylamine dissolved in sulphuric acid.

BEET.

Old tests by Miss Brown.—(1) 5 days, negative; (2) 33 days, reduced.

Tests in 1910 by Doctor Smith.—(3) 69-day-old culture by Miss Brown in her nitrate bouillon No. 600 (fluid strongly alkaline to litmus); on adding the nitrite reagents a copious blue, which does not shake out. (4) Plates poured and portions of 12 colonies transferred to the bouillon; tubes tested on the thirteenth day, when there were no strings or pellicle, but a white rim and a moderate amount of pinkish white precipitate; check and 6 inoculated tubes, negative; 6 tubes blue at bottom over the thick bacterial precipitate, but color shaking out of all.

COTTON.

Old tests by Miss Brown.—(1) 5 days, reduced; (2) 33 days, reduced.

Tests in 1910 by Doctor Smith.—(3) 69-day-old culture by Miss Brown in her nitrate bouillon No. 600 (fluid strongly alkaline)—copious blue color which does not shake out. (4) Plates poured and portions of 12 colonies transferred to as many tubes of nitrate bouillon. The tests were made at the end of 5 days when a good growth had taken place. Eleven tubes, negative. One shows a blue color at the bottom, but this shakes out. Presence of nitrate in these tubes was then determined by the diphenylamine sulphuric-acid test.

QUINCE.

Old tests by Miss Brown.—(1) 13 days, negative; (2) 5 days, reduced; (3) 33 days, trace of reduction, scarcely any growth.

POPLAR.

Tests in 1910 by Doctor Smith.—On July 25 three transfers were made from 3 subcultures to tubes of nitrate bouillon. The tests were made on the eighth day when there was a nearly clear fluid, a white pellicle covering the whole surface and not much precipitate, but some flocks and strings on one side next wall of tube. Result: Check and 3 cultures—all negative. Nitrate present in each tube. This was the organism called Flats poplar.

REMARKS.

A few of these contradictory results are to be explained on the hypothesis of contaminating organisms. When the blue color was merely local and shook out readily, the phenomenon would seem to be different in something more than degree from that ordinarily encountered. Whether nitrate reductions by bacteria are all alike and only a matter of degree, or whether there are two or more distinct mechanisms of reduction, is still an open question. Possibly one form is due to the direct action of sulphur compounds, while another depends on the activity of some enzyme.

The 1910 tests were made with 1 c. c. of opalescent boiled starch water (distilled water and starch prepared from potato in the laboratory); 1 c. c. of 1:250 fresh potassium iodide water, and 6 drops of 1:2 c. p. sulphuric-acid water. The checks were tested, the reagents were tested, and when results were negative the bouillon was also tested to see if nitrate was actually present. Frequently litmus tests were made and all of the cultures may be assumed to have been alkaline to litmus.

GROWTH IN BOUILLON OVER CHLOROFORM.

Inoculations from a 14-day peptone bouillon culture, examinations on the twenty-third day. All grew readily, except quince. Least growth in case of beet, chestnut, and newest daisy. To each tube containing 10 c. c. of +15 peptonized beef bouillon was added 5 c. c. of chloroform. The tubes were not agitated.

INVERSION OF CANE SUGAR.

The organisms were grown for 16 days in filtered river water containing 2 per cent Witte's peptone and 2 per cent c. p. cane sugar. On boiling with Fehling's solution (50 c. c. distilled water, 5 c. c. alkaline solution, and 5 c. c. $CuSO_4$ solution) the cultures fell into 3 groups as follows:

(1) Negative: Check tubes, sugar beet, new chestnut.

(2) Slight to moderate reduction. Apple hairy-root, new apple (slight), peach, grape.

(3) Copious reduction: Newest daisy, old daisy, new rose, hop, arbutus, alfalfa, Flats poplar, turnip.

All the organisms about which we felt any etiological certainty fell into class 2 or class 3.

TABLE X.—*Showing behavior*[a] *of crown-gall organisms in river water containing other stated ingredients, at room temperature.*

[Transfers from +15 peptone bouillon cultures.]

Organisms.	With 1 per cent Merk's asparagin added.		With 0.5 per cent each dextrose and glycocoll added (3-day-old culture).		With 0.5 per cent each dextrose and urea.		
					Inoculated Apr. 23 (3-day beef bouillon).		After 73 days (inoculated Mar. 5, 2-day beef bouillon).
	After 73 days (inoculated Mar. 5, 1910, 2-day-old culture).	Experiment repeated May 18, inoculating more copiously, (examined 43d day).	After 24 days.	After 2 months.[b]	After 24 days.	After 69 days.	
Daisy:							
Old strain	4		2	2	3	3	0
Newest	2. Thin fallen pellicle; does not break up easily.		2	2	2	2 (?)	[c] 2
Peach	4 or 5		2	2	3	3	4
Hop	4 to 5		0 (?)	2	0 (?)	0	0
Rose:							
Old	4 or 5						[d] 4
New	0	4	2	2	0 (?)	2 (?)	4
Apple:							
Old	0	0	0	0	0 (?)	0	0
New	3						0
Apple hairy-root	2		0	0	0	0	0
Alfalfa	0	2	0	0	2	2	0 (?)
Grape	0	3	0	0	2	2 (?)	2
Old chestnut	0		0 (?)	0	0	0	0 (?)
Arbutus	0	2	2	2		[e] 0	0
Beet	3		0	0	2 (ol·l)	2 (old)	0 (old)
Cotton	4		0	0	[g] 0 (?)	[f] 4	4
Quince	0	0	[h] 0 (?)	0	0 (?)	0	0

[a] Explanation of figures indicating growth: 0 = No growth; 2 = slight; 3 = fairly good; 4 = good; 5 = heavy.
[b] Possibly no use of glycocoll by any of the strains.
[c] No increase in an additional 69 days.
[d] Turbid; cloudy on shaking.
[e] Forty-four days.
[f] Forty-four days, another tube more copiously inoculated.
[g] No growth later.
[h] Plug wet.

TABLE XI.—*Showing behavior of crown-gall organisms in river water containing 2 per cent Witte's peptone and 1 per cent Schering's c. p. glycerine.*

[Transfers from peptone bouillon culture 3 days old; records made at end of 27 days at 23° C.]

Group.	Organism.	Character of growth.
1	Old daisy*, peach*, hop†, cotton*, new rose*.	Heavy white rim and dense pellicle; scant clouding of fluid; moderate precipitate. On shaking, a dense mass of coarse fragments fills the fluid; 10 to 20 times as much growth as in group 2.
2	Newest daisy*, apple hairy-root*, alfalfa†.	Moderate white rim and thin pellicle; slight clouding; some precipitate.
3	Arbutus*, grape*, old apple, quince, beet (chestnut did not grow).	Scant white rim, thin pelliele or absent; fluid moderately cloudy; some precipitate; about same amount of growth as in group 2, but distributed differently.

Tubes were now tested for indol. Those marked with a star (*) gave distinct indol reaction without heating; those with a dagger (†), on heating. The others were negative. (See Table IX, p. 147.) The indol reactions were not as deep red as in case of *Bacillus coli*.

EXPERIMENTS WITH LITMUS-MILK CULTURES.

AUGUST 2–3, 1910.

Experiments to ascertain the behavior of the various strains of crown-gall organisms in milk gave the results shown in Table XII:

TABLE XII.—*Showing reactions of crown-gall organisms in sterile lavender-blue litmus milk at 30° C.*

Strain.	Time.	
	3 days.	7 days.
Newest daisy	Bluer than check; no whey	Much bluer; no whey; strong pellicle.
Old daisy	do	Litmus now dulled to a uniform plumbeous; no whey.
Salsify	do	Much bluer; no whey.
Flats poplar	do	Uniformly much bluer; no whey; strong pellicle.
Old chestnut	Unchanged	Uniformly bluer than check; no whey.
Turnip No. 2	Bluer than check; no whey	Much bluer; no whey.
Arbutus	Pinkish at top; paler blue below; no whey.	Rose purple at top; mauve below; not coagulated; no whey.
Cotton	Bluer than check; no whey	Uniform color verging on plumbeous; no whey; pellicle.
Hop	do	Uniform dull blue, tending toward plumbeous; no whey; strong pellicle.
Apple hairy-root	Unchanged	Unchanged or nearly so.
Newest rose	Paler blue than check; no whey.	Uniform blue, verging to plumbeous; no whey; heavy pellicle.
Quince	1 cm. purplish whey; dulled purple below; curd dissolving.	Litmus color gone; whey translucent; curd digested (nearly). The only trace of color is narrow pinkish rim.
Old rose	Bluer than check; no whey	Uniform color, verging on plumbeous; no whey; strong pellicle.
Alfalfa	Bluer than check; trace of whey on top.	Uniform deep blue; no whey; heavy pellicle.
Beet	Bluer than check; no whey	Much bluer; no whey.
Peach	do	Uniform plumbeous; no whey; strong pellicle.
Old apple	Purple red throughout and whey separated (this also on second day).	Whey nearly colorless; pale pinkish firm curd at bottom.
Willow	Bluer than check; no whey	Much bluer; no whey.
Grape	do	Do.
Turnip No. 1	do	Uniform dull blue; no whey.
Newport poplar No. 1.	do	Much bluer; no whey.
New chestnut	Unchanged; i. e., like check	Slightly bluer than check; no whey.
Parsnip	Bluer than check; no whey	Much bluer; no whey.
Newport poplar No. 2.	Slightly bluer than check; no whey.	Nearly color of check; red rim.

AUGUST 13, 1910.

The litmus-milk cultures now fall into three distinct groups, as follows (temperature 28° to 30° C.):

(*a*) *Litmus a uniform gray;* milk fluid, no separation of whey, moderate rim, heavy white pellicle, moderate white precipitate: Flats poplar, peach, old rose, cotton, hop, old daisy, turnip No. 1.

(*b*) *Litmus distinctly reddened,* most at top: Old apple, quince, arbutus, Newport poplar No. 2.

In the first two the casein has been thrown down, the bulk of the fluid being whey which, together with the clot, is now nearly colorless, i. e., only pinkish, with more evidence of solution of the curd in quince than in old apple. In the other two the milk is still fluid, i. e., no separation into curd and whey, and not yet much reduction.

(*c*) *Milk decidedly bluer than check, no reduction;* in some a slight amount of whey on top of the fluid milk, in others no separation: Alfalfa, old chestnut, newest daisy, beet, parsnip, newest rose, new chestnut, grape, willow, turnip No. 2, salsify, Newport poplar No. 1. This group might be split again as follows:

(*a'*) Narrow pinkish rims and moderate pellicle over a uniformly deep blue fluid, and a white precipitate: Old chestnut, new chestnut, parsnip.

(*b'*) The same, but with a pinkish-yellow precipitate and no pellicle: Beet.

(*c'*) Heavy whitish pellicle, moderate white precipitate, uniformly dulled blue fluid: New rose.

(*d'*) Very wide white rim (1 cm.), heavy pellicle, and scanty white precipitate: Alfalfa.

(*e'*) Uniform deep blue with blue rims: Newest daisy, grape, salsify, willow, Newport poplar No. 1, turnip No. 2.

August 19, 1910.

Group *a*.—No change except in the color of the milk, which is now a muddy tan color, with the exception of Flats poplar, which is still gray.

Group *b*.—No change.

Group *c*.—(*a'*) Parsnip—no change; old and new chestnut—barely a trace of pink in the rims; no other change. (*b'*) Beet—no change. (*c'*) New rose—no change. (*d'*) Alfalfa—no change. (*e'*) No change.

August 26, 1910.

Group *a*.—All (including Flats poplar—see August 19, above) are a dirty brown (approximately broccoli brown, Ridgway). Milk fluid, no separation of whey.

Group *b*.—Quince: clot is being dissolved. Only slightest trace of color except in the rim, which is pink. Old apple—no change. Newport poplar No. 2—casein is being thrown down; very little reduction. Arbutus—milk coagulated, no separation of whey; no reduction.

Group *c*.—(*a'*) Parsnip: no change (milk fluid, bluer than check; no separation of whey). New chestnut—no pink in the rim, otherwise unchanged. Milk fluid, bluer than check; no separation of whey. Old chestnut—milk fluid, no separation of whey; but reduction is taking place. Milk approximately Ridgway's lavender gray.

(*b'*) Beet—milk fluid, deep blue; no separation of whey. Pinkish yellow rim and precipitate.

(*c'*) New rose—like old chestnut in color, but slightly bluer; otherwise as on August 13.

(*d'*) Alfalfa—no change except that milk is paler blue than on August 13 and 19.

(*e'*) Salsify and grape have white pellicles and are much paler blue than the others in this group (reduction probably taking place slowly); milk fluid, no separation of whey. No change in the others (all deep blue) except that newest daisy has a pellicle (whitish). Milk fluid in all, and no separation of whey. Apple hairy-root is exactly like check. Probably no growth.

The Flats poplar was tested in milk with results very different from those Brizi obtained with his *Bacillus populi:* Three tubes of sterile white milk were inoculated copiously on July 28, 1910, and kept at 28° to 35° C. In 28 hours there was no visible change. In 4 days no separation of whey or change in appearance of the milk. In 9 days a copious white bacterial pellicle, but still no separation of the whey or curdling of the milk.[a]

SILICATE JELLY.

The behavior of all the crown-gall organisms on silicate jelly was much alike (one test only). There was a feeble to moderate white growth divisible into two groups about as follows:

(1) Smooth surface, growth rather scanty: Old daisy, newest daisy, grape (? growth), new chestnut, arbutus, old apple, new apple, apple hairy-root, beet, quince.

(2) Surface papillate-rugose but smooth on the margins, growth more abundant: Cotton, alfalfa, old rose, Turnip No. 1, hop, new rose, Flats poplar.

INOCULABLE AND CROSS-INOCULABLE.

Whether the different behavior of galls on various individuals of different hosts, sometimes forming soft, rapidly developing spongy excrescences and sometimes hard, slow-growing, slightly elevated tumors, or abnormal clusters of roots, as for example in the apple, is due principally to individual differences in rate of growth or juiciness of the particular tissues involved, to the particular tissue first infected, or to some other cause, must be left an unsettled question. The writers are inclined to think that there are several races of the gall-forming organisms varying more or less in amount of virulence and in adaptability to various hosts. Starting from soft gall of the peach, hard gall was produced on apple; and in the

[a] On potato cylinders inoculated at the same time and from the same culture, the color of the slime at the end of 28 hours was white like that of the potato substratum. In 4 days the slime was thin and dirty white. In 9 days the bacterial layer, which had not increased much, was a dirty white, and the fluid slightly brownish. The slime was not yellow and there was no marked action on the starch. At the end of a month when tested with alcohol iodine the potato cylinder gave a strong starch reaction, but the color was purple instead of deep blue (check).

same way from soft gall of daisy the hardest of hard gall on daisy. But inoculating with an organism plated from hard gall of the apple into actively growing soft daisy stem a series of galls were produced more resembling the original hard gall of the apple from which the colonies came than any typical soft gall of the daisy (Pl. III). On the other hand, as already detailed, starting with an organism from apple hairy-root and inoculating into young apple tree roots one developed galls while the others developed hairy-root. From one of the galls, however, on the tree which developed only galls, an organism was plated out which looked typical for what was inserted, and this when inoculated into healthy sugar beet produced both galls and hairy roots, indicating that crown-gall and hairy-root are only two forms of the same disease. Clustered roots also formed on one gall on Brassica. This hypothesis is further borne out both by the fact that the hairy-root clusters often originate in slow-growing hard galls and by the observation that rootlets frequently appear on peach galls in early stages of their development, but do not persist. The same phenomenon, transitory for the most part, occurs frequently or occasionally in some other galls, i. e., daisy, grape, clover, alfalfa.

Attempts at cross-inoculation have shown numerous differences (Tables II and III) the explanation of many of which must be sought in further experiments. Strains taken from some hosts, e. g., daisy, peach, hop, were inoculated into other plants with great ease. The strain obtained from the rose was inoculated into other plants with difficulty, but inasmuch as tumors were not readily produced on the rose itself by such inoculations it may be only that we were unfortunate in the selection of our rose bushes or of the colonies for our subcultures, getting slightly virulent strains. It is certain from our experiments on the daisy that a virulent strain may gradually lose its power to infect when kept for several years under laboratory conditions, and it is very probable that in nature some strains are feebly infectious and others actively infectious.

But we know in case of certain bacterial organisms cultivated in the laboratory that lessened virulence can be restored by certain procedures and we are not warranted in assuming that such restoration may not also take place in the fields.

DISCUSSION OF QUESTION OF SPECIES, VARIETIES, AND RACES OF THE CROWN-GALL ORGANISM.

Have we to do with one species or several? The answer is not at hand. Indeed, to those who have read thus far, it must be evident that much further time will be required to decide positively whether it is best to regard all crown-galls as due to variations of one polymorphous species, or whether they should be separated into two or more

species. Certainly there are not as many species as there are host plants, and the ease with which in many cases cross-inoculations take place points rather to one collective species. The monotonous morphology also points in the same direction, but the evidence is not all in. In this connection the reader is advised to make comparisons with the literature on root tubercles of Leguminosae.

The differences we have observed may be noted by consulting the tables and these seem to be real differences, e. g., slight differences in color or amount or texture of growth, ability or inability to grow in Cohn's solution, reactions in litmus milk, toleration of acids, etc. The difficulty is we do not know exactly what these things mean in the microbian economy, nor what weight to give them as differential characters. All told, the points of resemblance or agreement so far as we have studied the subject are much more numerous and salient than the differences, and for the present at least we prefer to leave the group undivided, merely indicating the various cultures for purposes of convenience by the name of the plant from which derived, as daisy, peach, poplar, etc.

Certain very practical questions arise here, viz, would it be advisable in nursery and orchard practice to follow one galled crop by another crop subject to galls, e. g., peaches by apples or pears, raspberries by grapes or quinces, or roses by almonds? Admitting frankly that we do not yet know the extent of artificial cross-inoculability, much less that of natural cross-inoculability, and that many more observations and experiments need to be made before we can be quite certain in particular cases to what extent the galls are naturally cross-inoculable, the grower who reads carefully the evidence detailed in this bulletin will probably hesitate to take the risk.

LOCAL REACTION OF THE INOCULATED PLANT.

YOUNG VERSUS OLD TISSUES.

In general old and hard, slow-growing tissues are not favorable to the development of this disease, whatever the host species concerned. Inoculations into such tissues frequently failed. Inoculations into dormant tissues usually failed, even though such tissues began to grow in the course of a few weeks. Mature tissues are not suited for inoculation experiments.

The most uniform success was had when the inoculations were made into young and rapidly growing parts. In such cases it is often possible to obtain 100 per cent of infections (see Table II). Apparently it is sufficient to introduce the bacteria into any actively dividing tissues of root or shoot—cambium, xylem, phloem, bark, pith, or mesophyll. Whether the structure of the tumor tissue in such cases

is dissimilar and whether the metastases partake of the nature of the original tumor are subjects requiring much further study.

In sensitive tissues the tumor reaction begins at once, and can be seen as a slight elevation about the needle pricks as early as the fourth or fifth day, and in the form of perfectly developed, small, fleshy growths a few days later (daisy, peach, etc.).

STRUCTURE AND GROWTH OF THE TUMOR.

The gross appearance of these tumors when they occur on cruciferous plants somewhat suggests the "finger and toe" attributed to *Plasmodiophora brassicae*. It is not a remote inference that all phenomena of this character on the roots of crucifers should be attributed to bacteria, particularly as no clear-cut inoculations with Plasmodiophora have ever been obtained. By this is meant inoculations which would clearly exclude the possible presence of pathogenic schizomycetes. But the chances are against such being the fact. We have not made enough experiments to be able to say positively that crown-gall bacteria never occur associated with the Plasmodiophora, but in opposition to this view, and favorable to the autonomy of the finger and toe disease, is the structure of its tumor, which shows very little hyperplasia and a great amount of hypertrophy, especially of the cells occupied by the spores of the Plasmodiophora. Moreover, the phloem is a favorite point of attack in finger and toe. Probably the correct view is that these are two distinct gall diseases of crucifers. Writers on malignant animal tumors are correct in asserting that the Plasmodiophora tumor is anatomically quite unlike the tumors with which they have to deal.

The anatomy of the crown-gall having proved a much easier subject than the etiology, there is a considerable body of literature respecting the structure of the galls, the details of which need not here occupy much space, since we contemplate a special paper on the subject. Those who wish to know more may refer to the following authors for structure of the tumors of the plants specified: Almond and peach (Toumey); rose (Scalia); sugar beet (Briem); raspberry (Wulff); poplar (Brizi). The contentions of these writers agree in the main, and the principal facts set forth by them are not contradicted by anything we have observed.

Some additional observations and inferences may here find place. In crown-gall we may assume either (1) a direct stimulus to growth or (2) an indirect one through the removal of some normal inhibition. Probably the first is the true explanation. The tumors appear to be able to arise from any meristematic tissue, i. e., from any cells of the organism which are able to divide. They are not subject to any physiological limitation. In a way, of course, the growth that

takes place in crown-galls is like that seen in the regeneration tissue of wounds, but that growth is governed by a physiological need and ceases with the repair of the wound, whereas the gall tissue proliferates indefinitely, passes beyond the control of the plant, and becomes a wasting disease. So far as the tissues themselves are concerned, the chief difference appears to lie in the different distribution of the various elements, the overplus of parenchyma, the weakening of the conductive tissues, the persistent prevalence of meristematic (embryonic) cells, and of immature forms generally, e. g., defective vascular bundles. Crown-galls vary greatly not only in virulence, but in their structure from species to species and also from individual to individual within the species, depending, apparently, on where the tumor takes its origin, i. e., whether it begins in pith or bark or wood or on the lamina of a leaf.

Sometimes the tumors are very woody and hard, their structure consisting chiefly of twisted and contorted lignified vascular bundles and woody fibers mingled with more or less parenchyma. At other times, and very often, the structure consists mostly of rapidly proliferating nests of parenchymatous tissue of a round-celled or spindle-celled type (Pl. XXXII), intermixed with which are vascular bundles (conductive tissues), more or less lignified, but twisted out of their normal shape, with walls abnormally thin, the total mass of the conductive tissue being less by far than that encountered in normal tissue, i. e., there is an enormous excess of the rapidly proliferating parenchyma and a corresponding reduction of conductive tissues.

The cells of the hyperplasia are often much smaller than the cells of the tissue in which it originates, e. g., inoculated tumors in cortical parenchyma of tobacco stem (Pl. XXIX). There is never any enormous stretching of individual cells such as we find a common phenomenon in the galls containing Plasmodiophora and in those formed by the nitrogen tubercle organism of Leguminosae. On the contrary, the stimulus to division is so active that the cells do not have time to attain their normal size. The mechanism of division is a subject for further research. In young, rapidly growing daisy tumors fixed for that purpose we did not find many karyokinetic figures, but in a rose gall numerous double nuclei were observed lying close together in undivided cells. Toumey observed this in almond. In our pure-culture inoculations where several needle punctures have been made close together sometimes only one gave rise to a tumor; sometimes all or nearly all of them developed independent tumors which fused into one mass as growth continued.

A study of sections of the earliest stage of tumor development might lead to interesting results respecting the cells first infected. This we propose to undertake. The tremendous proliferation prob-

ably begins in a single cell or in a few cells and perhaps from a special tissue, but whether the impulse to division must always come from within the infected cell, this impulse being transmitted only to its daughter cells, and so on, or may also be external, influencing neighboring groups of cells, remains to be determined.

When this double phenomenon appears, to wit, overproduction of parenchyma and corresponding reduction of vessels, and it occurs very often not only in the daisy, but also in the sugar beet, peach, hop, and many other plants, the tumors do not appear to be able to obtain as much water and nourishment as is required to carry them beyond a certain point in growth, and portions of the morbid tissues slough off, necrosis following growth in the course of a few months. It is seldom that the primary necrosis involves the entire tumor; some portion of it, generally the margin, remains alive and proliferates more or less extensively the same season or the following season, forming additional tumor tissue, which subsequently extends the open wound by additional necrosis. Where the woody fibers are more abundant this phenomenon does not occur, or takes place at a more remote date. In other cases the tumor regresses and no new one appears.

SUGGESTED RELATIONSHIP TO ANIMAL TUMORS.

The writer can not help feeling that the phenomena displayed in a rapidly proliferating tumor of the type figured and described in this bulletin shows a likeness to certain tumors occurring in the animal body, namely, to sarcomata. These plant tumors often grow very rapidly, and when the plant is a small one either destroy it within a few months or greatly injure it. They seem to be much more nearly related to sarcomas than they do to inflammatory processes, to which some of the animal pathologists with whom we have talked have been inclined to liken them.

Exclusive of the presence of leucocytes, which do not occur in plants, and of swelling, which even in animals is not the invariable accompaniment of an abscess (e. g., abscess in bones), we have in plants phenomena quite like abscesses in animals, but these phenomena are in no way like crown-galls. They consist of the formation in the stems or other parts of plants of more or less extensive cavities or chains of cavities filled with fluid, broken down portions of the tissues, and a greater or less number of the bacteria which have caused the disorganization, together usually with saprophytes of various kinds. Such phenomena occur in bacterial diseases of potato, maize, sugar cane, cabbage, pear, etc., but they are purely disorganizations (areas of softening), not *abnormal organization processes*. In the abscesses no new organs are formed. The most the plant is able to

accomplish under conditions favorable to it is the formation by cell division of a protective cork layer about the diseased area somewhat as the tissues of the animal body for the same purpose inclose tuberculous nodules or syphilitic gummata with a fibrous mass of connective tissue. According to the current medical classification of tumors, crown-galls belong with the infectious granulomata apart from the true tumors, but it is not likely that such classifications represent anything more than a temporary phase of progress in pathology, because they rest largely upon absence of knowledge. One by one as the causes of tuberculosis, lepra, syphilis, actinomycosis, etc., have been discovered these diseases have been removed by medical writers from the domain of tumors and classed as specific inflammations, but logically if parasites should be discovered all the remaining malignant growths would have to be removed, leaving nothing but the empty pigeon hole for tumors.

Unlike teratomas, these tumors do not have a restricted growth comparable to a defective normal growth. Teratomatous growths are frequent in plants, but quite unlike the cell development here in question. Neither are crown-galls to be regarded as degeneration processes. We have in plants certain disease phenomena, namely, œdemas, which seem to be more like degeneration processes in animals than are the growths here described. In œdema, which is believed to be nonparasitic, we have swelling from excess of water supply and more or less enlargement of parenchyma cells, but it does not usually pass beyond simple hypertrophy and does not involve such heterogeneous hyperplasiac tissue changes as are conspicuous in the crown-galls. Some of our gum diseases of unknown origin show somewhat similar degenerations, formation of internal cavities, with enlarged cells in the walls. In crown-galls no abscess cavities have been observed.

Cancers occur in a variety of animals, and no good reason has been advanced why they should not occur in plants. These tumors are morbid new tissue developments tending to weaken and destroy the plant, and their structure does not suggest galls due to insects. Insect galls are usually of quite specific structure and definitely restricted growth, whereas the crown-galls are of indefinite structure and indeterminate growth. As a working hypothesis, we may regard insect galls as due to a localized and fleeting stimulus of a chemical nature not unlike the more generalized and prolonged stimulus which leads to cell division in crown-galls. The determination of the immediate cause of cell division in the one would probably throw much light on the other.

Certain resemblances to malignant animal tumors may be pointed out in more detail.

In the crown-gall there is not only enormous proliferation of the parenchymatic tissues (often in nestlike masses), but there occur in the tumor also all the other tissues normal to the organs attacked, although usually the woody tissues—the conductive ones—are greatly distorted and reduced very much in volume. They are there, however, permeating the tumor in various directions. Some portions of them are seen on sections as small ligneous islands and others as more or less lignified short tubes; but these fragmentary appearances are due simply to the direction of the knife cut, and careful dissection of the part shows that the ligneous conductive tissues arise from the base of the tumor and twist and branch in various directions through it, becoming reduced to widely separated single vessels or pairs or small groups of vessels in the remoter portions. This fact should, perhaps, count for more, in our judgment, as to the analogies of these tumors than the appearance of the rapidly proliferating parenchyma cells, which, however, strongly suggest malignant tumor tissue of animals, as may be seen by referring to Plates XXVI to XXX. (See also Plates VIII and XXI.) Undoubtedly many of the supporting elements in crown-gall, perhaps all, grow out of the substratum along with the growth of the tumor (Pl. XXVI, lower figure, *Y*). In some instances in large tumors it would seem as though some of the vessels were produced in place from the tumor itself, but of this we are not certain.

Another suggestive likeness is the fact that in this disease, and likewise in the olive-tubercle, there are well marked metastases, that is, secondary tumors arising from within, at some distance from the primary tumor, as the result of migrations, but as yet in the crown-gall we do not know the mechanism of this migration, i. e., whether the bacteria move independently through the tissues, setting up irritations in more or less remote places, or whether the migration takes place only within special host cells. In many plants cells push through small pits in vessel walls, forming in the interior of the vessels numerous rounded growths known as thyloses. These often contain bacteria. If they should become dislodged, they might then fall or be carried upward in the direction of the water current to become, if still able to divide, the center of a new growth elsewhere, quite after the manner of malignant animal tumors, assuming metastases of the latter to originate always in this way. Observations of Hunger on the brown rot of tomato and of the senior writer on the same and on mulberry blight show that thyloses are developed in vessels as the result of bacterial infection. Usually, in thyloses the irritation is temporary and tumors are not developed, although in case of the roots of old cucurbits it is common to find woody vessels compactly filled with a pseudo tissue composed of thyloses.

Whether this suggested mechanism of distribution actually occurs in the crown-gall must be left for further study. Owing to the absence of visible channels of infection and to the difficulty of staining the schizomycete in situ, we do not know where nor to what extent it occurs in a dormant condition outside of the tumor proper; but in a very interesting and destructive fungous gall of West Indian lime trees, studied in the Laboratory of Plant Pathology for several years, Miss Florence Hedges has demonstrated that the fungus may grow through the stems to a distance of several feet from the primary tumor before an internal secondary tumor develops. The distance in one case was so great that the writer of this paragraph supposed it to be a second external infection until proof to the contrary was obtained by tracing the internal mycelium through the wood from the primary to the secondary tumor. Here the bulk of the abnormal growth consists of wood. A bulletin on this subject is in preparation.

It is probable that the parasite in its migrations from one part of the plant to another does not make free use either of the vessels or of the intercellular spaces, at least we have not been able to find it in them. Rather we think it is imprisoned within the specially stimulated and rapidly dividing cells and is by the growth of these cells carried along. The location of the visible metastasis would then depend on where the most favorable conditions for rapid growth developed. There would then be a slight chain of morbid cells all the way from the primary tumor to the secondary one. Further studies are necessary.

Plate XXX shows a photomicrograph made through a young metastatic tumor in the petiole of a daisy leaf. The whole interior is a mass of rapidly proliferating morbid tissue (parenchyma cells and distorted bundles), but it has not yet ruptured to the surface, the outermost tissues being the normal tissues of the leaf stalk. Later such a tumor would tear apart these tissues and appear on the surface. The primary tumor in this case was some months older and situated lower down on the stem. There are some indications in this section that tumor cells are growing between and wedging apart larger normal parenchyma cells (infiltration?), and this phenomenon may be seen conspicuously in the section shown in Plate XXVII.

A third likeness which seems to us of some importance is the fact that a certain degree of immunity can be induced in the plants by repeated inoculations. When several inoculations have led to the formation of successive galls, it is then usually impossible to induce galls in the affected daisies by inoculating again with pure cultures of the same strain which produced the initial tumors. One set of

successful inoculations does not suffice to produce this quasi immunity, but several are required. Even then it is possible by inoculating with a more virulent strain to induce tumors on these plants, although as far as our observation goes, the tumors are slower to appear and generally smaller and less vigorous in their growth than on check plants (p. 177). Spontaneous recovery from the disease is quite frequent.

A fourth likeness is the tendency of the disease to appear in callous or scar tissue, e. g., on pruned roots or at the junction of stock and graft. This appears to be rather more than mere presence of wounds in these places. The wounds must probably exist, but the softer, modified character of the new tissue appears to invite both wounds and infections, just as it also invites secondary fungous and bacterial infections.

A fifth resemblance consists in the marked tendency of the galls to return after excision.

Sixth, the fact frequently observed by us that on agar poured plates made from tumors, especially those of some age, certain colonies which look like those of the right organism and which behave properly when transferred to peptonized beef bouillon either do not produce the overgrowth when inoculated into susceptible plants, or yield only very slow-growing, feeble, soon stationary hyperplasias, requires explanation and may be mentioned here. At first these results were interpreted by us as meaning accidental presence in the tumors of organisms resembling *Bacterium tumefaciens* on agar, etc., but unlike it in other respects. More recently we have come to the conclusion, or rather formed the working hypothesis, that these perplexing colonies, or at least some of them, must be nonvirulent strains of the gall-producing organisms, not other species. We do not know what constitutes virulence, but we do know that on culture media many organisms gradually lose this property, *Bacterium tumefaciens* being one of them. The question then arises: Why should not virulence often disappear from organisms buried inside the tissue of tumors? And is not the fact that the tumor has ceased to be active and the host has gained the ascendency evidence of this? It is certainly conceivable that either by the juices of the host or through their own by-products the bacteria might be so acted upon as to lose power to infect other plants when cultivated out, and this without losing their common cultural characters. The same phenomenon is believed to occur in cultures of the organism causing root tubercles of Leguminosae.

It is believed by us that we have here the beginning of a solution of the cancer problem in men and animals, or at least a most instructive border-line field. The chief objection raised by animal pathologists with whom we have talked to considering these tumors in the

light of cancers is the fact that we know them to be produced by a specific organism, hence they are granulomata. If we did not know them to be so induced, then they would be willing enough to consider them as tumors. This is shown by the fact recently called to our attention, i. e., after these pages were prepared for the printer, that in the International Conference on Cancer at Paris in October, 1910, Professor Jensen (of mouse-tumor fame), not knowing of our researches, presented without hostile criticism a paper (Von echten Geschwülsten bei Pflanzen) on the crown-gall of the beet, in which he maintained it to be not only a true neoplasm, but a genuine tumor for which he predicted a rôle as important in cancer research as the mouse tumor itself has played. His exact words are:

Obwohl meine Untersuchungen nur noch einen vorläufigen Charakter haben, glaube ich doch, aussprechen zu dürfen, *dass wir beim "Wurzelkropf" nicht nur mit einem echten Neoplasmus sondern gar mit einem Tumor zu schaffen haben, der in gewissen Beziehungen Aehnlichkeit mit den malignen Geschwülsten der Tiere darbietet; ja, ich bin geneigt zu glauben, dass er in der Geschwulstforschung eine ähnliche Rolle spielen können wird, wie jetzt die Mäusecarcinome.*—Page 248.

And again, on page 254:

Wir haben also in dem sog. Wurzelkropf *eine Geschwulstbildung* vor uns, *die auf einer andauernden, abnormen Proliferationsfähigkeit gewisser Zellen zu beruhen scheint, und die nicht nur dadurch, sondern auch durch ihre Beeinflussung des Wachstums der Rübe, ihre Fähigkeit zu rezidivieren und sich transplantieren zu lassen, so wie durch die abnormen chemischen Verhältnisse der Zellen so sehr an die malignen Tumoren der Tiere erinnert, dass ein näheres Studium der biologischen Verhältnisse der Geschwulst unzweifelhaft wohl angebracht wäre.*

The animal pathologists have not come to any agreement as to what is the cause of sarcoma, carcinoma, and similar tumors, some holding them to be due to organisms, either known or suspected, while others, now the majority, maintain that inasmuch as inoculations of certain ground cancerous tissues have not led to any infections and inoculations of uninjured tumor tissues of the same sort have led to numerous and repeated infections, therefore the disease can be transmitted only by the living proliferating cancer cells, e. g., experimentally by grafting. As clear a statement of this view as any, perhaps, is that given by Dürck:

The essential difference between infectious growths and genuine tumors is that when the former are reproduced by metastasis the parasite itself is conveyed in the blood and incites at the metastatic site new formation of tissue similar to that of the parent growth, whereas in the case of genuine tumors metastasis takes place by the transplantation of a part of the parent tumor, which then begins to proliferate independently at the new site.

But we are totally in the dark as to what originates cancer cells or causes them to proliferate.

It has been known for a considerable period, i. e., since 1900, that crown-galls could be inoculated into healthy plants by means of

pieces of tissue and that tissues so inserted would grow into a new tumor, and in that period we were in precisely the same condition as the animal pathologists of to-day, who reproduce mouse tumors and similar malignant growths by introducing pieces of the diseased tissue under the skin of healthy animals, but can not explain the reason why. We did not then know that such plant tumors were due to a specific organism, and a good many of us were very skeptical as to the existence of a parasite, because after repeated careful searches by a good many people no such organism had been demonstrated in situ by means of the microscope, and the things which had been plated out of such plant tumors and tested on healthy plants had produced in them nothing comparable to the growth from which they had been taken. Enough such experiments have been made and by a sufficient number of persons to show that unless one knows just how to set to work it is not at all easy to obtain the pathogenic organism from crown-galls. As stated in the beginning of this bulletin, we boggled away at the problem a couple of years before we were certain by isolations and inoculations that we had in a particular micro-organism the specific cause of the disease. Our troubles were of various sorts. First of all the tumor tissue when it has reached any considerable age is rapidly invaded by secondary organisms, i. e., saprophytes, and on the plates these are the ones that we commonly obtained. Isolation is also complicated by the fact that the organism which is the cause of the disease is a rather sensitive one, i. e., frequently is killed out quickly in the struggle with saprophytes. The problem was further complicated by the fact that on standard +15 nutrient agar, which was our common substance for poured-plate isolations, the initial growth of the bacteria taken directly from the interior of the tumor and distributed in the agar plates is extremely slow, so that often colonies visible to the naked eye are not seen before the fifth or sixth day, and sometimes not until the tenth or twelfth day or later. In other words, the saprophytes would come up quickly and be studied and the overgrown plates discarded before the right organism would appear at all.

The writers also believe that the organism in the tumors either multiplies very slowly or if growing at an ordinary rate is killed off rather rapidly by the chemical reactions of the plant itself, or by the by-products of its own growth. We have been led to this hypothesis by several facts. First, it is not easy to obtain stained preparations of the tumors in which the bacteria can be demonstrated. After six years we have to show not a single good preparation. We get numerous granules of the size of bacteria, and some of them of the general outline, but so far with few exceptions none which take a sharp stain leaving well-defined walls such as one looks for in order to be reasonably certain that he has bacteria in his preparation and not

something else, e. g., cell detritus. The fact that the organism as it occurs in the gall comes up slowly on agar poured plates, but grows as promptly as other bacteria in the same medium when transferred from cultures, may be coupled with the fact that irregularly shaped involution forms are common in this species when it is exposed to certain unfavorable conditions, e. g., cold or sodium chloride. If such involution forms were the common form also in the plant, it might explain many of our failures. Such club-shaped and branched forms occur abundantly in some of the nitrogen root nodules of Leguminosae.

If this were true, namely, that there is a pretty nearly even balance between the growth of the bacteria in the tumor tissue and the destruction of the same, then we might have the tumor rapidly proliferating as the result of the stimulus of enzymes, toxins, acids, alkalies, or other substances, dissolved out of the dead bacteria, and not in the tumor at any given time very many bacteria demonstrable by means of stains, because inactive and partially disorganized bacteria are proverbially difficult to stain.[a] Might not some phenomena of the kind mentioned be present in malignant animal tumors and thus complicate the determination of their etiology? We know in case of the crown-galls, even when we can not stain the bacteria in the tissues, that they are there, because by selecting small tumors no part of which has yet passed into a necrotic condition we can obtain therefrom cultures of the gall-producing organism, and have done it over and over again. All that it is necessary to do is to scrape thoroughly and wash the unfissured surface of the gall, then soak it in some germicide long enough to sterilize the surface (an hour or less in 1:1,000 mercuric chloride water usually suffices), dry it, and dig into the depths of the tumor (or in case of hard tumors into superficial rapid-growing portions) with sterile instruments, remove and crush some of this interior portion in sterile bouillon, and make poured plates in +15 nutrient agar. We are further led to the belief that living bacteria are not numerous in the galls by the fact that even when the melted cooled agar for plates is inoculated rather copiously, what one would ordinarily call very copiously (say, 1,000 or 10,000 or even 100,000 times too much) if he were dealing with other diseases, the colonies developing on the plates (which under the conditions mentioned are sometimes absolutely free from intruders) are not very numerous. Third, even this procedure will frequently fail to yield any colonies, unless one also takes the added precaution of allowing the living bacteria present in the partially

[a] Since this was written we have obtained numerous involution forms in agar and bouillon by adding weak acetic acid. These facts, coupled with the knowledge derived from the flask analyses, viz, that acetic acid is formed from sugar by this organism, makes it very probable that both acetic acid and involution forms occur in the tumor.

crushed tissue ample time to diffuse out into the bouillon by letting it stand for half an hour or an hour before the plates are made. This is additional proof that the number of viable and stainable bacteria in any given portion of the tumor tissue is rather small, so as to be hard to find, unless it be that the bacteria are abundant but intracellular to such an extent, i. e., so intimately mixed with the protoplasm, that they do not readily diffuse out of the partially crushed tissues. Certainly there is nothing in the crown-gall comparable to the phenomenon seen in the nitrogen tubercles of legumes where the hypertrophied cells become gorged with bacteria, easily seen as such whether stained or unstained. With good technique the right organism can be obtained by means of poured plates from almost any rapidly proliferating part of the crown-gall tumor, but often not at all if one tries from older portions of the growth, i. e., those more remote from the active centers of growth.

The isolations are also sometimes complicated by the fact that of two colonies on an agar poured plate looking just alike, one may be able to cause the disease and the other destitute of pathogenic properties.

The difficulties we have encountered in determining the etiology of these tumors make it only reasonable to suppose that similar difficulties would be encountered in isolating the parasites of animal tumors, admitting for the time being that they are due to organisms. This is also suggested by the past difficulties encountered in determining the cause of tuberculosis, lepra, and syphilis. A few suggestions may be offered for the consideration of pathologists who believe malignant animal tumors to be of parasitic origin, but have not been able to demonstrate the suspected parasite.

(1) All present cellular theories of cancer origin are incompetent to explain how such cells originate, i. e., become cancerous, or why they multiply; and in the light of the facts here presented we would suggest that renewed search be made for a parasitic organism or virus either independent of specific cells or confined to them and using them as a means of dissemination. It would seem that the initial cancer cell or cells in an organism must result from the action of a foreign organism or virus, whatever may be thought of the process of abnormal growth once established.

(2) If we may assume the suspected parasite to be present in the tissues in an active state in very small numbers only, owing to the nearly balanced struggle of the host against the invading organism, and the rapid destruction also of the causative organism in many parts of the tumor, owing to the invasion of saprophytes, then one might well have failed to produce the disease by injection of small quantities of ground-up cells without this being conclusive evidence

of the nonparasitic origin of the tumor, either no parasites being introduced or so few and these so reduced in vitality by long presence in the tumor or by exposure to the juices of the crushed cells, which we may suppose to be more or less germicidal, that they are overcome and destroyed by the normal activities of the body. It is possible also that there may be some special mechanism of infection. Here might also be pointed out that most of this evidence has been derived from mouse tumors, and that we are under no obligation to consider all malignant tumors as etiologically identical.

(3) A parasite might be present and not isolated because unable to grow on the media commonly offered to it, as in the case of syphilis and yaws. The most striking evidence of this nature the senior writer has had brought to his attention was the failure of a streptococcus associated with endocarditis to grow on media obtained from one of the best human pathological laboratories in the country, but which grew readily in slightly different media. The growth of the organism, as was afterwards determined by him, was inhibited by the presence of too much sodium hydroxide in the bouillon. This particular organism he also observed to be very sensitive to sodium chloride, so that a slight excess of sodium chloride in the agar or bouillon would also inhibit growth. Had only one bouillon or agar been used the experiment would have failed. This organism was isolated in +15 agar and bouillon, but would not grow in zero bouillon or agar (October, 1906).

In the light of these facts there can be little doubt that many of the blood tests in arthritis and endocarditis which have been described as negative by various physicians and surgeons are to be regarded as failures due to the use of improper culture media, rather than as proof of absence of organisms in the blood or other fluid tested. Why not failures of this kind also in other fields of animal pathology? In recent years but few serious attempts appear to have been made to isolate a parasite from malignant animal tumors.

The variety of difficulties encountered in obtaining cultures of the organisms causing tuberculosis, lepra, syphilis, rabies, etc., should also be considered; e. g., pathologists have been satisfied for a long time as to the cause of leprosy, being able to stain a certain acid-fast organism within the cells, but not until very recently has it been possible to grow it in pure culture and with subcultures therefrom reproduce the disease in mice (Duval: Jour. Exp. Med., 1910, Vol. XII, pp. 649 to 665).

(4) Failure to demonstrate the supposed parasite in stained sections might be due either to its scarcity, to its indifference to stains, to its lack of power to retain them during the washing, or to the fact that it may occur in the tumor in some very minute or unusual form,

e. g., in involution forms. In this connection see a paper by S. B. Wolbach and Tadasu Saiki on the presence of bacteria in normal livers, demonstrable by cultural methods but not by stains (The Journal of Medical Research, Boston, September, 1909, p. 274).

(5) The likeness of crown-gall to animal tumors might be thought at first sight to be lessened, owing to the fact that plants of many sorts can be made to take the disease by means of grafting or pure culture inoculation, whereas animal tumors are supposed to be very restricted in cross-inoculability. One reason for this difference may lie in the greater simplicity of plant structures, plants being much less highly specialized than vertebrate animals. It is possible also that the doctrine of non-cross-inoculability of animal tumors may be a sweeping generalization based on insufficient evidence. Recently Van Dungerm states that he has successfully inoculated sarcoma of the rabbit into the hare; and Sticker claims to have produced dog tumors in the fox.

(6) The most difficult thing to explain on any parasitic theory is the character of the metastases in cancer. These are so characteristic, and so like the tissues of the original tumor that from an examination of sections of the secondary tumor it is often possible to determine where the unseen primary tumor is located, whether, e. g., in the stomach or the ovaries. This, however, does not seem to be an insuperable objection. Vide Mühlman, Ueber Bindegewebsbildung, Stromabildung und Geschwulstbildung—Die Blastocyten Theorie (Archiv. f. Entwickelungsmechanik, 28 Bd., pp. 210–259).

It is not yet beyond dispute that a cell mother of one kind can never give rise to a cell of another kind when a changed stimulus is applied. Adami and several others maintain that particular animal cells forming a normal part of tissues, i. e., not in juxtaposition with the proliferating mass of morbid tissue, may become cancer cells.

METASTASES.

It had been noticed during the early part of our work with the gall organisms that when a daisy plant, never before affected, was inoculated and galls were produced, the disease did not confine itself to the inoculated part or its immediate neighborhood, but made its way to other parts of the plant. This was shown by the marked tendency of galls to form on leaves or parts of the stem other than that part on which galls developed as the result of our inoculations.

Some of these galls may have been due to accidental surface infections, but it seemed that all of them could not be ascribed to local surface infections for several reasons, i. e., because the check plants in the same house remained remarkably free from infection, because the hothouse was quite free from small animals likely to cause

wounds, and because some of these galls were observed to arise from the deeper tissues and to push up the sound superficial tissues (Plate XXX) several days in advance of any actual rupture of the latter.

When the first cuttings were made from the first galled plants, notes were kept of the behavior of these cuttings, and, of 33 made, 18 developed galls within six weeks. Some of the galls were underground on the base of the cutting, some were at the surface of the earth, and some were on the upper part of the stem.

Finally, experimental inoculations into the leaf-traces under the point of insertion of the leaf, caused, first, the appearance of galls on the stem where the needle entered, and subsequently at a distance, internal galls. These internal galls appeared along the line of the punctured leaf-traces in the petiole and on the midrib of the leaf, several centimeters from the primary galls, and gradually ruptured to the surface. The plants selected were sound and there could be no question of the secondary galls having originated *from within, and as a result of some stimulus due to the primary gall*, both because they appeared exactly where it was reasoned out in advance that they should appear, and because they were watched through all stages from the first slight elevation of some portion of the sound midrib until through stress of internal tensions it finally split open, showing the tumor tissue in the bottom of the cleft, which tissue gradually increased in size until it projected far beyond the borders of the crevice as a typical gall.

These growths developing from within outward must be due to migrations or growths from the primary tumor (of bacteria certainly, of host cells inclosing the bacteria probably), but we have not been able to demonstrate the channel of migration either in unstained or stained sections. Cuts made at various points between the primary and the secondary gall yielded nothing to the microscope, nor did we obtain bacterial colonies on agar poured plates made from such tissues, but this is not surprising considering the relative scarcity of the bacteria in the galls themselves. In the olive tubercle, which is superficially like the crown-gall, there are abscess cavities filled with the parasitic bacteria, and a distinct channel of infection can be traced from the primary tumor to the secondary (metastatic) tumors. This occurs in the wood following the path of certain spiral vessels situated at the inner border of the xylem next to the pith. Here distinct lesions occur. On cross section the path of migration in the stem can be seen with the naked eye in the form of small brown dots (lines on longitudinal section) from which under very favorable circumstances a white bacterial slime may be seen to ooze in minute quantity. Under the microscope this browned area is seen to be occupied by bacteria. The vessels and

surrounding tissues in which these bacteria lie are not only stained, but otherwise disorganized. Nothing of this sort occurs in the crown-gall. The subject is still under consideration.

The anatomy of one of these metastatic tumors in a very early stage of development is shown on Plate XXX. All the central portion of the section is occupied by the incipient tumor. The white lines on the margin mark off the extent of uninfected tissue. The tumor had not yet ruptured to the surface, but would have done so in course of a few days, on the upper part of the section, where the abnormal tissue is nearest to the surface.

CHEMICAL CHANGES.

EXCESS OF OXYDIZING ENZYMES IN THE GALL TISSUE.

The oxydizing power of extracts from crown-galls is greater than that of extracts from sound tissues. Toumey showed this for almonds. It was also shown by Miss Marian L. Shorey in some determinations made for the senior writer in 1908, using sugar beets. These beets had been inoculated for some months with the daisy organism and bore moderate-sized tumors. The black powder isolated and purified by repeated precipitations with alcohol was introduced by knife wounds into the crown of many growing sugar beets, but no tumors resulted. This excessive production of colorless substances oxydizing readily to dark compounds on exposure to the air is to be regarded as a host reaction, and is perhaps due to an increase in the oxidase content.

In 1909 (Blätter f. Zückerrübenbau, XVI Jahrg., Nr. 6) Reinelt mentions that Bartos had observed the gall substance in sugar beet to be somewhat darker than the rest of the beet, and says that he himself observed that when beets are placed in absolute alcohol or vapor of alcohol this difference in color becomes more pronounced, the gall becoming very dark, whereas the body of the beet is but little stained.

In some tests made in 1910 the senior writer observed the same difference *but only so far as regards the outer protected surface.* When the galled beets were thrown into alcohol the galled parts turned dark almost immediately, while the smooth part of the root (protected by a normal bark) remained white. No such contrast was observed, however, when the same beets were sliced so that the alcohol had an equal opportunity to act on all the tissues. These were the beets which served for the illustrations shown on Plate XXII.

OTHER CHANGES IN THE TISSUES.

The chemical analyses by Strohmer and Stift (Österr. Ungar. Zeits. f. Zuckerind. und Landw., II Heft, Wien, 1892) show in the

crown-galls of the sugar beet, as compared with the unaffected parts of the same roots, slightly more water, considerably less cane sugar, the presence of invert sugar, double the quantity of ash, and in all but one instance more than double the amount of raw protein. Six analyses were made and the calculations are expressed in per cents of fresh substance. They all agree, except that two blanks occur in the invert-sugar line, and two in the raw-protein line.

The same facts respecting cane sugar, invert sugar, pure ash, and raw protein are shown still more strikingly in a table where the amounts are calculated in 100 parts of the dry substance. No invert sugar was found in the normal parts of the roots, but 0.91 to 1.52 per cent in the galls. An average of the six analyses shows that the dry substance of the galls contained 50 per cent cane sugar as against 61 per cent in the normal parts of the roots. The average per cent of ash in the roots examined was 2.78 and in the galls 6.05. The average per cent of raw protein in the roots was 4.09 and in the galls 9.80.

ANALYSIS OF FLASK CULTURES OF BACTERIUM TUMEFACIENS.

Analyses of flask cultures of the daisy organism after some months' growth in 750 cc. filtered river water containing 35 grams c. p. calcium carbonate, 14 grams Witte's white peptonum siccum, and 35 grams Merck's c. p. dextrose were made for us by Dr. Carl L. Alsberg, with the following results:

Received July 26, from Doctor Smith, five flasks of the culture, labeled, "Daisy (newest strain)." The reaction of the culture medium [inoculated March 29, 1910] was distinctly alkaline; the bottom of the flask contained much calcium carbonate, which was filtered off. The filtrate was alkaline. A small portion, when acidified with acetic acid and treated with ammonium oxalate, gave a heavy precipitate of calcium oxalate, showing that a considerable amount of the calcium carbonate had been dissolved. The solution reduced Fehling's solution powerfully, showing the presence either of aldehyde or of sugar. Subsequent investigations showed the absence of aldehyde, so that this reduction must be attributed to sugar. Other flasks of the same lot, the analysis of which was taken one or two months later, still showed a large quantity of sugar present. The filtrate, which was alkaline, was preserved and examined. It did not reduce ammonium silver nitrate solution, and therefore can not have contained any aldehyde. It gave a powerful reaction with potassium iodide, resulting in the formation of considerable iodoform. Hence, the main constituent of the distillate was ethyl alcohol. The residue in the distilling flask was now acidified with sulphuric acid, and the distillation repeated. The distillate proved to be very acid, and had an odor resembling acetic acid. It was made ammoniacal and concentrated to a small bulk. The neutral solution resulting was treated with silver nitrate, yielding a crystalline precipitate. This was recrystallized in hot water, yielding large white needles; 0.3969 gram of this silver salt yields 0.2559 gram of silver, or 64.46 per cent of silver. Silver acetate contains theoretically 64.67 per cent of silver; hence the volatile acid can not be anything else than acetic acid.

The results obtained with this single culture flask were exactly duplicated with two other flasks.

Another portion of the culture was acidified and shaken out with ether. The ether was driven off, leaving a yellow, oily residue which contained a very small quantity of colorless, radiating, short prisms. These were insoluble in water, and had every appearance of fat. It was attempted to discover whether the residue contained any other acids, by preparing the barium salts and fractionating them by means of abstraction with absolute alcohol. No lactic or succinic acid could be detected. The residue from the ether seemed to consist mainly of a little fat and some fatty acids.

The calcium carbonate, which remained in the flasks, was removed from the cultures by filtration in hydrochloric acid and extracted with ether, and no acids passed into the ether extract, so that this precipitate does not seem to have contained anything besides the calcium carbonate.

Summary: A considerable quantity of acetic acid and ethyl alcohol was identified in the culture medium. No other fermentation acid could be detected. There seemed to be present a small amount of fat or fatty acids.

THE STIMULUS TO GROWTH.

All plant tumors are not due to the same parasite, but all the hyperplasias are due probably to the same chemical substance or to closely related substances, whatever the organism may be that produces these growths. This substance, which we shall eventually isolate, is probably a by-product of the growth of the intruding organism, possibly a complex colloid, or perhaps only some comparatively simple substance acting continuously in minute quantities. It is our hope finally to cause the crown-gall with specific products of the bacterial growth freed from the living organisms and from extraneous substances, and we have under way already certain experiments of this sort, but they are not yet ready to be reported on.

As a first working hypothesis we have assumed some salt of acetic acid, possibly ammonium acetate, to be the cause of the stimulus, either, (1) as the primary source of the irritation, or, (2) as the liberator of such an irritant from the protoplasm of the bacteria through its killing action on their membranes, which would render them permeable.

PHYSICAL CHANGES—EARLY DECAY.

The physical changes in the tumors are such as would naturally occur in any rapidly proliferating parenchyma imperfectly provided with conductive tissues. It would seem that beyond a certain point the soft tissues can not be supplied with water and food, and decay sets in with more or less sloughing of the tumor and the appearance of open wounds. The harder and more slow growing the gall the later this appears. A variety of saprophytic bacteria and fungi take part in disintegrating the overgrown tissues. Among these saprophytic bacteria there are several white forms closely resembling the gall organism as grown on agar poured plates, dendritic white forms, green fluorescent species, yellow species, orange species, pink species, etc.

The nonpathogenic white forms generally develop on agar plates somewhat whiter or creamier or denser colonies than the gall organism. They look more like the latter in early stages of growth than after some days. But some resemble it so closely on agar that cultures on other media are required. From old galls it is often difficult to isolate the parasite, the tissues swarm with such a mass of secondary and tertiary forms. So true is this that from such parts it is scarcely worth while to attempt isolations. These are best made from the youngest growing parts.

Their fleshy nature also tempts parasitic fungi and bacteria, mites, nematodes, and a variety of insects.

When the tumors are very fleshy decay sets in earlier than when they are woody.

EFFECTS OF THE DISEASE ON THE TISSUES NOT DIRECTLY INVOLVED.

PHYSICAL EFFECTS.

The necrosis of gall tissues already mentioned affords opportunity for the entrance of rain water and many sorts of insects, bacteria, and fungi, which bring about more or less destruction of supporting tissues not involved in the original tumor. In this way the pear-blight bacillus and facultative wood and bark parasites of various sorts may enter, causing serious stem and root injuries. If the plant is an orchard tree it may be weakened by this decay of the wood so as to be easily broken off by animals or blown over by the wind. This often occurs in the peach and almond; rarely in the apple. Plate XXXI shows the bacterial apple blight (*Bacillus amylovorus*) originating in a hard gall.

PHYSIOLOGICAL EFFECTS.

The immediate and remote physiological effects of these tumors vary from species to species and also within the species and are generally less pronounced and certainly less speedy than we might expect from their size and vigor of growth. The plant, however, is less specialized than the higher animals, especially by absence of a nervous system, and in this connection it might be interesting to speculate on what would be the outcome of malignant animal tumors if the depressing influence of pain were removed and the consequent greater or less disturbance of all the functions of the body.

In many instances the tree shows no material injury even after a series of years. This is especially true of the apple, according to Hedgcock, Stewart, and others. In other cases, and this is true even of the apple, the attacked tree is dwarfed in comparison with its unattacked fellows. Peaches and almonds show this dwarfing to

a greater extent than apples, and roses in hothouse culture are still more conspicuous examples of it. Unfruitfulness has also been observed in the last three species and in the grape. A large part of this phenomenon is perhaps attributable to simple abstraction of food and water. In case of the daisy this often proceeds to such an extent that individual branches projecting beyond well-developed galls present a starved appearance and die prematurely.

This disease never induces premature development of blossoms and fruit so far as observed, but on the contrary retards development—rose, daisy, apple.

It is a difficult matter to determine whether the substances elaborated in the tumors by the parasite or by the saprophytes which follow it are absorbed and act as slow poisons on the remoter tissues, but there is some warrant in the appearance of the plants for this assumption.

Death of galled cuttings may occur within a few months, but ordinarily on well-rooted plants it either does not occur at all—i. e., the the plant outgrows the disease—or it occurs only after a lingering illness of many months or several years, and then frequently as the result of secondary infections due to other organisms.

In many of our inoculated daisies we have observed what we have interpreted as increased resistance due to the long-continued growth of tumors on the plants, and consequently there would appear to be reactions set up in the plant which are possibly comparable with some of those observed in the animal body. We do not yet know to what substance this increased resistance is attributable. The subject is dealt with more fully in the following chapter.

EXPERIMENTS SHOWING INCREASED RESISTANCE OF THE HOST DUE TO REPEATED INOCULATIONS AND ALSO DECREASED VIRULENCE OF THE BACTERIA.

While the work with the different gall organisms was being carried on extensively, a group of plants of the Queen Alexandra daisy or progeny of the same was used constantly for inoculating, and the diminishing size of the galls that formed in comparison with those of the first inoculations and also the longer period of time required for their formation drew attention to the fact that either the organisms used were less virulent than when they were first isolated or else that a change was taking place in the plants themselves. To determine which hypothesis was the correct one fresh daisy galls were taken and the organism plated out to get a strain which had not become attenuated through repeated transfers on culture media. The new strain was inoculated into cuttings made from galled plants which themselves had been cuttings from previous galled ones.

The results of the inoculations seemed to indicate that the change must be in the plant itself, for the galls that formed from the presence of this newly isolated organism were also slow growing and did not reach half the size of those galls produced when the first daisy plants were inoculated.

The idea then began to take shape that this failure of the organism to form a gall of the usual size when inoculated into the most favorable growing daisy tissue might be due to some substance developed in the plant for protective purposes, and experiments were planned to determine if daisy plants could be made immune to this disease through repeated inoculations into the same plant or into rooted cuttings made therefrom.

In the following tests the plants used were taken at their most favorable age—that is, they were inoculated when the tissue was young and tender, so that the organism would have the best possible opportunity to produce the disease. Because cuttings did not grow well in the winter months the work was confined generally to the spring and summer.

(1) In March, 1907, a dozen daisy plants of the Queen Alexandra variety were inoculated with the daisy gall organism. These plants had never been known to have galls and had not been inoculated before. In two months' time good-sized galls had formed at all the points of inoculation.

(2) Cuttings (first set) were made from the preceding plants in May, 1907. The cuttings were growing well in July and then a second series of inoculations were made on them. A dozen plants were used this time. Galls formed which were as large as those of the first series.

(3) In November, 1907, cuttings (second set) were made from the plants of the second series, but they did not grow well at first and it was decided to wait until growth had started up well in the spring before further work was done with them. The inoculations (third inoculations) were made in April, 1908. Galls formed at each inoculated place, but they were much smaller and grew very slowly. In August they were less than half the size of the galls of the first series.

(4) Twenty-five cuttings (third set) were made from these diseased plants on August 17, and inoculations (fourth series of inoculations) were made November 18, 1908, on a dozen plants two and three shoots each. In the meantime a new strain of the Queen Alexandra daisy was purchased from a florist and the virulence of the organism checked up on these new plants which had never been affected with the gall. Large galls formed on the new daisies in a month, but there were none on the third set of cuttings. This was the fourth time that strain had been inoculated.

At the end of a month (December 22, 1908), as there was no trace of a gall starting to form, the same 12 plants were inoculated again (fifth series of inoculations) further to test the case. These plants were watched carefully but no galls formed. In a few cases the tissue at the points of inoculation was raised a little as though the presence of the organism had had some little effect. As galls formed at every point of inoculation on the check plants the organism used for the inoculations was proved to be all right. However, four months after this last inoculation of the third set of cuttings, the plants were examined again, and a gall was found on the root of one of them and one on the stem of another where a cutting had been taken.

(5) In March, 1909, cuttings (the fourth set) were made from the plants which seemed to be immune, and on May 20 they were inoculated as follows (sixth series of inoculations), some with the daisy organism which had been used through the entire test (strain B), some with the peach-gall organism, and some with a daisy organism recently plated from a gall and proved up by other inoculations. Six to 8 shoots on each of 6 plants were inoculated with the old-daisy organism; 4 plants including a like number of shoots on each were inoculated with cultures of the crown-gall of peach organism; and 6 plants with cultures of the daisy-gall organism recently plated out. In all there were over a hundred inoculations, i. e., groups of punctures.

There were no daisy plants available for controls, so young sugar-beet plants about 6 inches tall were inoculated at the crown with the same cultures. Two beets were inoculated with cultures of the old daisy, 2 with the new daisy, and 2 with the crown-gall of peach organism. Sugar beets were used because they had been found to take the gall very readily.

On June 18, 1909, there was not a trace of gall formation on any of the daisy plants inoculated May 20. The checks of the peach gall and of the old daisy (both on the sugar beets) had good-sized galls, but those beets inoculated with the new daisy had none. These plants, however, were in a shady place and had not made much growth since the time of inoculation. The galls on the 4 sugar beets were accounted sufficient proof that 2 of the 3 strains were able to produce galls in susceptible plants.

The same day (June 18, 1909) some of the same daisy plants were inoculated again with the crown-gall of peach organism, 16 groups of punctures being made (seventh inoculation). The plants were growing very well. Five young sugar beets were inoculated at the crown with the same cultures as checks on the daisies.

On July 6, 1909, the plants were examined and no galls were found on the daisies; 2 of the 5 sugar beets had small galls which bade fair to increase in size as the beets grew.

This last set of cuttings (fourth set) in which two sets of inoculations had been made already was subjected to one more test. A fresh strain of the peach-gall organism which had been isolated in April, 1909, from some trees grown in Virginia was used for these inoculations. This organism was selected because it had produced galls very rapidly on a daisy plant which had never been affected with this disease. In July, 1909, 43 inoculations (eighth series of inoculations) were made. Five young sugar beets were inoculated at the crown with the same cultures used on the daisies. On August 30 the last inoculations of the daisy were examined and no trace of a gall was found on any. Of the 5 sugar beets only 1 had a gall; the beets had grown scarcely at all since they were inoculated, so they were repotted and left to develop. October 4: These beets never grew to any extent, but 1 other bore a tiny gall.

On September 20 all of the plants included in the fourth series of cuttings were taken from the pots; the soil was washed from the roots, after which they were examined thoroughly. Four out of the 16 plants had galls on the roots, only 1 of which was of any appreciable size.

(6) Cuttings were again made, this being the fifth set from the original galled plant. For checks, new daisy plants of the Queen Alexandra variety were purchased from a Boston firm and grown under the same conditions, so that both sets of plants would be about the same age when inoculated.

A fresh strain of the daisy organism was obtained in November, 1909, by plating from a gall, and inoculations were made December 1 on 31 of the supposedly resistant cuttings which were growing well and on 16 of the new daisies from Boston never before inoculated to be held as checks. The first subcultures from the poured plate colonies were used for the inoculations.

On December 14 (two weeks' time) galls had formed on 14 out of the 16 daisies of the new strain, but none whatever on the resistant strain.

On December 21 a gall had formed in one of the resistant cuttings; it was very tiny, but unmistakably a gall. By this time (end of third week) galls had formed on all the check plants and were from half an inch to an inch in diameter.

On January 6, 1910, 14 out of the 31 resistant cuttings had small galls starting to form. Some of these were merely a slight swelling. This was thirty-seven days after inoculating, and it will be remembered that all but 2 of the check plants had galls within two weeks.

On January 18 (forty-nine days) the supposed resistant cuttings were examined again and 23 of the 31 found with galls. None of the galls were larger than a small pea, however.

On February 9 all of the resistant cuttings had small galls, except 4, and 2 of these showed indications of swelling. This was seventy days after inoculating, and nothing comparable with this has been known to follow the inoculations of a daisy plant which had never before been inoculated with the gall organism. The beginnings of gall formation have been seen on daisy as early as the fifth day after inoculating, but the usual time for decided evidence is ten days or two weeks and always within three weeks.

On March 10, 1910, galls were forming on the 4 resistant cuttings which were still free from galls on February 9.

In July, 1910, all of the resistant plants bore large galls, i. e., growths 1½ to 2 inches in diameter.[a]

(7) Cuttings were made from these plants in August, 1910 (sixth set), and inoculations were made on these in November, December, and January, after they were well rooted and growing rapidly. The results are not yet ready to be reported upon.

So far as we have gone, loss of virulence may account for some of our failures to infect, but not, it would seem, for all, since in some of the experiments already described the check plants contracted the disease promptly, while the others did not. The results now under way ought to settle the question.

The following results are believed to be due, in part at least, to loss of virulence, but in part also to increased resistance. The weak point in the reinoculations is the almost complete failure of the checks.

In September, 1909, about 200 rapidly growing young daisy plants (rooted cuttings from old plants) were inoculated in the top of the shoot with young slant agar cultures of the old daisy gall organism (strain B).

No galls resulted. Thinking this complete failure might equally well be attributed to increased resistance on the part of the plants, since all of the cuttings had been taken from plants already twice and thrice successfully inoculated, the plants were repotted, top pruned, forced into rapid growth, and reinoculated.

The first reinoculations were on December 6, using young agar subcultures from several typical-looking colonies recently derived from a daisy gall by Miss Lucia McCulloch. The bacteria were pricked in. A small part only of the plants were inoculated. Checks were kept. All failed.

On December 13 to 17 the entire 200 plants were reinoculated by needle pricks, rather more than 400 groups of punctures being made on young branches. For this purpose young agar subcultures were used. They were derived from a colony recently isolated from a

[a] A comparison of No. 6 with earlier results seems to indicate that even when first isolated from a gall some colonies are more virulent than others.

daisy gall by Miss Brown and believed to be the right thing because it behaved typically on agar. The inoculations were made by the senior writer, assisted by Miss Bryan. Five days were devoted to the work, and, as 85 check plants were held, interesting results were anticipated, but no galls ever formed. The check plants (with two exceptions, 1020 and 1056),[a] also remained free, although they were in a growing condition and derived from plants never before inoculated and not long in the hothouse. The experiment must, therefore, be set down as a lost one without knowing quite why. Probably the failure must be ascribed to the use of a nonvirulent colony.

The plants stood in 10-inch pots, occupying the whole of a 125-foot, well-lighted greenhouse bench, and made throughout a good growth. They were of two susceptible varieties.

When the final examination was made in August, 1910, the plants were large and had been in bloom all summer. Occasional shoots showed a slight knobbiness where the needle pricks entered, and often there was more than the usual amount of corkiness in the pricked areas, but not a single tumor resulted from the inoculations. That these plants were still subject to infection (given a sufficiently virulent organism) is indicated by the fact that 13 of them bore natural tumors on the stem at the surface of the earth. Six of these tumors were large; the others were less than 1 inch in diameter. The parents of all of these plants (about 21 large daisies) all bore similar natural (and large) tumors on the base of the stem at the time the cuttings were made, and, as already stated, the plants from which they in turn were propagated had been (they or their progenitors) several times artificially inoculated with the production of galls. Cuttings were now made (August 5, 1910) from a large number of these plants for a second large experiment, and cultures were plated from the most favorable looking (youngest) of the 13 knots, with a view to obtaining a more virulent strain with which to make subsequent inoculations.

In November, December, and January inoculations were made on these plants as follows:

(1) With subcultures from a colony on a plate poured from the most favorable of the 13 tumors just mentioned.

(2) With subcultures from a colony on a plate poured from a daisy tumor occurring on a "nonresistant" plant.

Both these sets failed to produce tumors. Not only was this true of the "resistant" plants, but also of the check plants never before inoculated.

(3) Isolations were now made from a gall growing on one of Miss Brown's resistant plants (sixth series), and subcultures from two of

[a] These had very small galls in the inoculated places at the end of a year.

the colonies thus obtained proved to be actively virulent. When these were inoculated into the control daisies tumors soon appeared and are now growing rapidly. Numerous "resistant" plants were inoculated at the same time. All of these have developed small hyperplasias; but it is too early for comparative statements, and furthermore a correcter test, and one we have not yet been able to make (owing to the failure mentioned above), would be to inoculate checks and resistant plants with a virulent organism taken from a tumor on some plant *which had never before borne tumors*. This would remove the possibility of a heightened virulence in the organism used.

LOSSES DUE TO CROWN-GALL.

In consideration of the slow progress of this disease on many inoculated plants, the question has arisen whether crown-gall is really a serious disease or only to be regarded in the light of a minor disturbance, i. e., something comparable to warts or benign tumors in the higher animals.

Inasmuch as our exact experiments have not continued in all cases for a long enough period of years to give comprehensive results the most that can be done here in many instances is to summarize the opinions of growers and others who have given most attention to the disease as it prevails in the field, supporting these as best we may with our own observations, already detailed, in great part.

THE DAISY.

The plants are dwarfed and disfigured but only rarely killed outright or at least not for a long time. They are more or less stunted according to the size and rapidity of growth of the gall. Cuttings are injured worse than old plants. The New Jersey grower mentioned earlier is the only one who has made complaint to us.

THE ALMOND, THE PEACH, AND OTHER STONE FRUITS.

Toumey described this disease as serious on the almond in Arizona, and showed photographs of a 40-acre orchard ruined by it. Speaking of this orchard, he says:

> In the Glendale orchard some of the trees were diseased when planted. The actual number, however, that had galls upon them was very small. After the expiration of eight years, less than 1 per cent remained unaffected. * * *
>
> With each succeeding year a greater number of trees died outright or broke off at or just beneath the surface of the ground, where developing galls had gradually weakened the stem. A very conservative estimate would place the losses in this one orchard at at least ten thousand dollars. Probably the losses to the deciduous fruit and grape growers of Arizona from this disease amounts in the aggregate to from forty to seventy-five thousand dollars annually.

In reply to an inquiry, F. H. Simmons writes as follows (1910) concerning crown gall in Arizona:

There were 40 acres in the tract [probably Glendale orchard described by Professor Toumey]. I think they were set in the fall of 1889, and I took charge in 1899. The crown-gall was very bad on them, and in spring of 1897 there were cut and gathered three wagonloads of the gall. The trees were treated with bluestone on all cut surfaces. This treatment was followed up each year with less galls until spring of 1902 there was less than a bushel basket of galls cut. The drought by this time having made inroads on the trees the treatment was abandoned and part of the orchard pulled out, scarcely a gall being found. * * *

Trees badly affected seemed to have lost power of growth. There were practically on the mesa 125 acres in all. With the exception of 10 acres, all the orchards were badly affected, and about the year 1900 were practically out of business as a paying proposition, and have been nearly all pulled out.

Selby, of Ohio, reported to Toumey as follows:

From observations made in Ohio there seems no reason to believe that peach trees affected with crown gall at transplanting age will ever come to successful fruiting. * * *

One orchard in Lawrence County, containing 200 trees purchased in New Jersey, was grubbed out at seven years of age without having borne a single profitable crop, although other trees of like age situated near them had yielded fruit. These trees were badly affected when delivered, and were nearly all of them diseased at the time of removal. * * * Another parallel case occurred in Ottawa County.

In 1908 Selby made the following statement:

I do not recall a single instance out of many observed and recorded in which, the tree surviving transplanting, the removal of the galls by excision served to prevent the formation of new galls upon the same tree. Excision appeared to exert no influence whatever in the way of suppressing the trouble, and this irrespective of the location of the excised galls; whether but a single gall upon a small root or more than one gall on stem or root or both were removed and the wounds rubbed with sulphur, the new galls constantly appeared later. This may be taken as showing a diseased tendency of the plant tissues and this condition, this diathesis as it may be called, can scarcely contribute to the longevity of the tree independent of cutting off the water supply.

Earle reported to Toumey as follows:

Crown-gall is very abundant in Alabama on the peach and is sometimes found on the plum. I consider it a very serious peach disease in Mississippi and Georgia, as well as in this State.

In 1892 Wickson, of California, wrote as follows:

For some time many nurserymen followed the practice of removing the knots from the trees as dug from the row, but this was abandoned when it was found that the knot commonly reappeared after planting in the orchard. At present no reputable nurseryman sells such trees; they are burned at the nursery.

Probably during the last twenty years hundreds of thousands of such trees have spindled and died in the best soil and with the best treatment.

Woodworth, of California, reported to Toumey as follows:

The crown gall occurs in California on all our deciduous fruit trees and on grapes. It has been abundant and serious.

Toumey wrote:

In California, where the fruit industry is many times what it is in Arizona, the losses must be correspondingly great.

In Pennsylvania on fruit trees in the nursery, according to Butz (Ann. Rep. Pa. State College, 1902, p. 405):

There is little warning of the presence of the disease in a block of trees while they are developing into salable stock, but when they are taken up it is frequently discovered that from 20 per cent to 80 per cent of them are affected at the roots with crown-gall, rendering them unsalable.

Butz also cites from correspondents as follows:

We have known peach blocks in New Jersey to be entirely destroyed. * * * One year ago we had it bad in peach and threw away thousands.

APPLE TREES.

Whitten, of Missouri, reported to Toumey as follows:

I have seen it on a few apple trees in the nursery, but it was not severe enough to impair their growth.

Concerning the injury done to orchard trees, Butz has the following as the result of one of his experiments.

On November 21, 1898, 11 apple trees were planted upon the station grounds. These trees were donated by a Pennsylvania nurseryman, and all of them bore galls at the crown varying in size from a hickory nut to an unhulled walnut. The root system of these trees was apparently most excellent, having an unusual amount of fibrous roots. But owing to the fact that these fibrous roots proceeded mainly from and about the galls it was evident that the galls were the inciting cause of the unnatural development. The trees were three years from the graft, and but for the galls were excellent trees for planting in the orchard. Five of these trees were York Imperial and six were Ben Davis, the two varieties of apple which are most susceptible to crown-gall and the most extensively propagated and planted in Pennsylvania. Records taken in April, 1901, after the trees had made two seasons' growth, show immediate injury due to the galls. Two trees of York Imperial had died, and the other three had made only weak and slender growth. * * *

Of the Ben Davis trees, all grew, though the growth made was in all cases short and weak. The length of the best shoots made in the second season varied from 4 to 10 inches. After another year's growth these trees are still living, making some new wood each year, though it is not as strong as it should be. An examination of the galls at the roots (June, 1902) by removing the ground about them shows that they are increasing in size, and in some cases more completely girdling the trees than when they were planted. The effect of this gall development is shown in the heavy production of sprouts from the stock roots below the gall and the consequent weakness in the graft head. * * *

A peach grower in Franklin County in Pennsylvania is now having a similar experience with peaches. He wrote me in November, 1900, that he suspected something wrong with a block of 1,000 peach trees in an 80-acre orchard, and digging at the roots discovered an enlargement which was identified at this station as crown gall. The trees came from an Alabama nursery and were planted in the spring of 1899. The growth during the first two years was excellent, but now as the trees reach fruiting age they indicate a weakness that can not be overcome.

He also cites from a correspondent as follows:

It is more prevalent in apple than in anything else. On the block of apple trees which were 2 years old when you were here, we did not find a single tree affected, while on our trees, now 2 years old, we find 30 to 40 per cent affected with crown-gall and we will sustain a big loss. At the time these 2-year trees were grafted, I grafted 30,000 for a neighbor for his own orchard planting and on the trees taken up he has found but 2 or 3 per cent affected, though the source of stocks and grafts was the same. This looks as if the disease was in my ground.

The conclusion of this nurseryman is entirely correct; the cause of the disease is in his ground.

A former colleague, Mr. P. J. O'Gara, who has had a very wide experience on the Pacific coast, has observed the disease to be seriously injurious to Spitzenberg apples in Oregon, and also to pears, dwarfing the trees and reducing the size of the fruit. He states that hold-over blight (*Bacillus amylovorus*) is very apt to find lodgment in the galls when they occur above ground and that root-rot begins commonly in the galls when they are underground (oral communication). He is also our authority for the statement that crown-gall has seriously injured peach growing in Colorado. The disease seems to be worse in dry climates, where irrigation is practiced.

In 1910, after conversation with Mr. O'Gara, the following letter was received from him:

I am inclosing a photograph of crown-gall (hairy-root type), taken in my office at Medford, Oreg. This tree is 7 years old, but is no larger than a good 3-year old and certainly not so vigorous. This tree is exactly like 50 trees in the same apple orchard, the variety being Esopus Spitzenberg. Crown-gall, either *hairy*, *hard*, or *soft* types, certainly injures apples if the infection starts with the seedling or the graft. If a tree is several years old before becoming infected, serious injury is not so liable to be the case, as the vigor of the tree somewhat counteracts the effects of the gall. But Spitzenberg apples infected on bodies or crowns often become so "warty" that growers cut them out. Besides, crown-gall above the ground always permits the entrance of fungi, and in susceptible varieties like Spitzenberg, *Bacillus amylovorus* gets in its deadly work through the gall. Anyone having experience on the Pacific coast knows that a crown-gall above the crown of a Spitzenberg means blight infection sooner or later.

Later Mr. O'Gara sent on a blighted apple limb from Medford, Oreg. (Pl. XXXI), with the following note:

I am sending you under separate cover a specimen of Spitzenberg apple limb which has a bad crown-gall, through which pear blight infection entered. Crown-gall on the body or crown of a Spitzenberg apple is very dangerous, from the blight standpoint. The past year I have seen hundreds of blight infections through these galls. For this reason every crown-gall must be removed, and our inspectors enforce this regulation to the letter.

In 1898 Selby cited the case of a grower of nursery stock who found part of a block of apple trees badly affected with gall about the year 1893. The trees were dug up and the ground left to rest a year,

then peach trees were planted. In that portion where the apple trees had been diseased most of the peach trees became affected with galls, and were worthless.

Quite opposite views are expressed in the following citations, the first one of which is from Mr. F. C. Stewart, of the experiment station at Geneva, N. Y. (Proc. 53d Ann. Meeting, West. N. Y. Hort. Soc., Rochester, Jan. 22 and 23, 1908, p. 98):

In this connection it should be mentioned that the crown-gall of apple, although resembling crown-gall of peach and raspberry, is an entirely different thing.[a] There is abundant proof that the apple crown-gall is not communicable from one tree to another. Moreover, in New York, at least, apple crown-gall is an unimportant disease. Although common in our nurseries, it is rarely found in orchards. In 1899 C. H. Stuart & Co.,[b] Newark, N. Y., set out an experimental orchard of 500 trees, mostly Baldwins, all affected with crown-gall. The trees have now been set nine years. Under date of January 20, 1908, Mr. Stuart writes as follows: "These trees to-day show as good a growth as the trees planted the same time and free from crown-gall. The bark is smooth, healthy in appearance, and the trees look thrifty and vigorous." An experiment made by the station bears on this point. In 1901 we planted 22 apple trees affected with crown-gall to determine the effect of this disease upon the growth of the trees. The trees were 3 years old. The galls varied in size from 1 to 2 inches in diameter and were located mostly on the taproot, but in a few cases on lateral roots. Some of the trees had several galls each. We believe the galls were typical of those commonly found on apple trees in New York nurseries. Five of the trees were dug in 1903, 5 in 1905, and the remainder in 1907. In no instance was there any evidence that the galls had increased in size or number, or that they had been in any way injurious to the trees.[c] Probably apple trees bearing large galls should be rejected, but unaffected trees from the same lot may be planted without fear of bad results.

Mr. Barden also writes as follows to Mr. George G. Atwood, chief bureau of horticulture, Albany, N. Y., concerning this same orchard:

Referring to yours about crown-gall on nursery trees that have been planted in orchard for several years, I would say that the Stuart orchard on the Bailey farm 3 miles north of Newark is the only one that I have had any knowledge of. In company with Mr. Stuart I drove to this farm last fall [1909] and carefully studied the different trees, every one of the 400 [d] having been planted with a large crown-gall on it. These trees have now been planted eight years, and, with the exception of a few that were girdled by mice several years ago, are in a vigorous and healthy condition.

The growth has been even, no stunted trees, and it would certainly he hard for an orchardist to condemn a tree on account of crown-gall after seeing this orchard.

Doctor Hedgcock also regards crown-gall as of small consequence to the apple, especially if the root-grafts are well made. His field experiments on the apple have been extensive (mostly in the Mississippi Valley), and cover a period of five years. Mr. Güssow has expressed similar views.

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[a] See note under raspberry.

[b] Nurserymen.

[c] The location of a gall perhaps may determine its injuriousness, i. e., whether on crown or root. Butz's trees bore galls on the crown. So far as known, no comparative orchard tests have been made.

[d] Five hundred in Mr. Stewart's statement. Were 100 lost during these years? And if so, how many by crown-gall? No checks appear to have been held for comparison.

THE QUINCE.

The galls of the quince (*Cydonia vulgaris*) occur on the stems, and are warty in appearance. Often an entire limb will be covered by these broad irregular outgrowths. Whole orchards in California have been attacked by these galls and quince trees in other western States are known to be affected. Mr. Hedgcock has received diseased specimens also from Ansted, W. Va. Doctor Trabut sent specimens of quince gall from North Africa (Pl. XXXV). Lounsbury reports a quince gall which appears in the form of "rough, lumpy growths" as common in South Africa.

THE RASPBERRY AND THE BLACKBERRY.

The disease appears to be quite prevalent on the red raspberry in various places in the United States, and must be regarded as injurious, although some nurserymen are of a contrary opinion. The extent of injury to black raspberry and to the blackberry is not known. Mr. P. J. O'Gara has observed one apple and pear nursery in Oregon where practically all of the young trees were galled. This nursery was set on the site of an old berry patch in which the crown-gall had prevailed (verbal communication).

The following similar statement is taken from the report of the Dominion Botanist (Güssow) (1 George V, Sessional Paper No. 16, A. 1911, p. 273):

> One prominent grower had a small area planted with raspberries. These on being taken up showed many "root galls." The plants were destroyed and no specimens were sent us for examination. The grower then planted a large area to young peach trees, the rows of which passed through the land formerly occupied by the raspberries on which the root galls were discovered. He then observed that the peaches growing on this latter area were not doing well and finally failed, while all the other trees did exceedingly well. On taking up the failing peach trees, their roots showed plenty of root galls, while the others growing outside the raspberry area were free from it. The same facts were recorded by other growers. There could hardly be given a more typical example of an infectious disease. But, unfortunately, we were not acquainted with any of these observations until it was too late to make any investigation. If these facts as related are correct, and we have no reason to doubt them, there is still a considerable amount of research necessary.

Selby is on record as long ago as 1898 to the same effect. He says that 16 per cent of some healthy peach trees planted in a badly galled raspberry plantation became affected with the gall.

Wulff's statements (Studien über heteroplastische Gewebewucherungen am Himbeer- und am Stachelbeerstrauch, Arkiv für Botanik, Bd. 7, No. 14, Upsala, 1908) are equally explicit. He says respecting the appearance of the raspberry gall in a garden near Karlshamn (South Sweden):

> On an area of 33 by 4 paces were about 100 raspberry bushes, all very badly affected by the disease. * * * From the time of their planting in 1901 to the summer of

1907, inclusive, the bushes were always sick, and have during the whole time borne either no fruit whatever or a very scanty crop.

These plants were an ever-bearing variety from Denmark.

In August, 1907, Wulff also found a bad outbreak of the disease in middle Sweden near Orebro:

Here about 800 bushes of Red Hornet and about 100 of Superlative were attacked. The first-named bushes were planted in 1901, had borne very well during the first years, and appeared entirely normal. In 1906 the first symptoms of the disease were discovered, and in consequence of this no crop was borne in the summers of 1906 and 1907.

In the next paragraph Wulff speaks of the disease as "very injurious to raspberry culture" everywhere in Sweden where it has appeared. He also brings forward evidence to show that frost injuries have nothing to do with its appearance, and cites similar statements by Blankenhorn and Mühlhäuser (vide Sorauer I, 596) with respect to the grape gall. Wulff's own statement is:

Bei meinem Untersuchungen der Himbeerkallose habe ich niemals auch nur die geringsten Andeutungen von Frostbeschädigungen entdecken können.

Concerning the origin of the disease neither in this paper nor a second one (Weitere Studien über die Kalluskrankheit des Himbeerstrauches, Arkiv für Botanik, Bd. 8, No. 15, Upsala, 1909) does he reach any positive conclusion, other than that he has not been able to find in the fresh overgrowths any parasitic organism and is inclined to ascribe them to excessive nitrogen nutrition and excessive water supply.

Lawrence (Some Important Plant Diseases of Washington, Bull. No. 83, 1907) shows a very interesting figure of blackberry canes split open by the growths arising from within and says that in the State of Washington the disease is very destructive to the Snyder, and that occasionally Kittatinny and Himalaya Giant are badly infected, while Erie, Early Harvest, and Evergreen are not seriously injured.

He has also observed the disease to be severe on the red raspberry, especially the form growing on roots and crowns.

Güssow has attributed a gall on the blackberry in England to a fungus, *Coniothyrium tumefaciens* n. sp.

THE ROSE.

Occasionally the disease is very prevalent on the roots of roses grown in the hothouse, and skilled gardeners are generally of the opinion that the galls are seriously injurious, reducing the size of the plants, the amount of foliage, and the vigor of the flowers. Here again exact comparative studies are wanting. It must be obvious, however, in the case of a small plant like the hothouse rose, that the

energy used up in the production of the galls, which are often large, must be abstracted from the general needs of the plant, which as a result must either yield an inferior product or blossom for a shorter period.

The following statements were received in 1909–10 from a rose grower who had much of the gall in his houses:

> Our houses of 10,000 plants seem all to be affected, and it looks [October 23] as though we would have to throw the plants out.

The disease was definitely identified as crown-gall by the writers, who received numerous well-developed specimens (Pl. XX, fig. 2) and recommended substitute crops. Nematodes were not observed. This man was asked later in the season for more definite information concerning his losses and replied as follows:

> Replying [February 22] to yours of 16th instant, would say that after consultation with other growers of roses who had had experience with crown-gall and eelworm, we decided to keep our plants in and get what we could from them, rather than take a chance on some other crop so late in the reason.
>
> All the plants are affected more or less—some not as bad as others—while perhaps 200 or 300 have been killed outright.
>
> The great loss is shown when we come to cut the buds. At a time when we should have been cutting 1,500 to 2,200 a day, we were cutting but from 400 to 600, and the average loss for the season thus far has been on a conservative estimate 67 per cent.
>
> We will cook our soil this year and hope for better results another season.

In December, 1910, this grower wrote as follows:

> Replying to yours of 9th instant, would say we did cook our soil last spring, as we wrote you we should, and that we have had *no trouble* with crown-gall this season.
>
> Our plants are very fine this year, and we have been cutting some very fine blooms. Just now we are off crop, but plants are breaking in good shape and the future looks very promising.
>
> Our commission house sent us word early in the winter that they had not seen finer specimens of Bride outside of the flower show than the ones we were shipping.

THE GRAPE.

European observers have generally regarded the scab of the grape as a serious disease.

Delacroix (1908) states that the attacked shoots grow feebly for a year or two and then the parts above the galls dry out and die.

The statement of Cavara respecting rachitic growth has already been quoted (p. 15).

In Italy, in 1906, in the Po Valley (near Modena), the senior writer saw cases of *rogna* on large vines and was informed by competent viticulturists that the disease was becoming more and more prevalent, mostly on the flat irrigated lands, but to some extent also in the hills, and that the life of an attacked vine seldom extended beyond four years. In sections of Italian rogna of the grape preserved in 10 per

cent formalin the senior writer saw bacteria in the browned outer crevices much like those described by Cuboni (1.5×0.3 to $0.5\ \mu$), but less numerous and not likely to be the parasite.

RED CLOVER.

Galls have been found on roots of red clover (*Trifolium pratense*) in Kentucky and Alabama. It is not yet known how destructive this organism is when it gains entrance to a clover field.

ALFALFA.

Roots with tubercles other than the nitrogen-fixing nodules have been found on alfalfa plants (*Medicago sativa*) in Kentucky, Maryland, Pennsylvania, Alabama, and New York (?). The galls are found on plants in fields where the stand is very poor and also an occasional gall is found on plants in very good fields. The plants affected do not grow to full size, but it is not yet known whether they are killed directly by the work of the gall organism or not, although large portions of fields die and the roots are found more or less affected with galls.

COTTON.

The crown-gall of the cotton plant (*Gossypium* sp.) occurs rarely (so far as our information goes) and is not known to cause any trouble whatever to the growers of cotton. It has been found in Texas and also on the crown of cotton plants growing in the greenhouse in Washington.

HOPS.

The reports of hop growers on the Pacific coast indicate that this disease may do considerable damage, particularly as the galls often reach a diameter of one's double fist. Some believe that an attack of two years' duration is sufficient to kill a plant. According to Dr. W. W. Stockberger, of this Bureau, the disease occurs on hops not only in Washington State and Oregon, but also in the Sacramento valley in California: "There I have seen acres of hops in which scarcely a hill could be found which did not show these tumors, some of them being larger than my fist."

SUGAR BEETS.

A crown-gall also occurs naturally on the sugar beet both in this country and in Europe. While rather rare in the United States, it appears to be widely distributed, and more common some seasons than others. We have received specimens from localities as widely separated as Virginia, Michigan, South Dakota, Utah, California,

and Washington State. In general it is easily distinguished from the attacks of nematodes (Pl. IV, fig. 1). It is less easily distinguished from what we have called tuberculosis of the beet. The latter occurs in Kansas and Colorado. It appears to be most prevalent in Colorado where at least one field was badly injured. According to one of our correspondents it is on the increase. Should this disease become widespread the yield of sugar would be greatly reduced.

Crown-gall seems to be rather infrequent in Germany, judging from Dr. Reinelt's paper in Blätter für Zuckerrübenbau (Berlin, 31 März, 1909), since with the assistance of various sugar-beet men he obtained only 47 specimens for his studies.

According to Dr. Kølpn Ravn, of Copenhagen (oral communication), the gall occurs on sugar beets in Denmark, but does not injure the crop, only about one beet in a million showing it.

Of 3,247 beets dug in November, 1910, in Virginia (Arlington Experimental Farm), 5 bore tumors.

The galls on the beet often grow to large size, e. g., Reinelt mentions some as large as a child's head or larger (weight 1.5 kilos), others which caused thickenings of the whole or a great part of the root, and still others which were small as peas, but set close together over the whole surface of the root.

This gall we believe to be due to the crown-gall organism. Three times prior to 1910 typical looking colonies on agar poured plates were obtained from the interior of beet galls from California and once from Virginia. The Virginia colonies were not transferred to subcultures, and the two or three colonies selected from the California plates proved nonpathogenic to sugar beets; no additional opportunity for making poured plates occurred until November, 1910. (See pp. 81-85.)

Reinelt failed to isolate bacteria from the inner tissues and comes to the conclusion that bacteria are not present. He used various sorts of gelatin media. His technique of surface sterilization appears to have been proper and the source of his failure appears to have been (1) that he selected improper material (too old), (2) that he did not wait long enough for the bacteria to appear on his plates, or (3) that he diluted his infectious material too much. The period the plates were under observation is not stated. He should have held his plates for at least ten or fifteen days; he should also have mashed up the fragment of beet and inoculated copiously from the first tube, whereas he did not crush his material but only allowed the small cube to remain in the bouillon for a short time and then made his inoculations from a third transfer (third tube). Judging from our own experiments, on daisy galls, the third tube of bouillon prepared in the manner he describes would ordinarily contain very few living

bacteria—often none, or less than 1 per loop (see p. 168).[a] If he had mashed his cube in the first tube of bouillon, allowed the contents of the crushed cells to diffuse for an hour, and then inoculated directly from this first tube, *rather copiously*, e. g., with several 3 mm. loops of the fluid, he would probably have had colonies of the

[a] As the result of poured plates made in 1910 by Lucia McCulloch, using a sound old hop gall received from the Pacific coast, it would seem that there were less than 500 living bacteria per cubic inch of the material used. The right organism was plated out and tumors obtained with it on sugar beet and daisy, but two of the three colonies selected were noninfectious.

Plates of +15 nutrient agar, poured by Miss Brown in the fall of 1910 from tumors on sugar beet, gave the following results:

(1) First set of Arlington (Va.) plates. Two c. c. of a rather old and tough tumor were mashed in 10 c. c. of bouillon. Eleven plates were poured, all from the original tube, inoculating as follows:

3 plates each five 3 mm. loops.
3 plates each four 3 mm. loops.
2 plates each three 3 mm. loops.
2 plates each two 3 mm. loops.
1 plate one 3 mm. loop.

Five favorable colonies appeared on this set of plates.

(2) Second set of Arlington (Va.) plates made from another tumor—material good. Three c. c. were mashed in 10 c. c. of bouillon. Eight plates were poured. The first six were from the original tube, the other two from the first dilution. The inoculation was heavy, viz:

2 plates with five 3 mm. loops.
2 plates with four 3 mm. loops.
2 plates with three 3 mm. loops.
1 plate with three 3 mm. loops.
1 plate with two 3 mm. loops.

Fifteen favorable colonies appeared on this set of plates.

(3) First set of Blissfield (Mich.) plates.

Of this tumor 3.4 c. c. were mashed in 10 c. c. of bouillon. Eight tubes were poured, the first six from the original tube, the other two from the first dilution, inoculating as follows:

3 plates each with three 3 mm. loops.
2 plates each with two 3 mm. loops.
1 plate with one 3 mm. loops.
1 plate with two 3 mm. loops.
1 plate with one 3 mm. loop.

Five colonies resembling gall colonies came up on this set of plates.

(4) Second set of Blissfield plates (same tumor, next day), using 0.5 c. c., which was mashed in 10 c. c. bouillon. Eight plates were poured, all from the original tube, inoculating as follows:

4 plates each with four 3 mm. loops.
1 plate with three 3 mm. loops.
1 plate with two 3 mm. loops.
2 plates each with one 3 mm. loop.

No gall colonies appeared on this set of plates.

(5) First set of Fairfield (Wash.) plates. A smooth tumor 3.5 to 4 cm. in diameter was selected and about one-half of it (possibly 10 c. c.) was mashed in 10 c. c. of bouillon for the plates. All of the eight plates were poured from the original tube, inoculating as follows:

5 plates each with five 3 mm. loops.
2 plates each with four 3 mm. loops.
1 plate with two 3 mm. loops.

No gall colonies appeared.

(6) Second set of Fairfield plates (same tumor). About one cubic centimeter was mashed in 10 c. c. of bouillon. Eight plates were poured, all being inoculated copiously from the original tube, viz:

4 plates each with five 3 mm. loops.
2 plates each with three 3 mm. loops.
2 plates each with two 3 mm. loops.

Four colonies looking very much like the gall-forming organism grew on these plates.

(7) Plates were also poured in December from a gall on another Arlington beet which had been transplanted to the hothouse for six weeks. These yielded only one colony resembling *Bacterium tumefaciens*, and this gave no positive result when inoculated into sugar beets.

Of these 30 colonies, as already stated, only 5 have proved infectious and all of them are possessed only of feeble virulence.

(For a quantitative study made by the senior writer in November, 1910, see under "*Sugar beet*," p. 81.)

gall organism in all of his plates, provided nothing was wrong with his culture medium or the galls themselves were not too old. We have not used gelatin media for isolations from galls, but ordinary +15 peptonized beef-bouillon agar.

Dr. K. Spisar has also investigated the sugar-beet gall and reaches the conclusion that it is not due to animal or plant parasites of any sort (Zeits. f. Zuckerind. in Böhmen, Prag., Aug., 1910). Bacteria do not occur in all the galls and with those he cultivated out he could not reproduce the disease. He, therefore, ascribes it to wounds, but does not advance any satisfactory reason why it should arise in some wounds and not in others.

Since the above paragraphs were written we have plated what we believe to be the right organism from natural tumors on the sugar beet and with subcultures therefrom have obtained small slow-growing galls on beet (Pl. XXXVI, fig. 1), tomato, and daisy. Most of the colonies tested were noninfectious.

TUBERCULOSIS OF BEETS.

In the autumn of 1910 beets from Colorado and Kansas were found commonly attacked by a yellow schizomycete capable of causing cells to proliferate in a nodular growth. On section the attacked parts showed as small, water-soaked, brownish areas (Pl. XXXIV, fig. 2). Under the microscope great numbers of bacteria were observed therein and the center of the spot was seen to be disorganized into a small cavity. Often the surface of the nodules bore small central radiating fissures (Pl. XXXIV, fig. 3). The appearance of these cracks suggested the possibility that they preceded the infection. In some instances these brownish areas of softening were traced from the galled portion of the beet into the ungalled part. The diseased parts appeared mucilaginous—stringy when touched.

This disease, which was at first supposed to be crown gall, is only superficially like the latter, because, as in the olive tubercle, the bacteria are abundant and easily detected and produce areas of softening and central cavities. The disease has been reproduced on sound sugar beets in the department hothouses by pure-culture inoculations (subcultures from poured plate colonies).

From these artificially produced tubercles the organism has been reisolated and successfully reinoculated into other sound beets. Up to this time cross-inoculations on other plants (daisy, tomato, etc.) have failed.

Description of Bacterium beticolum n. sp.—This organism, which may be known as *Bacterium beticolum* n. sp., is a rod with rounded ends, single or in pairs, chains or clumps. Clumps and chains frequently occur, especially in pellicles. It measures about 0.6 to 0.8

by 1.5 to 2.0μ. It is flagellate by means of several polar flagella. No spores have been observed. It has a capsule. It liquefies gelatin slowly, but not Loeffler's blood serum. Gelatin stabs at 18° C. required a month for complete liquefaction. It reduces nitrates. It grows readily in peptonized beef-bouillon containing 9 per cent sodium chloride. In ordinary peptone bouillon there is uniform clouding and a copious pellicle, which falls easily. It is killed in beef-bouillon by 10 minutes' exposure in the water bath to 51° C. It grows at 37° C., but not so well as at room-temperature (bouillon). It also grows slowly at 1° C. in bouillon. In milk the growth occurs mostly on the surface. It forms a yellow rim and pellicle and slowly solidifies it, but the whey separates very slowly. The fluid is viscid. Litmus milk is blued, and subsequently reduced (1 month). After boiling, the color returns red. It does not grow in Cohn's solution. It grows readily in Uschinsky's solution, making it viscid, like *Bacterium pruni*. In this fluid rods with enormously thick-walled capsules occur. It makes a moderate growth on potato. It does not convert the fluid around the cylinder into a solid slime. There is a copious starch reaction with iodine even after many weeks' growth.

For experiments in fermentation tubes a basic solution was made of river water containing Witte's peptone. In this the following carbon compounds were tested: dextrose, saccharose, lactose, maltose, mannit, and glycerin. The organism grew readily in the open end of all the tubes and clouded the closed end except when lactose and glycerin were offered to it. No gas was produced from these carbon compounds. It did not produce gas in any culture medium, except possibly sparingly in beef peptone gelatin. It should be tried for gas formation in presence of inosit. On thin sown agar plates the colonies may become 1 cm. in diameter. Often they are smaller. These colonies are circular, smooth or wrinkled. The colonies are similar on gelatin and finally form saucer-shaped liquefactions, or if the plate is thickly sown the whole becomes fluid. It grows well the whole length of agar stabs, and sometimes sends out small brush-like projections. Growth is much paler in cane sugar agar, but becomes yellow with age. Indol is produced in 2 per cent peptone water, but less abundantly than by *Bacillus coli*. It grows readily in bouillon over chloroform. It is not killed by drying (fourteen days). It stains well by Gram. It is yellow or becomes yellow on all ordinary culture media.

SHRUBS, SHADE TREES, AND FOREST TREES.

We have no means of determining the amount of injury done by crown gall to nut trees, shade trees, etc. The disease is common on the chestnut and the gray poplar in the eastern United States, and is said to occur frequently on the Persian walnut in California.

Under date of September 24, 1909, Mr. Frank N. Meyer, agricultural explorer for this Department, sent from Angers, France, a young plant of *Arbutus unedo* bearing root galls. From these galls bacterial colonies resembling the daisy organism were plated out and galls produced on sugar beet by pure-culture inoculations (Pl. XXIV, *A*).

Lounsbury has reported it, or something closely resembling it, as prevalent and injurious on the willow in the Transvaal and Cape Colony, South Africa, where it appears to be a new trouble, having come to scientific attention first in 1899. He sent some of these willow galls to us and from one of them a colony was plated which produced slow-growing galls on the daisy and upon weeping willows (Pl. XXXV, fig. 1).

HOTHOUSE PLANTS.

Other than those already mentioned we have found what may be this disease on roots of lettuce (Pl. XXXVI, fig. 2). Our attention was called to this by Mr. W. W. Gilbert, a Bureau colleague, who turned the material over to us with the statement that he could not find any nematodes in the root swellings. We also failed to find them. Thereupon poured plates were made.

The plants shown on the plate were photographed natural size. They had been growing nearly three months and were badly dwarfed. There were many such plants in the hothouse and all had similar galls on their roots, and no other assignable cause for their stunted appearance, since the roots of those lettuce plants in the same house which had made a good growth were free from these nodules. The only other disease in the house was an occasional case of the *drop*.

Agar-poured plates were made from one of these galls after properly sterilizing the surface and colonies obtained which resembled those of *Bacterium tumefaciens*. With subcultures from half a dozen of these colonies inoculations have been made into the roots of young sugar beets, but no galls have appeared to date (13 days).

BEST METHOD OF DEALING WITH THE DISEASE.

Up to this time the best method of dealing with this disease remains the old one of strict inspection of nursery stock and the condemnation of all trees and shrubs found diseased. In individual cases this undoubtedly works hardship to the nurseryman, but, on the other hand, to allow him to sell galled trees injures the fruit grower, serves to distribute the infection broadcast, and tends to destroy his own reputation. The nurseryman's remedy lies in careful methods and the abandonment of infected soils.

By no amount of special pleading can it be made to appear that an infectious disease should be tolerated on nursery trees offered for sale simply because it is rather prevalent and is inconvenient to deal with. Before the nurseryman can be allowed to sell such trees without restriction he must establish conclusively that it is not injurious, and not transmissible to susceptible species.

We are disposed to include apple trees also in this recommendation. While these seem to be less subject to crown-gall in a serious form than some other plants, frequently they do not make good trees, and our cross-inoculations suggest, at least, that they may serve to carry the disease to other plants and into localities previously free from it. Moreover, even when the apple gall does not itself seriously injure the tree it may serve, as we have seen, for the entrance of other parasites.

In some cases the inspector will be in doubt whether to condemn stock or pass it, particularly when the trees have been carelessly grafted and show more than the ordinary amount of callus. He may then either refer the specimens to some more experienced pathologist or refuse to take chances. Until we know to the contrary excessive callus should be regarded as incipient gall. Ordinarily there will be no difficulty in determining whether or not a given lot of trees has crown-gall or hairy-root, except when the nursery stock has been dishonestly pruned before shipment to remove signs of the disease, and then usually some traces will be left. In case trees are improperly condemned there is always a remedy at law.

SYNOPSIS OF CONCLUSIONS RESPECTING CROWN-GALL.

(1) Crown-gall is a disease common in nurseries on the roots and shoots of various plants, and likely to continue on the plants when they are removed to orchards, vineyards, gardens, and hothouses. It also occurs on various field crops. This name is used for the disease whatever the situation of the galls on the plant.

(2) When we began our studies the cause of crown-gall was unknown, and by them it has been determined.

(3) Bacteria were seen in crown-galls of the daisy in 1904, and the studies then undertaken have been pursued continuously to date, and are here first offered in complete form.

(4) The first successful isolations and infections were obtained in 1906, and the biology of the bacterial organism derived from the daisy has been determined more carefully than that from galls on other hosts.

(5) Hundreds of pure-culture inoculations on daisy have removed the subject from the domain of speculation and shown that the galls

on Paris daisy are due to a white schizomycete named *Bacterium tumefaciens* (April, 1907).

(6) This organism is a short rod multiplying by fission and motile by means of polar flagella. It can be grown in many sorts of culture media, but does not live very long upon agar. It forms small, round, white colonies in agar or gelatin poured plates. Under unfavorable conditions of growth it readily develops involution forms.

(7) This schizomycete differs from many bacterial organisms in not producing open cavities in the plant. It appears to occupy the living cells in small quantities, causing rapid proliferation.

(8) We have not been able to stain it in the tissues, at least not satisfactorily.

(9) It is readily plated from young sound galls, i. e., those not fissured or decayed, often in practically pure culture, but it comes up slowly on +15 nutrient agar, and generally not very abundantly. It grows, however, promptly on agar when transferred from cultures.

(10) It produces galls most readily in soft, rapidly growing tissues. Ordinarily, resting tissues can not be made to produce galls. Turnips seem to be an exception.

(11) Cross-inoculations to plants of other families have shown the daisy organism to be capable of inducing tumors on many species in widely separate parts of the natural system (Compositae to Salicaceae), these galls varying somewhat in appearance.

(12) Some species of plants were not infected (onion, fig, olive) and possibly are not infectable, but further experiments should be made.

(13) For purposes of comparison natural galls have also been studied on the following plants: Peach, apple, rose, quince, honeysuckle, *Arbutus unedo*, cotton, poplar, chestnut, alfalfa, grape, hop, beet, salsify, turnip, parsnip, lettuce, and willow.

(14) From all of the preceding, by means of Petri-dish poured plates on agar, schizomycetes have been isolated closely resembling (as grown on agar) the *Bacterium tumefaciens* obtained from the Paris daisy.

(15) With eight of these organisms tumors have been produced on sound specimens of the species from which obtained. With these eight and two others (not tested on the host) tumors have been produced on daisy and various other plants, thus tending to show a wide range of natural cross-inoculability.

(16) On pages 133 and 137 the reader will find tables summarizing all the results of the inoculations.

(17) These organisms have been studied comparatively as to their morphology and cultural characters and found to differ only slightly from each other, and from the organism isolated from the daisy, i. e., the agreements are more conspicuous than the differences.

(18) The beginnings of the galls are visible in some cases as early as the fourth day after inoculation by needle prick, and they often reach a large size in one to two months, but frequently on woody plants they continue to grow for several years. On the contrary, sometimes they have been very slow to develop.

(19) Some cross-inoculate less readily than others, but in general the monotonous morphology, the cultural uniformities, and the ready cross-inoculability (daisy, peach, hop, grape, poplar, alfalfa), point to one polymorphic species rather than to several distinct species, but further studies should be made.

(20) The galls are often rapidly invaded by saprophytic bacteria, especially the softer galls. On agar poured plates many of these bacteria are readily distinguished from the parasite by differences in form and color, but others are distinguished therefrom with great difficulty, cultures on other media or inoculations being requisite.

(21) The galls also invite various parasites—nematodes, fungous root rot, fire blight of apple and pear, etc., and some of these are able to cause great damage.

(22) We have not been able to distinguish etiologically between *hard* galls and *soft* galls. Even the hardest crown-galls are due to bacteria which closely resemble those found in the softest.

(23) Overfed plants are more subject to the disease than those making a moderate growth.

(24) The size of the tumor, other things being equal, depends on how rapidly the plants are growing, i. e., the state of nutrition. Actively growing plants usually developed large tumors when inoculated, and slow-growing plants none at all or small ones; but, as in apple, small slow-growing galls may finally become large. This long-continued growth would not be possible if there were not a very nearly even balance between the stimulus of the parasite and the response of the host.

(25) The apple hairy-root, hitherto a disease of unknown origin and supposed to be noninfectious, has been shown to be due to bacteria which culturally and morphologically differ, if at all, only slightly from the crown-gall organisms.

(26) This causal organism is located not in the hairy roots themselves but in the flattened tumor from which such roots arise.

(27) Typical hairy-root has been produced on sound apple seedlings by pure-culture inoculations, and in the same way on sugar beet both galls and hairy-roots have been obtained.

(28) These abnormal growths which we have designated indifferently as tumors or galls are believed to be like malignant animal tumors in various particulars: Permanent and very rapid new growth containing all the tissues of the part attacked; enormous round-

celled or spindle-celled hyperplasia; great reduction of amount of conductive tissues; early necrosis, especially of the more fleshy tumors, with renewed growth at the margins; frequent recurrence after extirpation; extension of the disease to other parts by metastases, etc.

(29) The disease is one which progresses slowly, stunting the plant first and finally destroying it, unless removed by extirpation or by the development of increased resistance on the part of the plant.

(30) The continuation of rigid State inspection with rejection of diseased nursery stock is recommended.

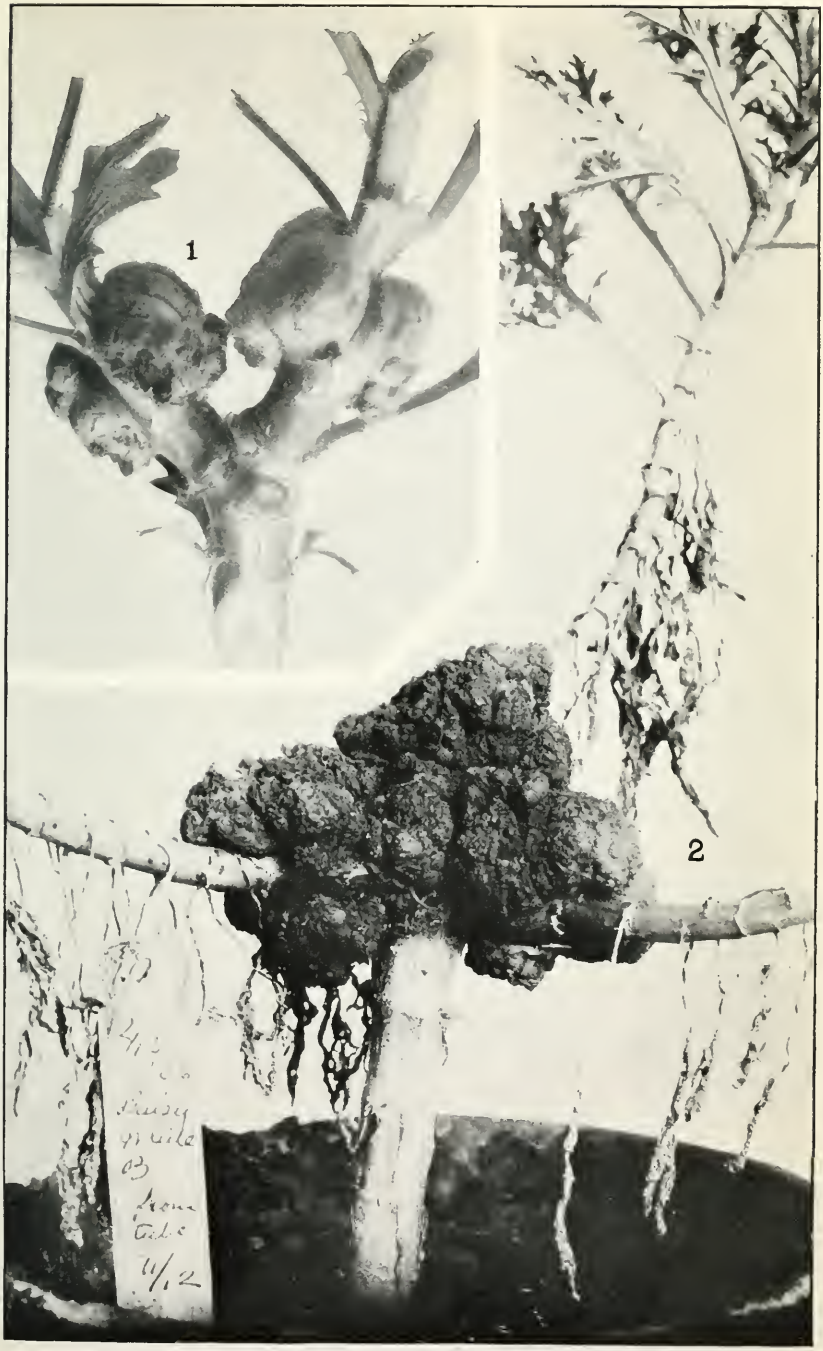
(31) The organism is moderately susceptible to germicides but can not be reached in the galls. Moreover, germicidal treatment, after excision of the galls (p. 184), can not be depended upon in all cases because of the tendency of the organism to form metastases.

(32) The organism from the daisy loses virulence on culture media, and in some cases is believed to lose it also in the tumor itself (daisy, hop, sugar beet).

(33) The organism is believed to occur inside the rapidly proliferating cells, which by its presence are stimulated to divide with formation of the tumor.

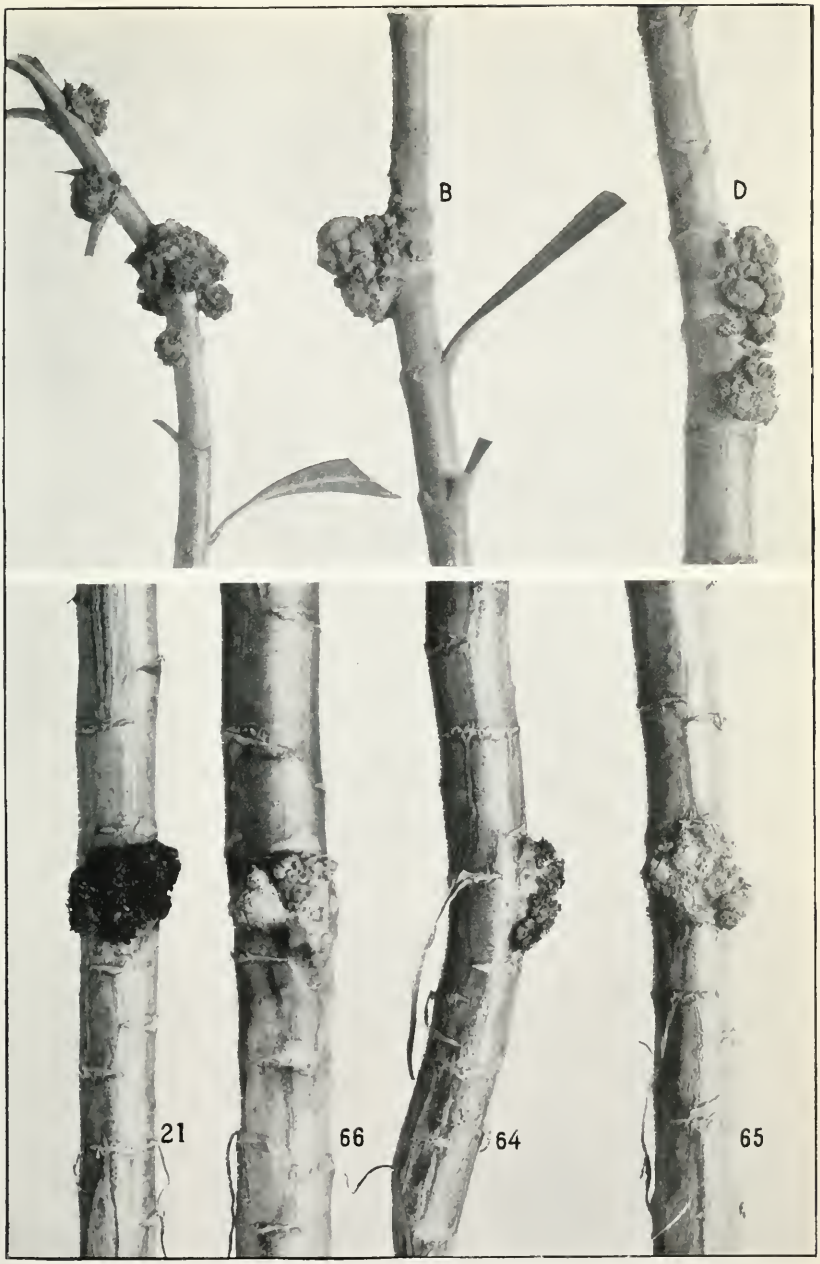
(34) During the progress of our studies a new disease of the sugar beet has been discovered. This disease, which is liable to be confused with crown-gall, causes overgrowths of a coarse nodular nature which soon disintegrate. It appears to be a more serious enemy to the sugar beet than crown-gall, and is one to be greatly feared should it become generally disseminated. We have called it tuberculosis of the beet, and have designated the yellow organism causing it *Bacterium beticolum* n. sp. (p. 194).

PLATES.



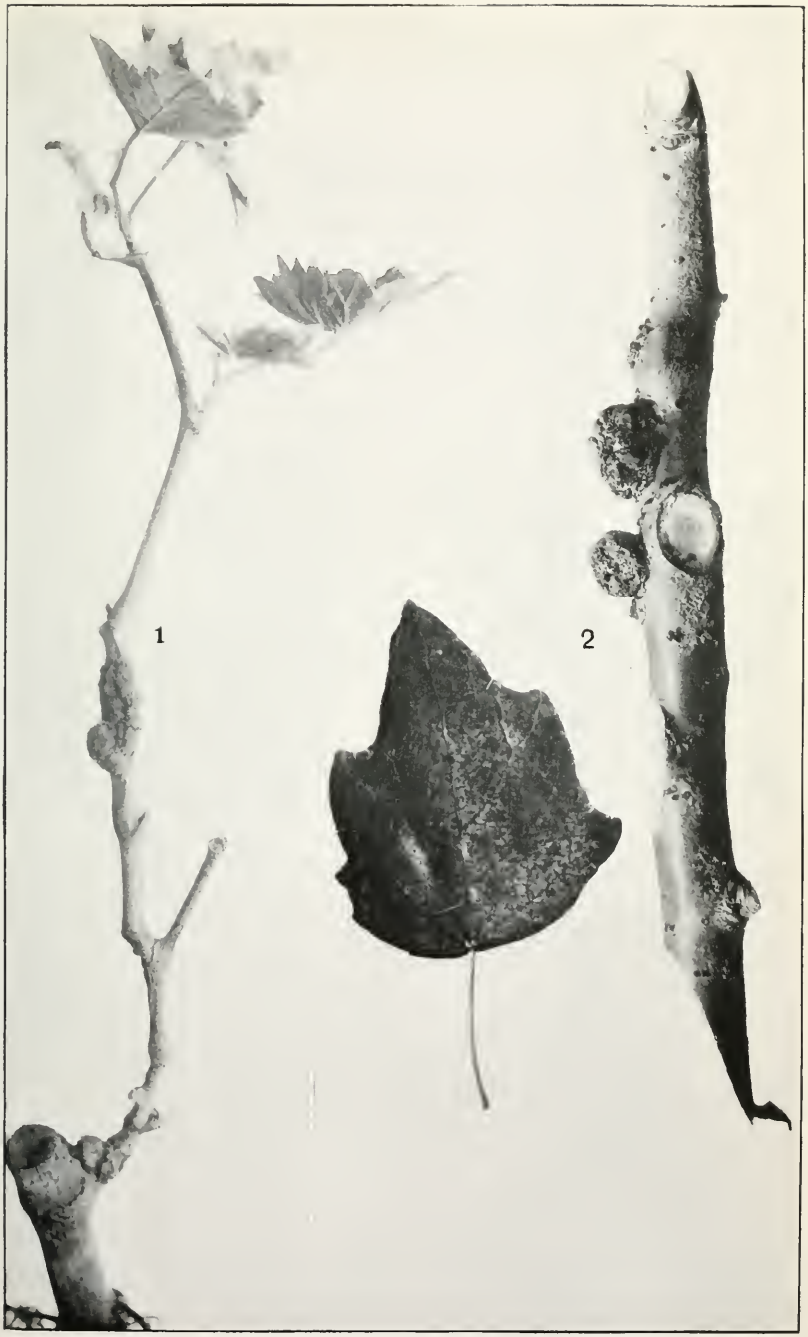
(1) Daisy on daisy. Natural size. Inoculated Dec. 13, 1906. Time: 2 months 10 days.
(2) Daisy on daisy. Three-fourths natural size. Inoculated Dec. 13, 1906. Time: 7 months.

(1) Peach on rose; inoculated Jan. 15, 1908. Time: 3 months.
(2) Apple on apple. Galls at x, x. Time: 2 months.
(3) Hop on tomato; inoculated Nov. 21, 1908. Time: 2 months 26 days.
(4) Chestnut on sugar beet; inoculated Nov. 13, 1908. Time: 33 days.
(5 a, b) Daisy on potato; inoculated Mar. 27, 1907. Time: 26 days.
(6) Rose on sugar beet; inoculated Dec. 3, 1908. Time: 19 days.



Top: At right (B and D): Daisy on oleander; inoculated Mar. 12, 1908. Time: 6 months 9 days.
At left: Natural gall on oleander from California.
Bottom: Hard gall of apple on daisy; inoculations of Nov. 9 and 18, 1908. Time: 8 months.

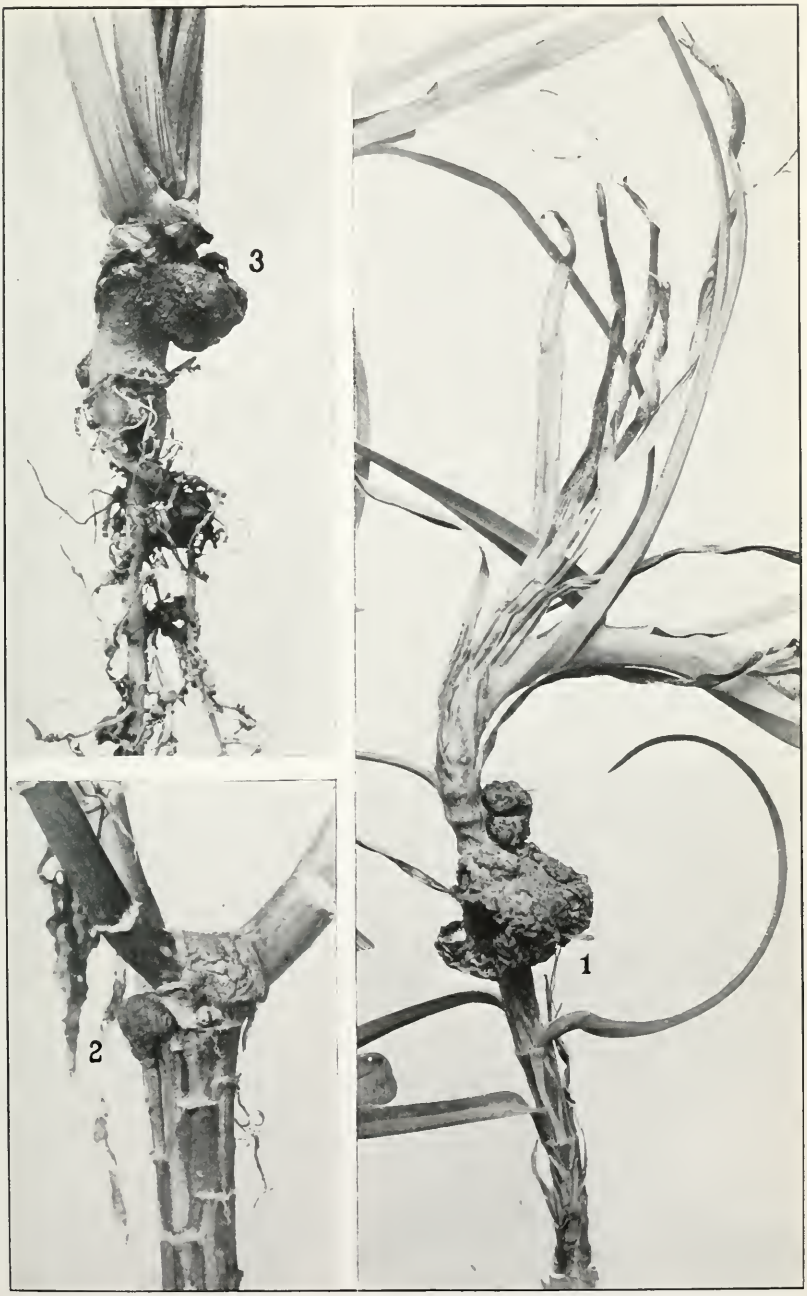
(1) Nematode gall on sugar beet, from Chino, Cal., 1909.
(2) Daisy on red radish; inoculated Apr. 26, 1907. Time: 3 months.



(1) Daisy on grape; inoculated Apr. 3, 1907. Time: 2 months 24 days.
(2) Daisy on gray poplar; inoculated June 9, 1908. Time: 6 months 15 days.

(1) Daisy on peach; about two-thirds natural size; inoculated Mar. 11, 1907. Time: 10 months 18 days.

(2) Peach on sugar beet; inoculated Mar. 11, 1908. Time: 54 days.



(1) Daisy on carnation; inoculated Mar. 2, 1907. Time: 6 months 16 days.
(2) Rose on daisy; inoculated Mar. 21, 1909. Time: 5 months 23 days.
(3) Alfalfa on sugar beet; inoculated June 14, 1909. Time: 2 months 9 days.

Daisy on sugar beet. Both plants from same series. Pure-culture inoculations of Dec. 4, 1909. Time: 4 months.

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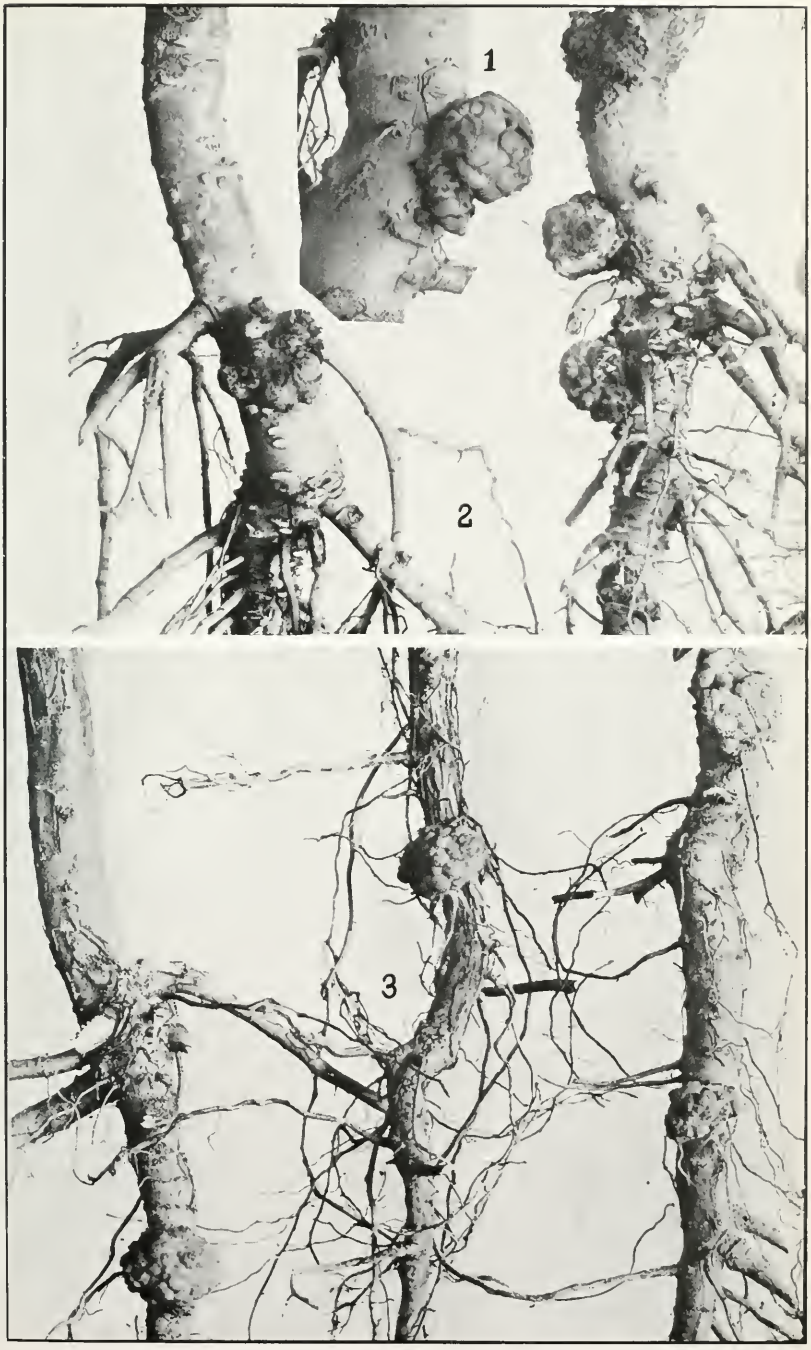
(1) Daisy on hop; inoculated Apr. 10, 1907. Time: 3 months 15 days.
(2) Daisy on cut surface of raw turnip in covered Petri dish in laboratory.
(3) Grape on almond; inoculated June 28, 1910. Time: 31 days.

(1) Grape on grape; inoculated Aug. 31, 1909. Time: 43 days.
(2) Grape on daisy; inoculated Aug. 31, 1909. Time: 4 months 19 days.
(3) Grape on daisy at the crown; from same series as fig. 2. Time: 7 months 19 days.



(1) Peach on peach; inoculated Dec. 5, 1907. Time: 41 days.
(2) Daisy on peach; inoculated Apr. 6, 1907. Time: 3 months 6 days.
(3) Peach on peach, second series; inoculated Jan. 13, 1908. Time: 50 days.

(1) Hop on sugar beet; inoculated Apr. 17, 1908. Time: 31 days.
(2) Soft gall of peach producing hard gall on apple. Three-fourths natural size. Time: 2 years.

Peach on daisy: (1) Inoculated Dec. 4, 1907. Time: 5 months 12 days. (2) Inoculated Feb. 3, 1908, with colonies plated from one of the growths shown in fig. 1. Time: 4 months.

Peach on geranium (Pelargonium). Slightly under natural size. Inoculated Oct. 13, 1908. Time: 3 months.

(1) Apple on daisy; inoculated Oct. 22, 1908. Time: 10 months.
(2) Hop on almond (one gall on crown, one on stem above crown); inoculated Apr. 16, 1909. Time: 7 months.

(1) Chestnut on daisy; less than natural size; inoculated Nov. 13, 1908. Time: 4 months 10 days.

(2) a, Alfalfa on alfalfa; inoculated Sept. 7, 1909. Time: 2 months 25 days. b, Ordinary nitrogen-fixing nodules of alfalfa, introduced for comparison.

Hairy-root of apple on sugar beet: (1, 2) From one series of inoculations; the normal lateral roots are shown at x, x; inoculated Dec. 22, 1908. Time: 3 months 19 days. (3) Enlarged from another series; inoculated Nov. 11, 1909. Time: 4 months 27 days.

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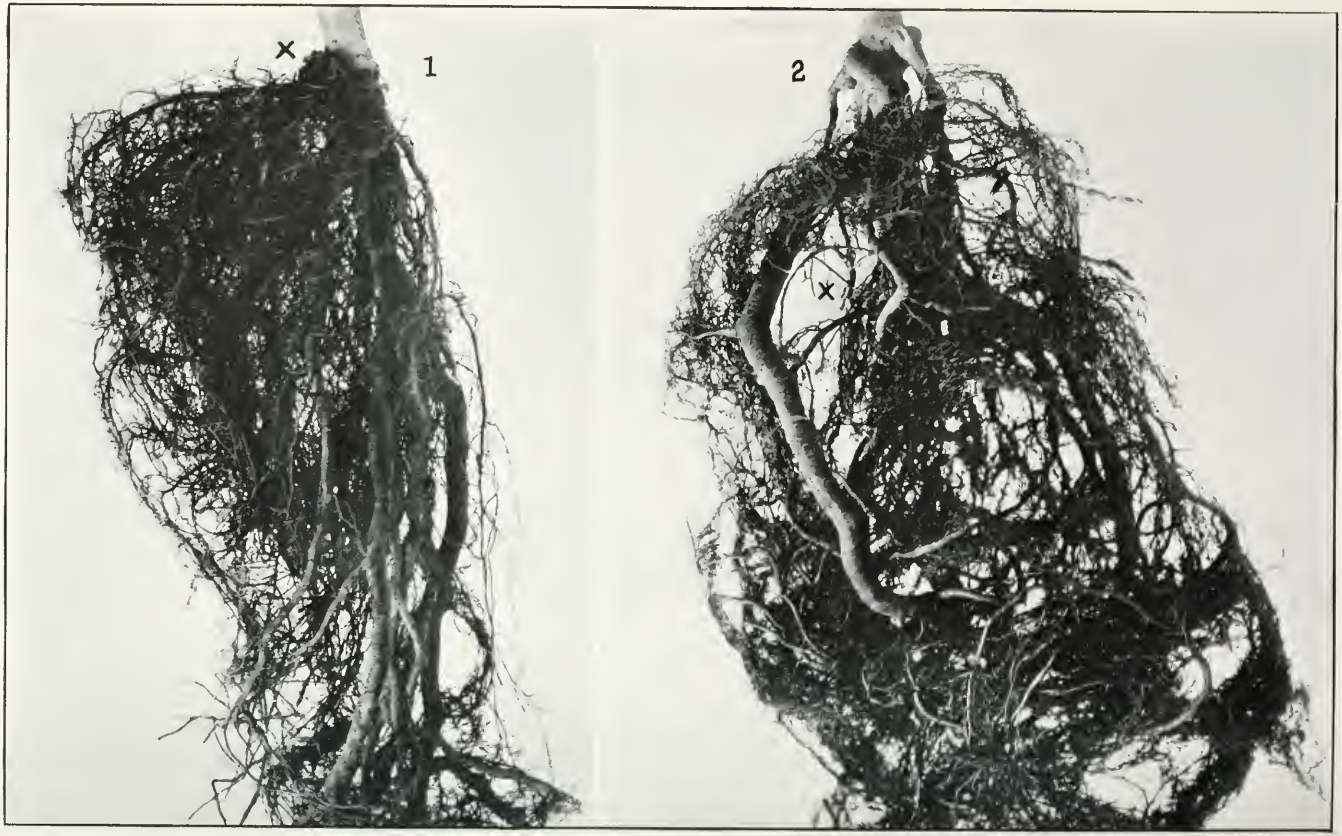
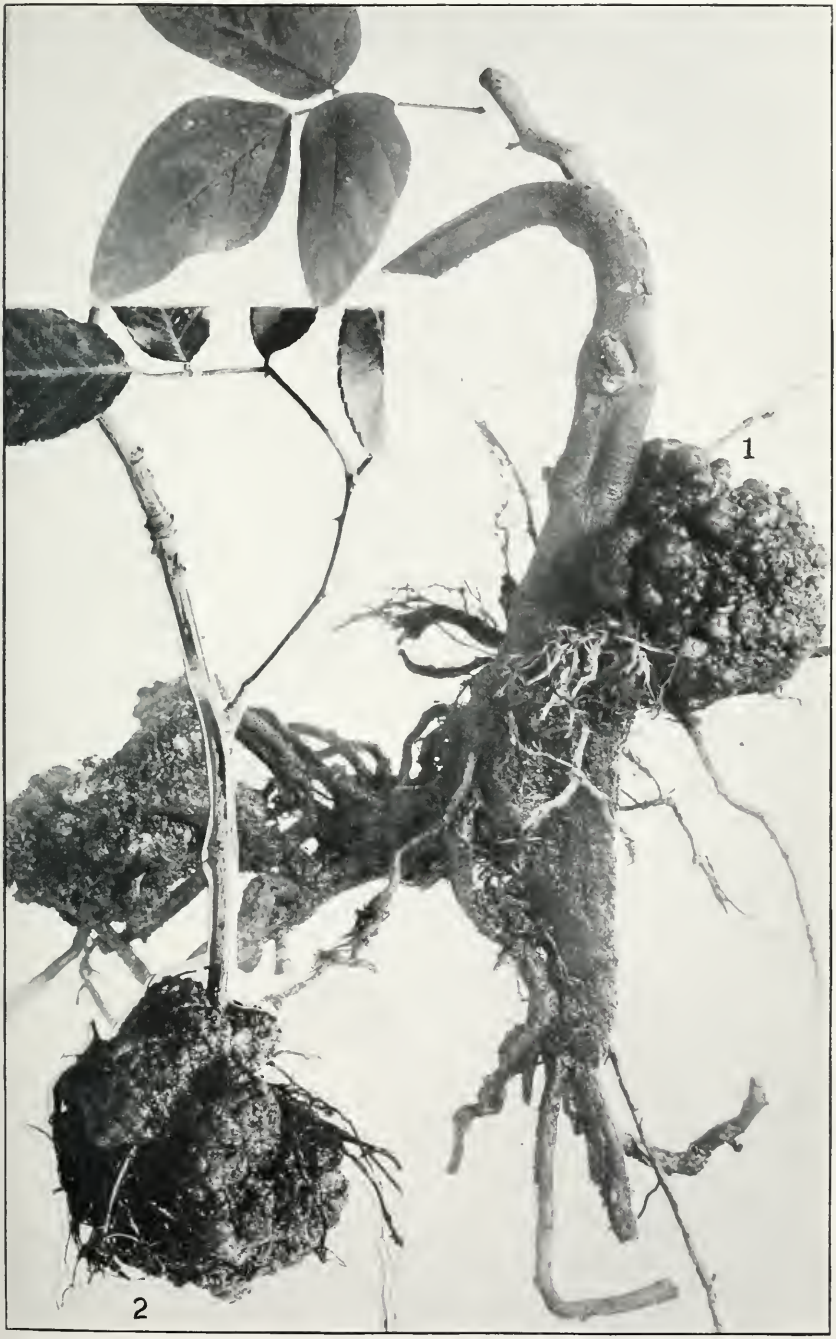


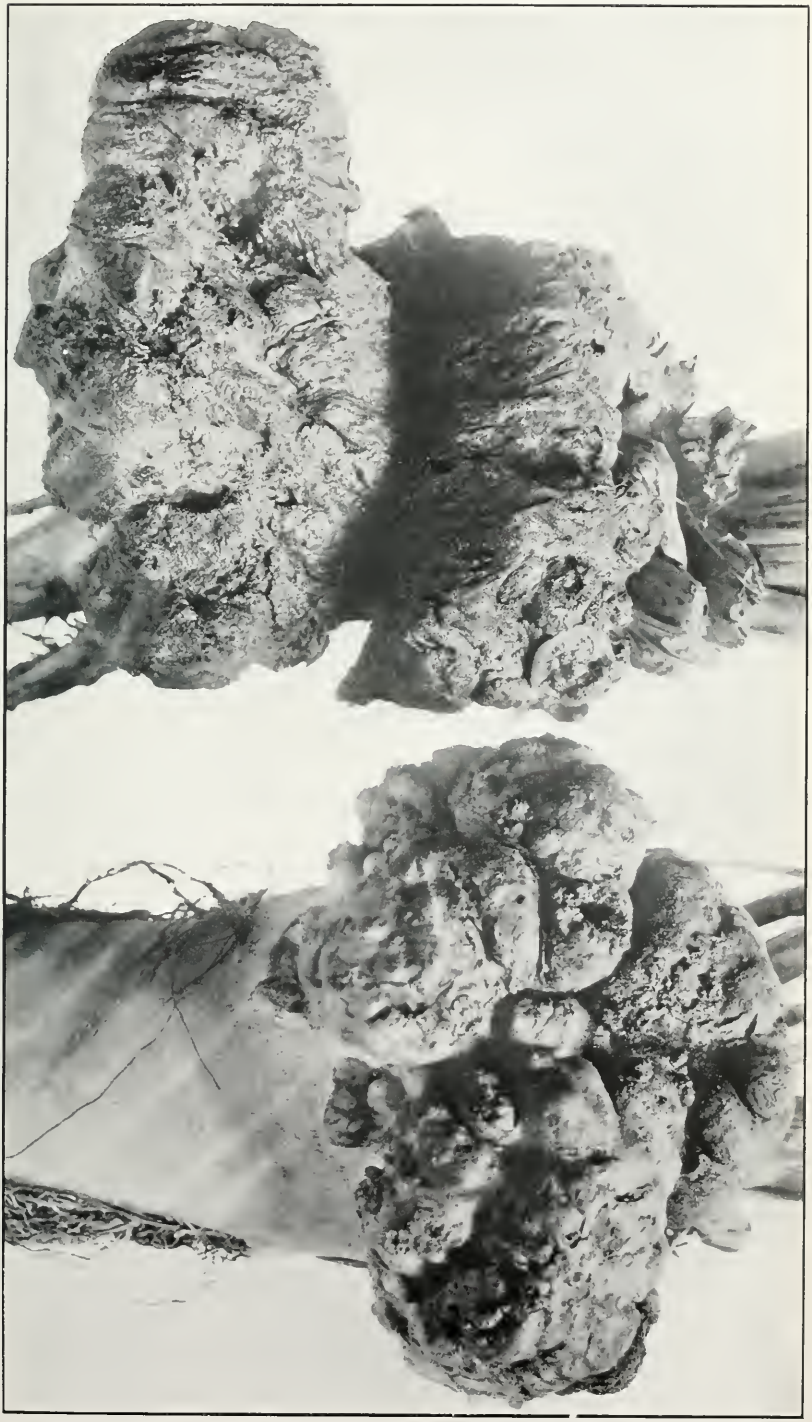
Apple hairy-root inoculated on young apple trees, both photographed after several months in alcohol: (1) Hard gall at x. (2) Typical fleshy roots at right of x. Inoculations of Apr. 5, 1909. Time: 4 months 8 days.

Hairy-root of apple on sugar beet. Two sides of the same beet enlarged twice to show small galls with clusters of roots originating therefrom. Inoculated Nov. 11, 1909. Time: 4 months 27 days.

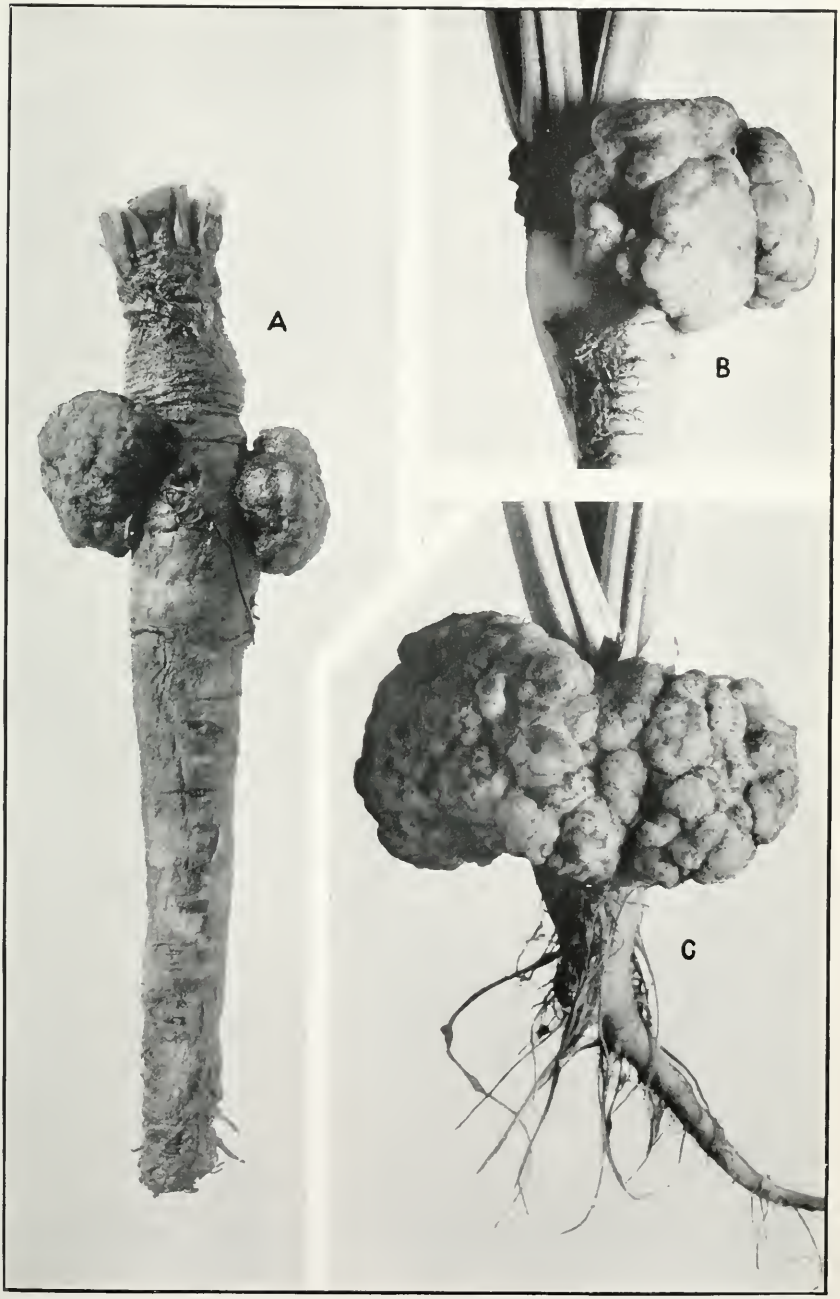


(1) Stizolobium pruriens S. P. I. No. 21300. A nematode infection occurring in the hothouse and supposed at first to be crown-gall; young gall on crown, old decaying gall on root at left.

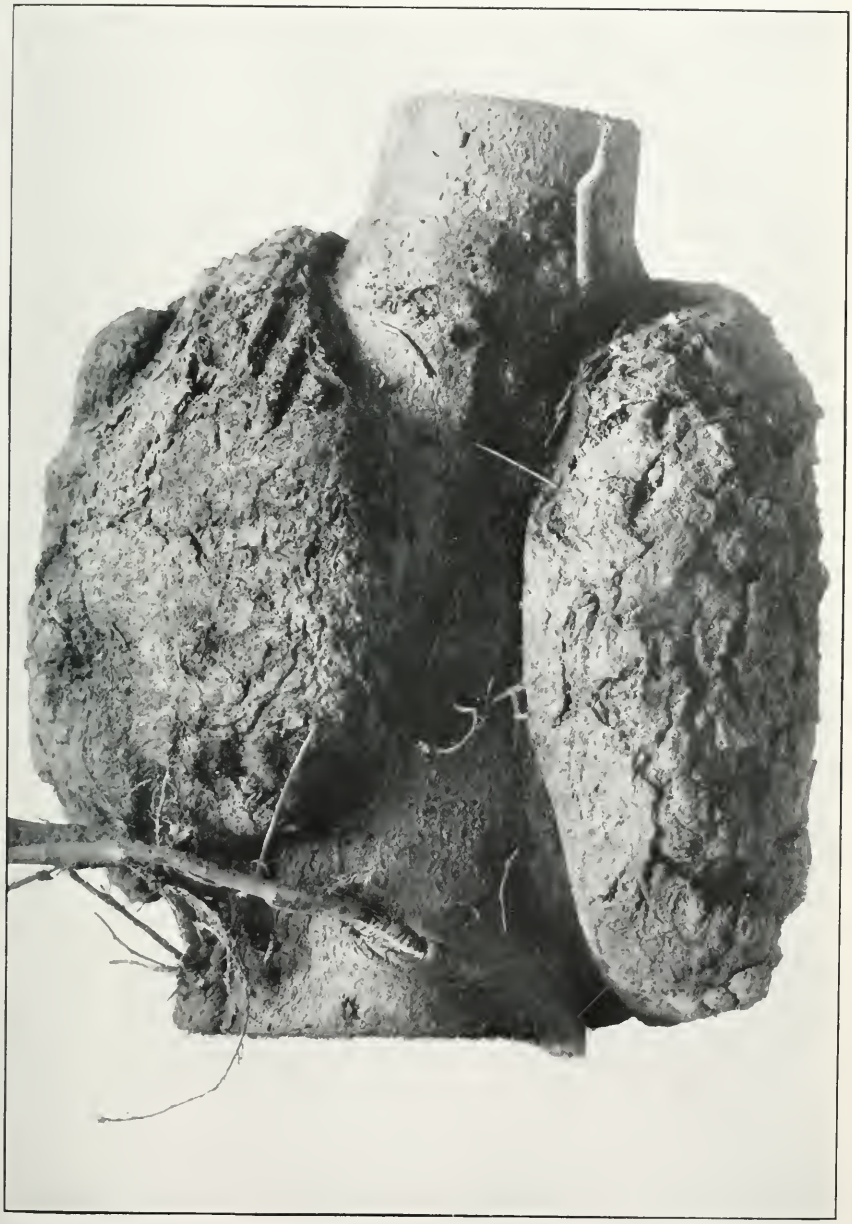
(2) Natural crown-gall infection of young rose, from a hothouse in New Jersey.



Hop on sugar beets. About four-fifths natural size. Inoculations of Mar. 7, 1910, from twenty-sixth subculture. Time: 2 months.



(A) Daisy on salsify. Pure-culture inoculations of Feb. 27, 1908, at the points where the galls developed. Time: 2 months 9 days.
(B, C) Poplar on sugar beet. Pure-culture inoculations of June 4, 1910. Time: 31 days.



Crown-gall on white poplar from Newport, R. I. Three-fourths natural size. Stem above the gall much dwarfed. Except that part here shown, the gall entirely surrounded the stem.

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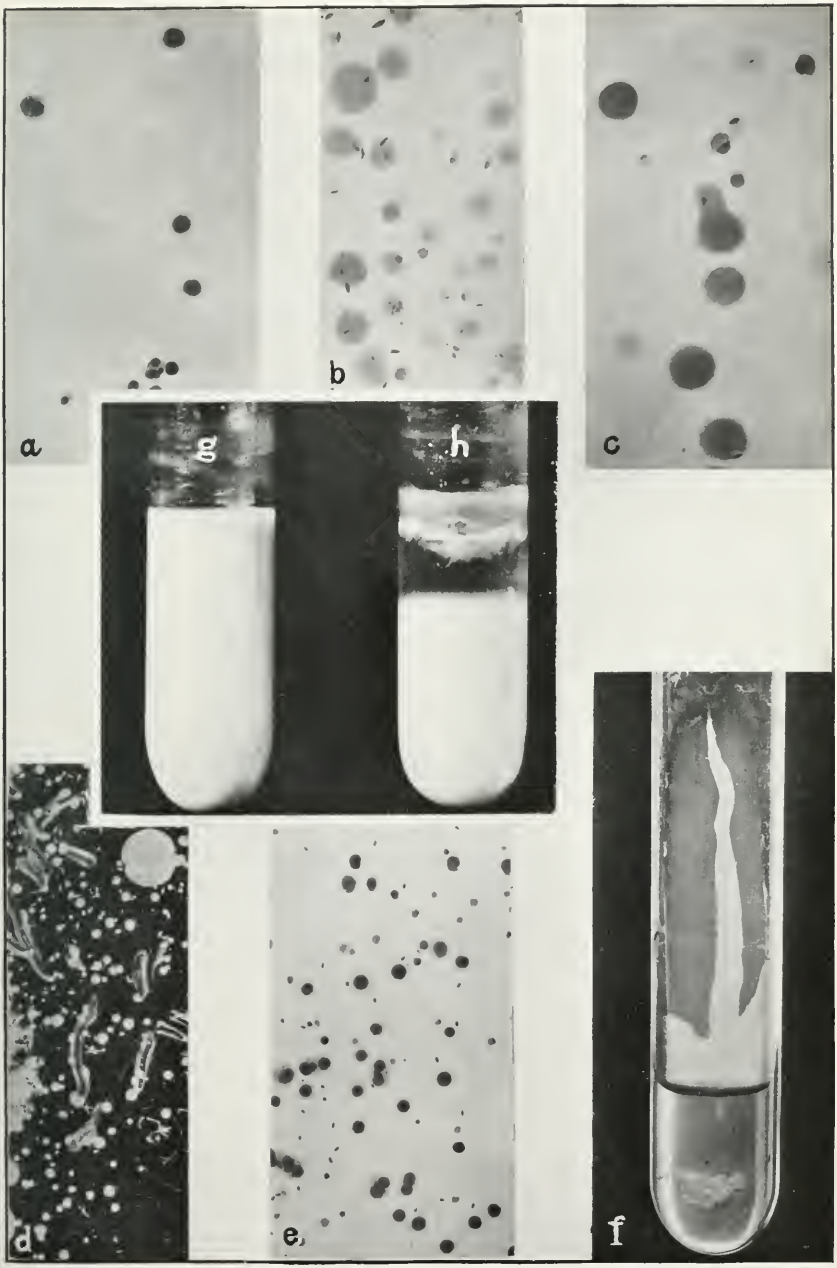
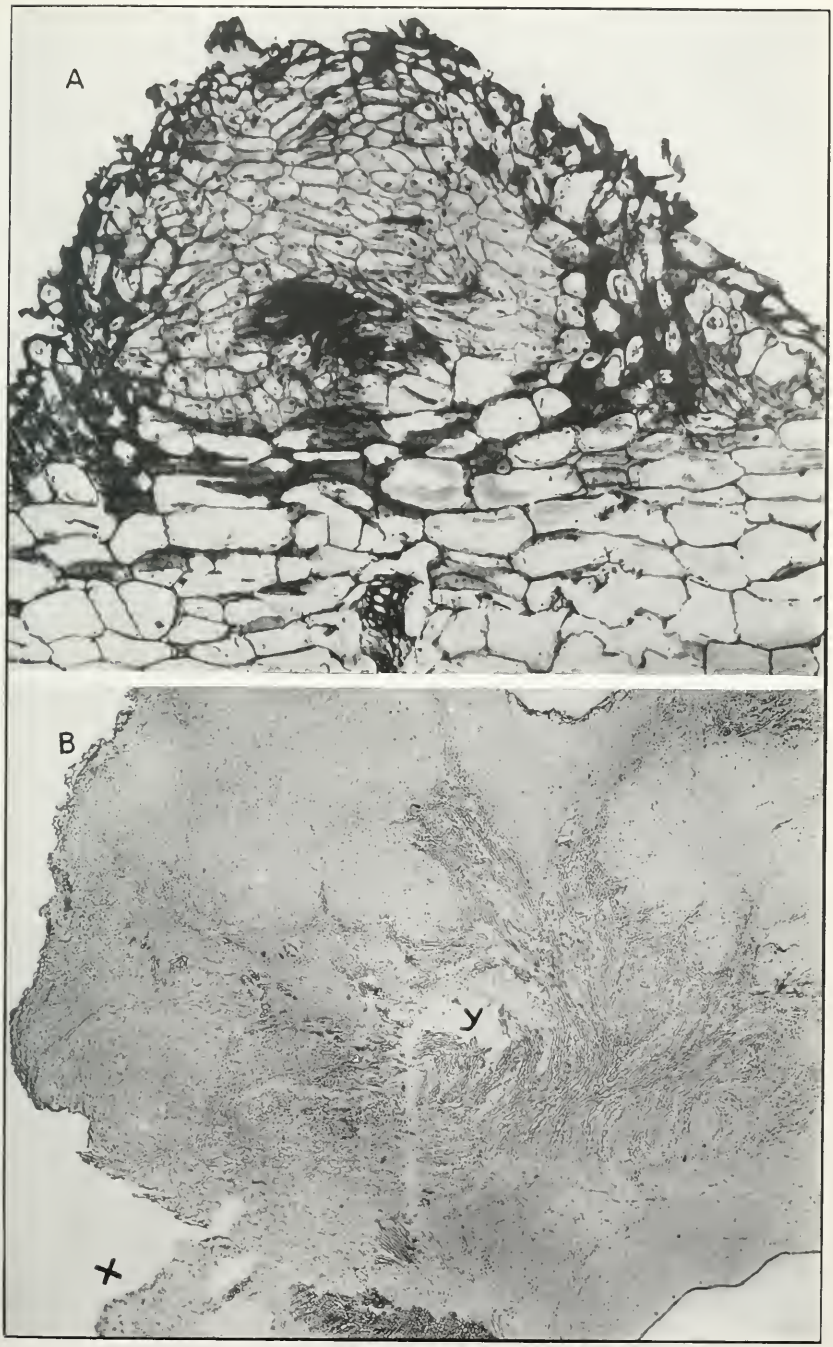
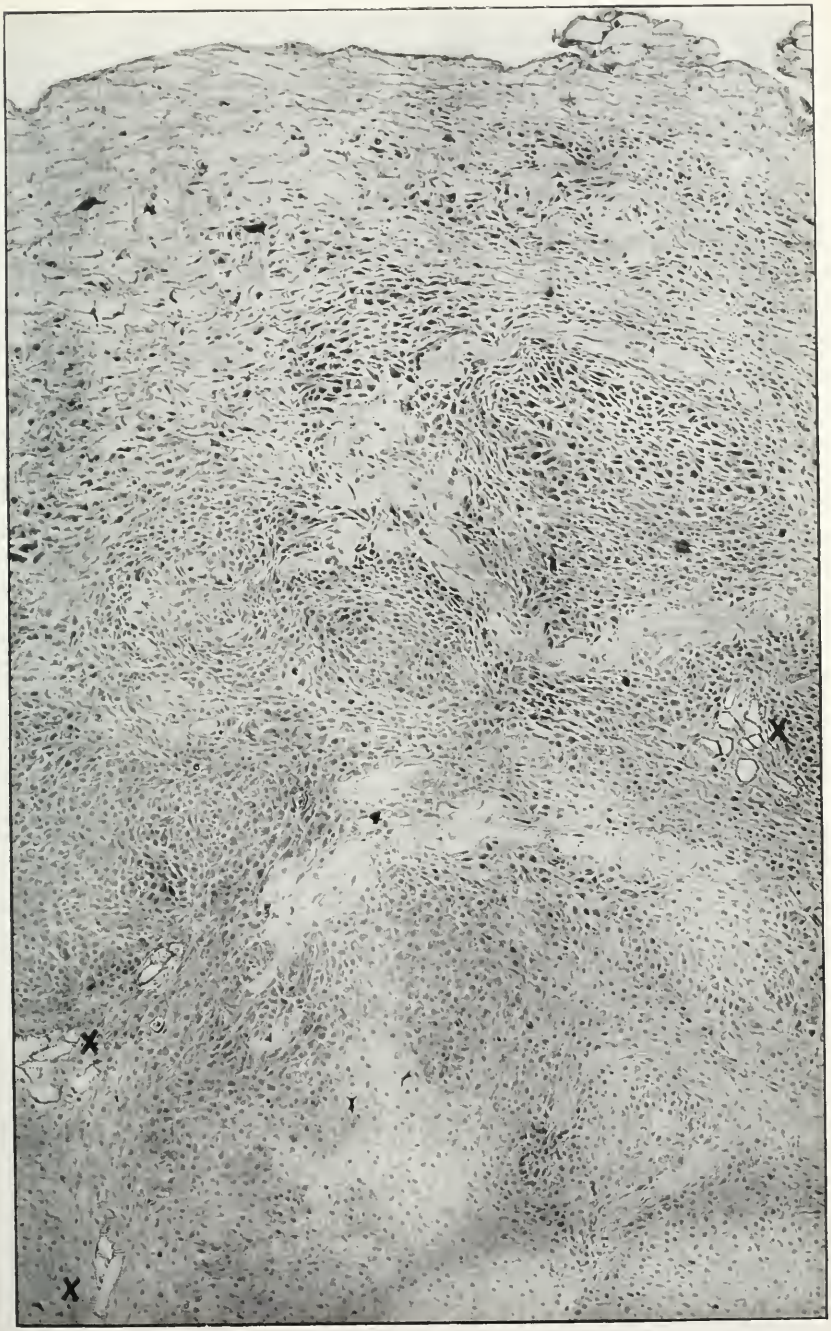
(A) Arbutus on sugar beet. Tumor partly decayed. About three-fourths natural size. Inoculated Nov. 8, 1909. Time: 7 months.
(B) Grape on sugar beet. Inoculated May 7, 1910. Slightly less than natural size. Tumor actively proliferating. Time: 47 days.
(C) Flats poplar developing on immature grape stem. Inoculated June 4, 1910. Every one of the punctures gave a tumor. Time: 1 month 14 days.



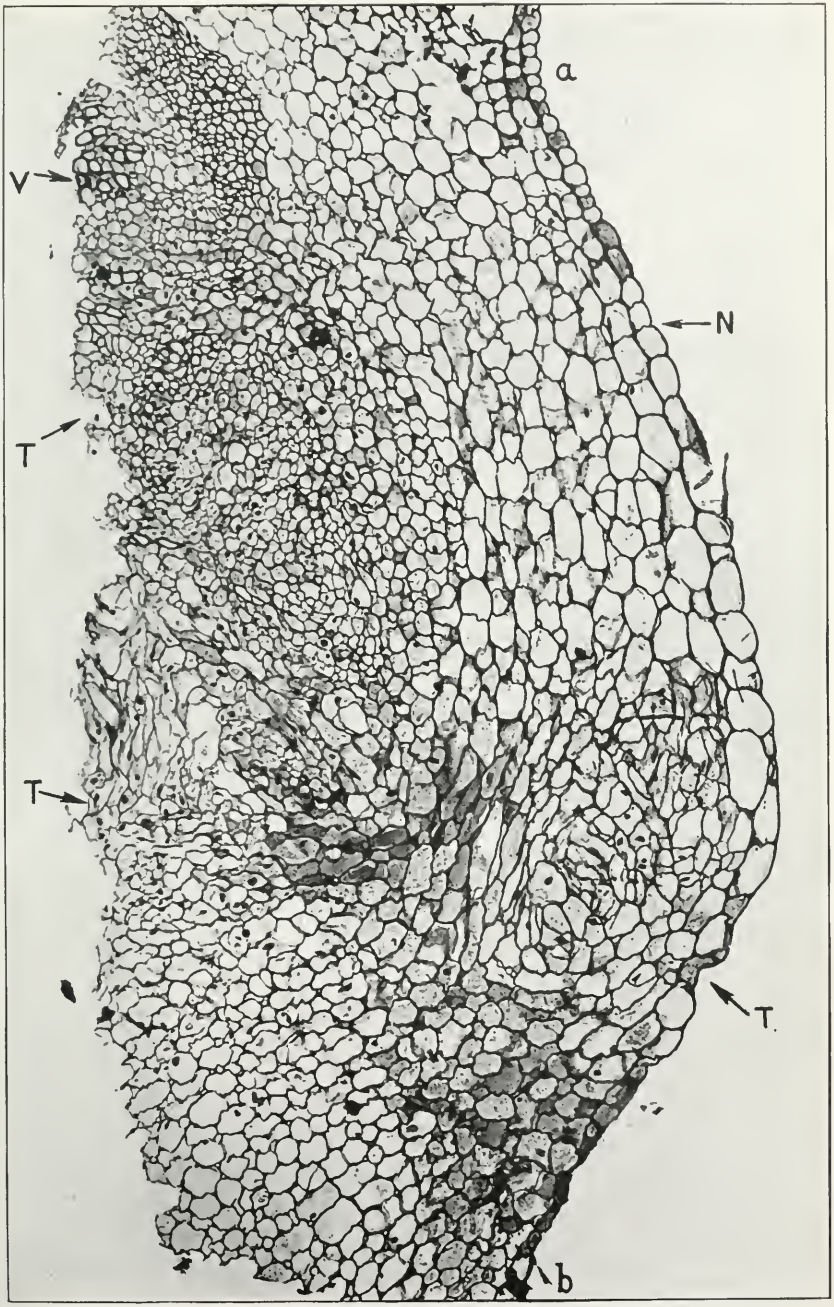
Bacterium tumefaciens on culture media: (a) Daisy organism; agar plate from bouillon; incubated 4 days at 22 to 25° C. (b) Daisy organism from a tumor; agar plate 8 days old. (c) Gall organism from a peach gall; agar plate 14 days old. (d) Hard gall of apple on agar poured plate at end of 5 days after being used for inoculations. The large colony is an intruder. (e) Same as a. (f) Needle stroke of daisy organism on slant agar, photographed after some days. (g) Old tube of sterile milk. (h) Similar tube inoculated 2 months with daisy organism, showing the pellicle formed and slow separation of whey from casein which remains undigested and fluid.



(A) Photomicrograph of section through a very young daisy gall on rib of daisy leaf. The most of the cells of the leaf rib are not yet involved in the abnormal growth.
(B) Photomicrograph of section of small daisy gall, showing supporting stroma and abnormal conductive tissue at y. A small portion of the nearly normal tissue is shown at x.



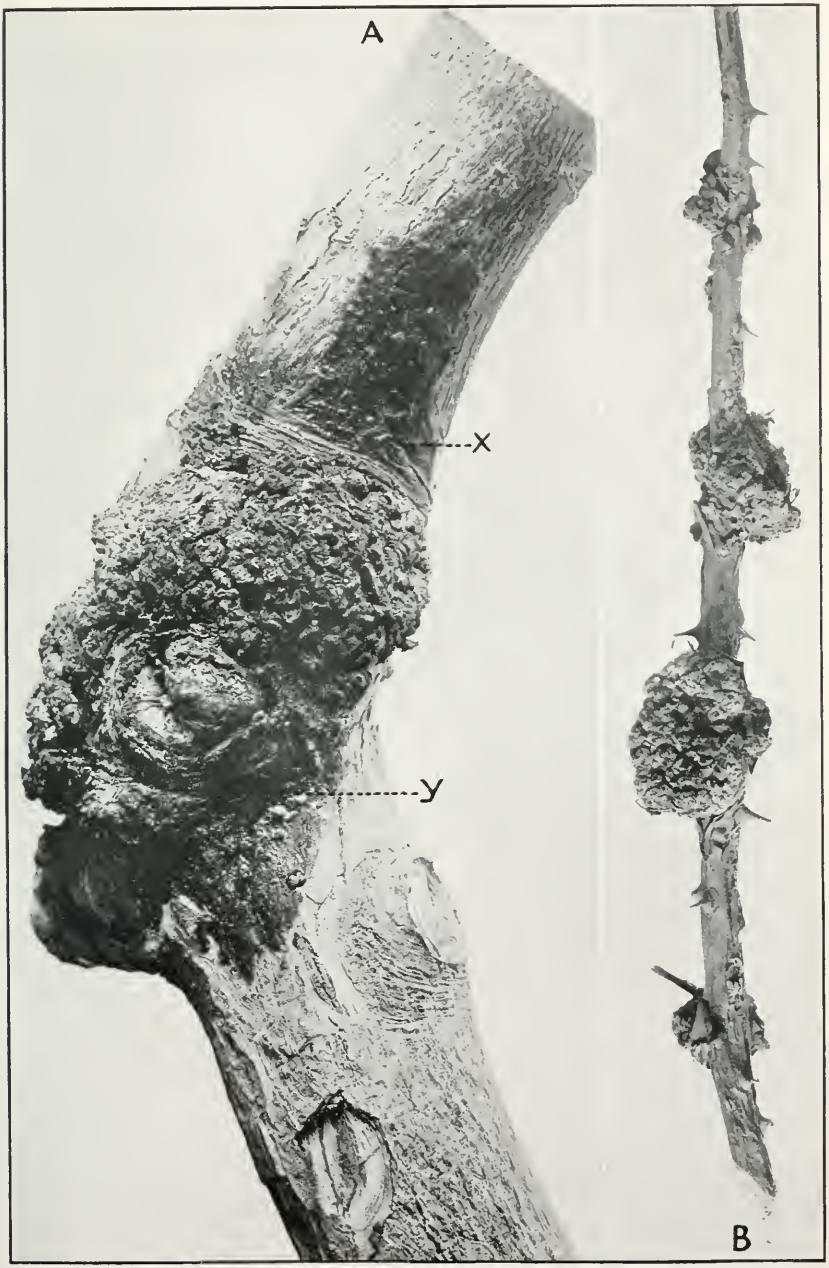
Photomicrograph of section through a rapidly proliferating gall on tobacco. The centers of most active proliferation may be seen crowding the older parenchyma cells out of place. Here and there (x, x, x) may be seen small groups of abnormal vascular bundles. Margin at top. Toward the left at top is nearly unchanged parenchyma.



Cross section of a daisy stem 10 days after inoculation with Bacterium tumefaciens: N, Normal epidermis and cortical parenchyma. V, Vascular bundles nearly unchanged. T, T, T, Rapidly proliferating tumor tissue at a distance of 1 or 2 mm. from the needle puncture. The tissue is pushed up over this hyperplasia from a to b, indicating location of the future gall.

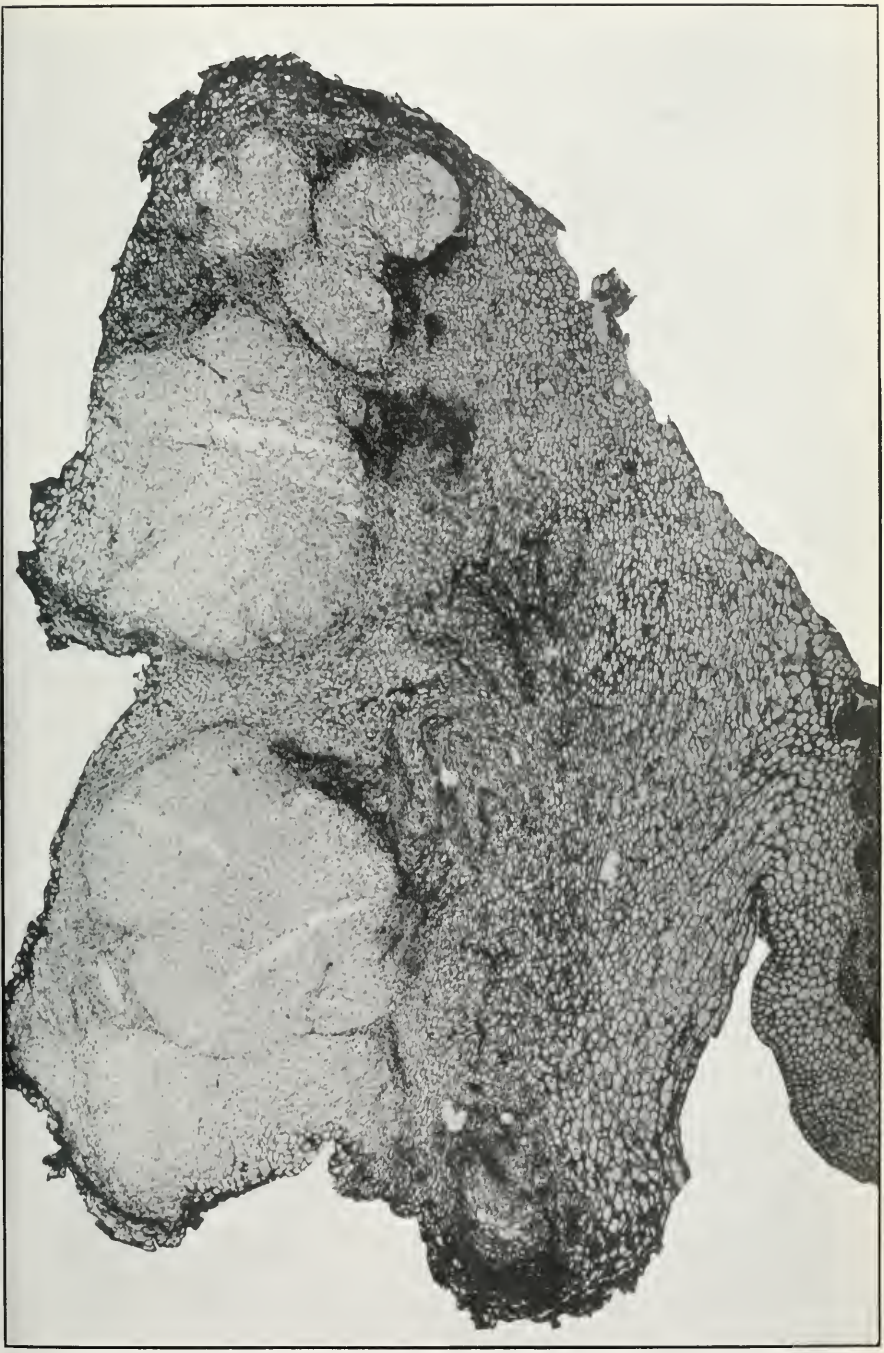
Cross section of outer part of a tobacco stem. The lower half of the plate shows normal cortical parenchyma; the upper (outer) half, rapidly proliferating small-celled tumor tissue resulting from an inoculation. In the upper right-hand corner (in cross section) is a recently developed vascular bundle.

Radial section through a daisy petiole showing the internal origin of a small metastatic tumor. The normal tissues are bracketed, the epidermis is not yet ruptured, and the tumor includes all kinds of tissues peculiar to the petiole.

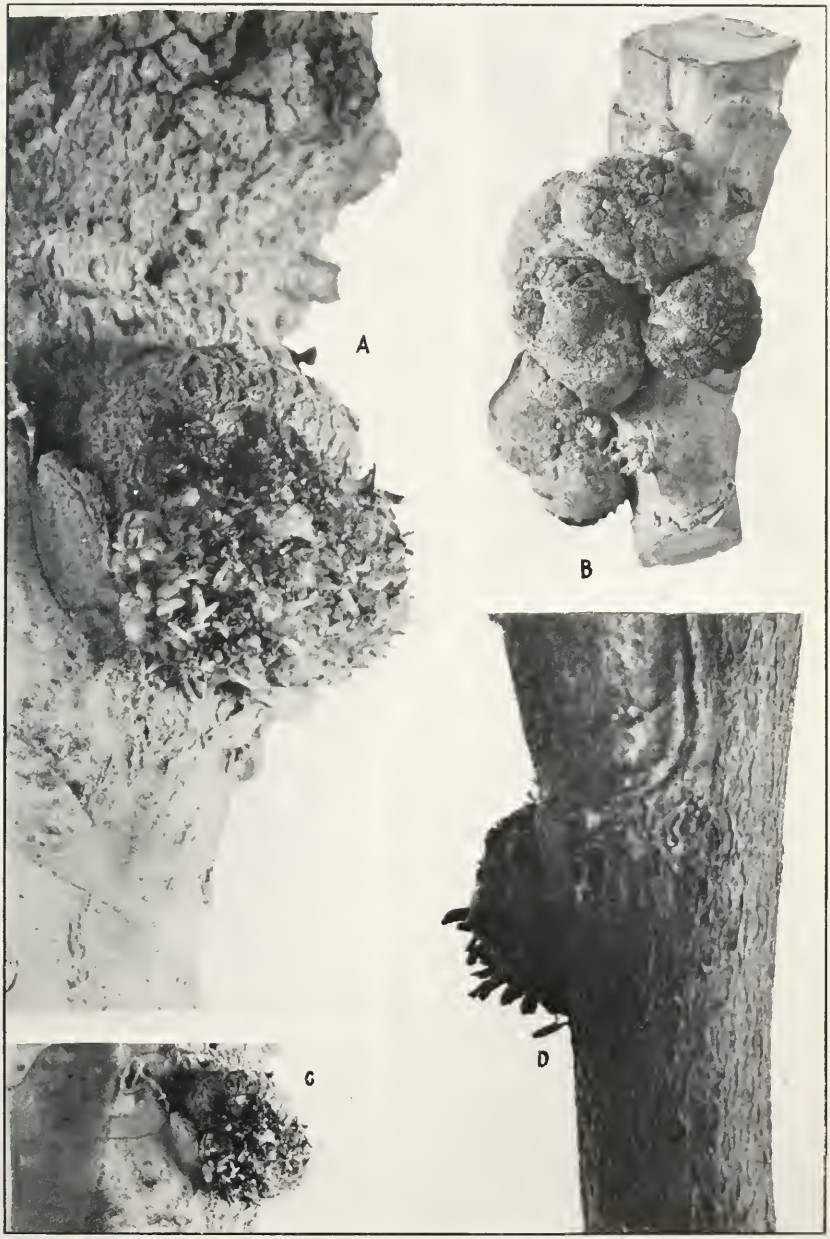


(A) Limb of Spitzenberg apple from Oregon attacked by a hard gall. Introduced to show a secondary infection by the pear-blight organism (B. amylovorus) radiating from the gall. x, y, Blighting areas covered by the bacterial exudate.

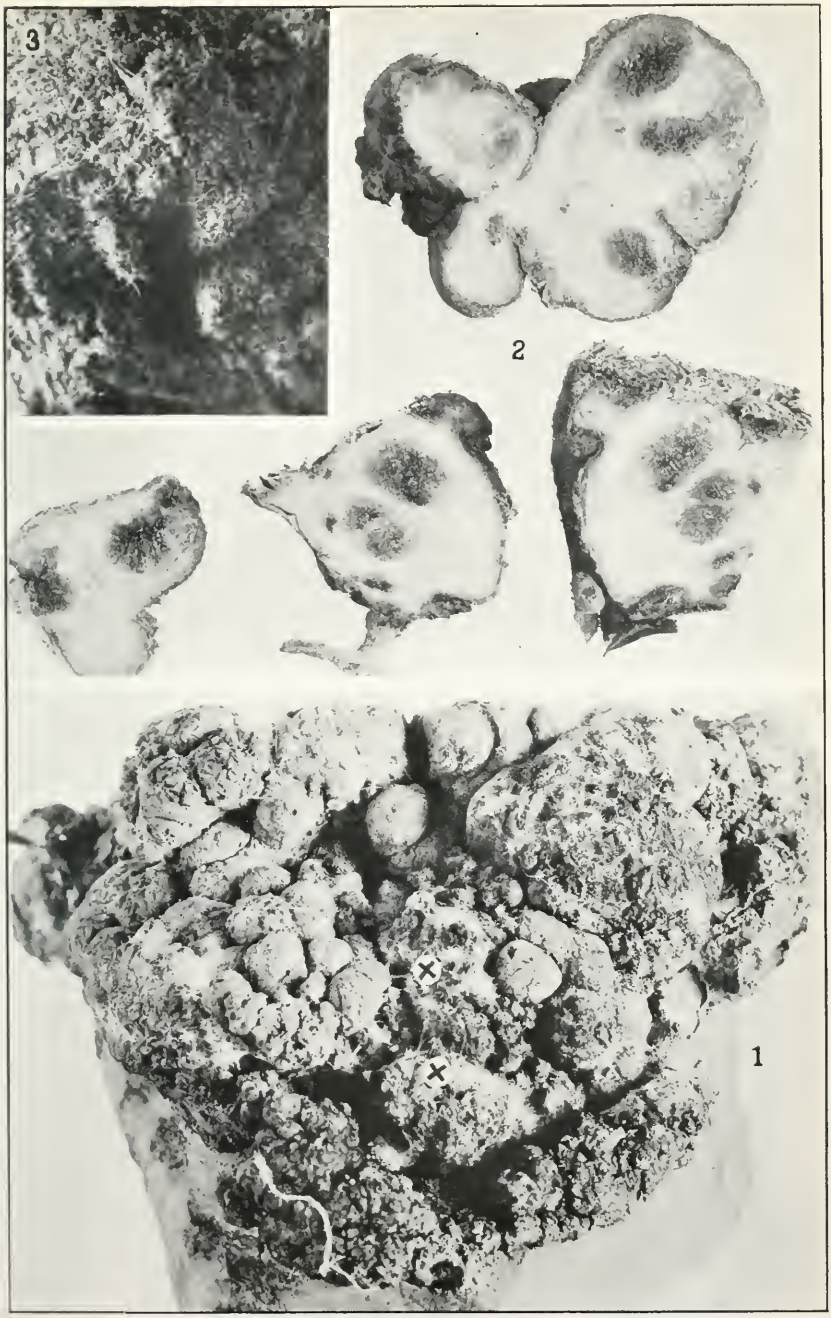
(B) Destructive galls on blackberry received from Prof. L. R. Jones, Madison, Wis. Autumn of 1910.



Photomicrograph of cross section of a daisy stem including a portion of a gall. Introduced to show centers of rapid proliferation. At the right is a portion of the normal stem—wood, bark, epidermis.



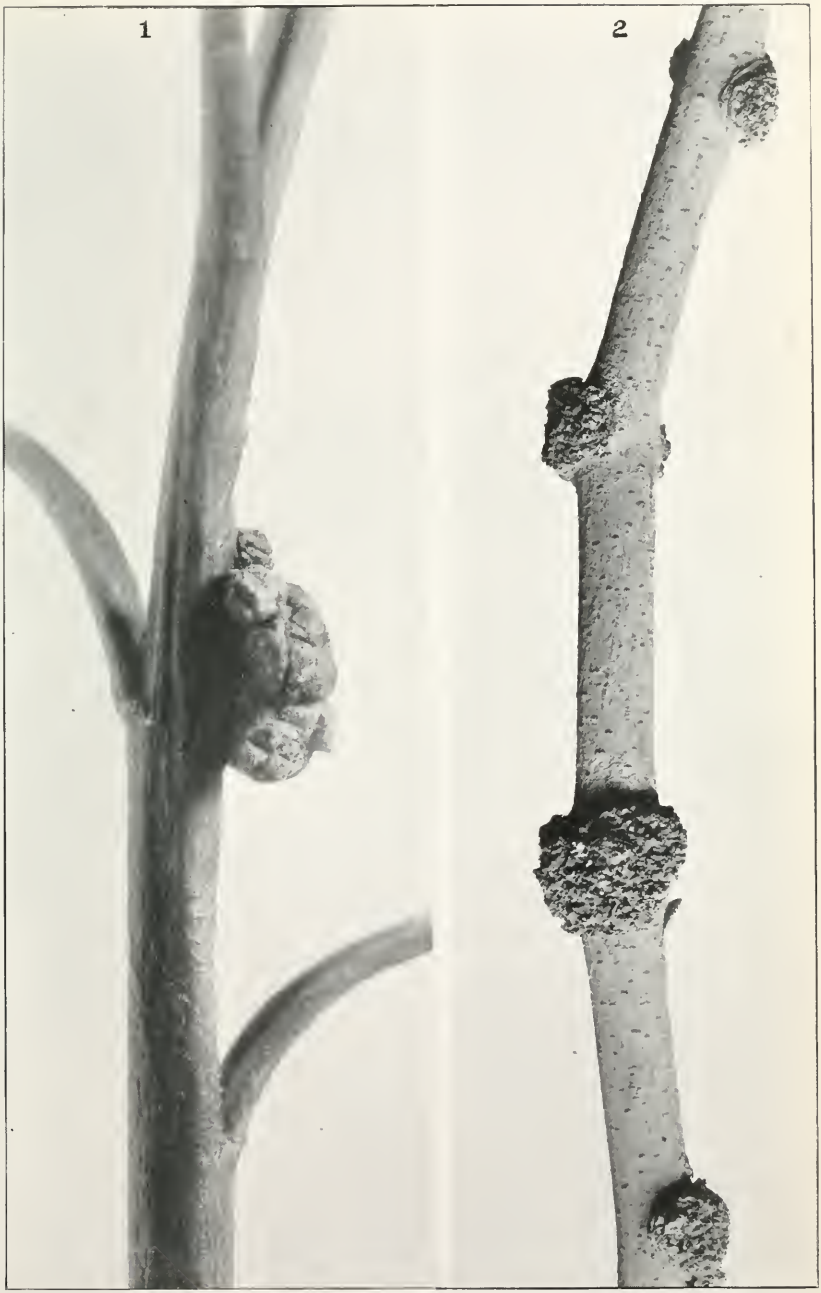
Crown-galls on Brassica due to inoculation: (B) Cabbage. (C) Collard. (A) Enlarged view of C, showing clusters of roots (hairy-root) growing out of the lower half of the gall; year, 1910; time from needle pricks to photograph: 3 months. (D) Hairy-root of apple on quince. Time: 19 months.



(1) Sugar beet from Colorado, showing bacterial tubercles distinct from crown-galls, attacked by fungi, at x, x.

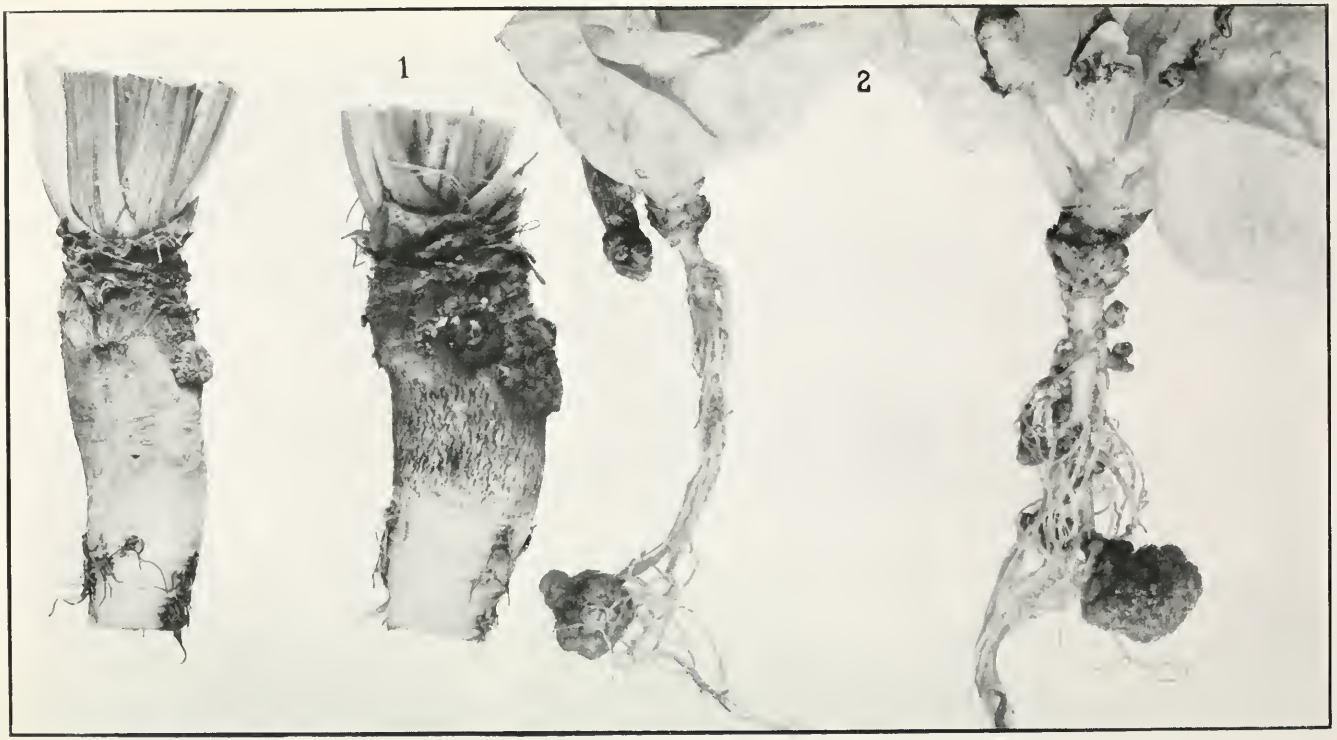
(2) Sections of some of the upner nodules showing the central brownish water-soaked bacterial areas surrounded by white flesh.

(3) The surface of one of the nodules much magnified, to show the small central rifts in the tissue, referred to in the text.



(1) Gall on Salix babylonica induced by needle prick introducing pure subculture of Bacterium tumefaciens plated from a South African willow gall. Enlarged.
(2) Galled quince stem from Dr. Trabut in Algeria for comparison with Plate XXXIII, fig. D.

PLATE XXXVI.



(1) Beet on beet; inoculations of December, 1910. Time: 44 and 52 days.
(2) Hothouse lettuce, Maryland, January, 1911. Badly dwarfed.

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U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 214.
B. T. GALLOWAY, *Chief of Bureau.*

THE TIMBER ROT CAUSED BY LENZITES SEPIARIA.

BY

PERLEY SPAULDING,
Pathologist, Investigations in Forest Pathology.

ISSUED JULY 21, 1911.

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Perley Spaulding, *Pathologist, in Charge of Forest Nursery Diseases.*
Carl Hartley, *Assistant Pathologist.*
C. J. Humphrey, *Scientific Assistant.*
E. P. Meinecke, *Expert.*

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., February 21, 1911.

Sir: I have the honor to transmit herewith a paper entitled "The Timber Rot Caused by Lenzites Sepiaria," by Dr. Perley Spaulding, Pathologist in the Office of Investigations in Forest Pathology of this Bureau. I recommend that it be published as Bulletin No. 214 of the series of this Bureau.

This paper summarizes and brings up to date our knowledge concerning this serious wood-rotting fungus. It contains new information concerning its life history and gives practical methods of preventing its ravages. It is designed as a contribution toward our knowledge of the fundamental facts of forest pathology in this country. At the time the manuscript was first prepared there was no adequate publication upon this form of timber rot in any language; recently, however, such a paper, Falck's "Die Lenzitesfäule des Coniferenholzes," has been issued in Germany. So far as the two papers cover the same ground they agree in essentials, but vary in minor details. The fields and conditions are so different in the two countries that nothing else could be expected.

Dr. Spaulding is indebted to the custodians of the following herbaria for the privilege of collecting data from their collections: Missouri Botanical Garden, University of Wisconsin, New York Botanical Garden, New York State Museum, Harvard University, University of Vermont; also to Mr. E. T. Harper, for allowing similar work in his private herbarium.

Respectfully,

Wm. A. Taylor,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE TIMBER ROT CAUSED BY LENZITES SEPIARIA.

INTRODUCTION.

The value of the total timber and wood cut in the year 1908[a] in the United States was slightly more than $1,000,000,000. About three-fourths of this immense production was supplied by the coniferous species of trees. A large proportion of the timber used in heavy construction, such as bridges, railroad ties, trestles, etc., is coniferous. One may obtain a slight idea of the enormous quantity of coniferous timber that is required from the fact that untreated coniferous railroad ties last only from 1 or 2 to 10 years, according to their conditions of use, the average length of service being about 7 years. Because of the great aggregate values involved, any factor which influences the length of service of this timber becomes a matter of primary importance. This is especially true at the present time when we are threatened with a shortage of timber of all kinds. The most important factors affecting the length of service of coniferous timber when exposed to the weather or in contact with the soil are the wood-rotting fungi. These greatly shorten the period of usefulness of such timber and thus help to increase the already too great demands upon our forests.

While there are dozens of wood-rotting fungi which attack coniferous wood, certain ones are especially prevalent and destructive in their action. *Lenzites sepiaria* (Wulf.) Fr. and *Lentinus lepideus* Fr. are probably the most widespread and injurious in this country. The former is most destructive in the southern part of the country, while the latter is very prevalent in the northern part, although both are widely distributed in both sections. *Lenzites sepiaria* is common wherever coniferous timber grows or is used. In the north the climatic conditions are such that peeled timber will season before the fungus can get well started in its growth, but it succeeds in causing the decay of unpeeled timber.

In spite of the economic importance of this fungus, not only in America but in Europe, no publication, so far as the writer knows,

[a] Bureau of the Census, Forest Products, vol. 10, p. 5, 1909.

adequately considers the decay caused by *Lenzites sepiaria*.[a] Practically all the literature concerning this species and the timber rot caused by it consists of short notes on occurrence and short paragraphs upon the damage caused.

ECONOMIC IMPORTANCE OF LENZITES SEPIARIA.

The damage inflicted in America alone by *Lenzites sepiaria* is enormous. This fungus, together with several others, destroys a large proportion of all untreated coniferous railroad ties and telegraph and telephone poles which are in service in the country. Probably one-fourth of this damage is done by *Lenzites sepiaria*.

The valuation of the railroad ties and telegraph and telephone poles furnished by the coniferous species of trees in 1908[b] was in round numbers $32,500,000. If the above estimate of damage done by *Lenzites sepiaria* is anywhere near correct, this would mean that timber worth about $8,000,000 annually has its length of service seriously shortened by this fungus. Under present methods of American railroading it is probable that an average length of service of an unrotted coniferous tie would scarcely be more than 12 to 15 years—that is, the tie will be worn out by the end of this period. The actual average service of untreated coniferous ties can hardly be placed at more than 5 to 8 years. Thus, we find the wood-rotting fungi practically diminishing the service of this timber by one-half. Moreover, there are vast quantities of timber in the form of piling, bridge timbers, trestles, sidewalks, fence posts, etc., which are also destroyed by this fungus.

DISTRIBUTION AND HOST WOODS OF LENZITES SEPIARIA.

[The location of certain cabinet specimens is indicated by arbitrary signs as follows: (*), In the herbarium of the Missouri Botanical Garden; (†), in the pathological collections of the Bureau of Plant Industry; (‡), in the herbarium of the New York Botanical Garden; (§), in the cryptogamic herbarium at Harvard University; (‖, with number), in the forest pathological field collection; (¶), in the private herbarium of E. T. Harper; (**), in the herbarium of the New York State Museum; (††), in the Frost Herbarium at the University of Vermont; (‡‡), in the herbarium of the University of Wisconsin.]

GEOGRAPHIC DISTRIBUTION.

The Fungus in Foreign Countries.

Lenzites sepiaria has been reported from and collected in the following countries:

EUROPE.

England: Berkeley (1836, 1860), Cooke (1871, 1883, 1888–1890), Smith (1891), Sowerby (1814), Stevenson (1886).
Norway: Blytt (1905), Fries (1849), Karsten (1882).

[a] Since this manuscript was prepared the writer has first seen Falck's Die Lenzitesfäule des Coniferenholzes, issued as part 3 in Möller's Hausschwammforschungen, 1909.

[b] Bureau of the Census, Forest Products, vol. 10, pp. 66–67, 107, 1909.

Sweden: Fries (1849, 1863), Karsten (1882), Murrill (1904, 1908), Persoon (1799, 1801), Vleugel (1908), Wahlenberg (1820, 1826, 1833).

Russia: Lapland—Karsten (1876), Sommerfeldt (1826), Wahlenberg (1812). Finland—Karsten (1876, 1881, 1882, 1889, Fung. Fenn. No. 88), Thesleff (1894). Moscow—Bucholtz (1897). St. Petersburg—Perdrizet (1876).

Denmark: Fries (1849), Hornemann (1837), Rostrup (1902).

Germany: Bachmann (1886), Fuckel (1869), Hennings (1898, 1903), Hoffmann (1797–1811), Magnus (*), Pabst (1876), Rabenhorst (1840, 1844), Röhling (1813), Winter (1884). Baden—Jack, Leiner, and Stizenberger (Krypt. Badens, No. 936). Bavaria—Allescher (1884), Allescher and Schnabl (†), Britzelmayr (1885), Magnus (1898), Schaeffer (1800), Schrank (1789). Brandenburg—Hennings (1903), Sydow (Myco. March. No. 716). Hessen-Nassau—Von Braune (1797), Gärtner, Meyer, and Scherbius (1802). Prussia—Nitardy (1904). Saxony—Brick (1898), Krieger (Fung. Saxon. No. 69), Von Thümen (Myco. Univers. No. 2202). Silesia—Aderhold (1902), Schroeter (1888). Thuringia—Hennings (1903a).

Austria-Hungary: Winter (1884). Bohemia—Bodenath (‡) Corda (1842). Karnten—Jaap (1908). Lower Austria—Strasser (1900). Transylvania—Barth (‡). Tyrol—Bresadola (see Murrill, 1904), De Cobelli (1899), Von Dalla Torre, Von Sarnthein, and Magnus (1905), Von Höhnel (1909), Jaap (1901, 1908), Kerner (Fl. Exsicc. Austr.-Hung. No. 761), Von Sarnthein (1901). Voralberg—Von Dalla Torre, Von Sarnthein, and Magnus (1905).

Servia: Ranojevic (1902).

Italy: Rome—Lanzi (1902). Venice—Pollini (1824), Saccardo (1879).

Switzerland: Fries (1828), Murrill (1904, ‡), Neuweiler (1905), Ruffieux (1904), Schenk (‡), Secretan (1833).

Holland: Oudemans (1867, 1893).

Belgium: Kickx (1867).

France: Arnould (1893), Bigeard and Jacquin (1898), Clerc (1902), Desmazières (Pl. Crypt. Fr. No. 2155), Gillet (1874), Gillot and Lucand (1888), Guillemot (1893), Matruchot (1902), Paulet and Léviellé (1855), Persoon (1799), Quélet (1886, 1888), Roumeguère (Fung. Gall. Exsicc. No. 855).

Spain: Colmeiro (1889).

ASIA.

Siberia: Hennings (1898), Von Thümen (1878).

East Indies: Java—Kops and Van der Trappen (1849).

AUSTRALIA.

Victoria: Cooke (1892), McAlpine (1895).

SOUTH AMERICA.

Argentine Republic: Spegazzini (1899).

Brazil: Rick (1904).

NORTH AMERICA.

Canada: Dupret (see Lloyd, 1906a, 1908b), Fowler and Langton (see Lloyd, 1909), Macoun (see Murrill, 1904).

British Columbia—Hill (‡).

New Brunswick—Hay (§).

Nova Scotia—Somers (1880).

Ontario—Dearness (‡), Macoun (‡).

Newfoundland: Robinson and Von Schrenk (§).

Mexico: Egeling (‡).

These reports indicate that *Lenzites sepiaria* is present throughout Europe; that it is prevalent and probably widely distributed in Australia and the neighboring islands, including the East Indies, and is widely distributed in South America. In North America it is undoubtedly present in Canada and Newfoundland throughout the coniferous forests; and it is probably equally prevalent in the coniferous forests of Mexico.

The Fungus in the United States.

Lenzites sepiaria has been reported from and collected in the various States of this country as follows:

Alabama: Earle (1901), Earle and Baker (‡), Humphrey (‖ No. 5295), Von Schrenk (see Murrill, 1904, ‡), Underwood and Earle (1897).

Arizona: Burrall (‖ Nos. 1089, 1156, 1162, 1163, 1168), Hedgcock († ‖ No. 4889).

Arkansas: Humphrey (‖ No. 5646).

California: Harkness and Moore (1880), Hedgcock (‖ No. 1889), Palmer (§).

Colorado: Baker (‡), Baker, Earle, and Tracy (†), Bethel (‡), Harper (¶), Hartley (‖ Nos. 1641, 1678, 1775), Hedgcock (‖ Nos. 576, 577, 618, 619, 620, 651, 852, 889, 921, 1606, 1626, 1632, 1634, 1635, 1930), Hedgcock and Hartley (‖ Nos. 609, 692, 694, 697), Hodson (‖ No. 1177), Knaebel (see Lloyd, 1908a), Underwood and Selby (‡, see Murrill, 1904).

Connecticut: Spaulding (‖ No. 2275), White (1905), Miss White (see Murrill, 1904).

Delaware: Commons (†).

District of Columbia: Spaulding (‖ No. 106).

Florida: Britton (see Murrill, 1904, ‡), Calkins (†), Fisher (see Lloyd, 1907), Noble (see Lloyd, 1902).

Georgia: Humphrey (‖ Nos. 5047, 5059, 5090, 5091, 5117, 5194).

Idaho: Hedgcock (‖ Nos. 877, 978, 979, 4452, 4725, 4741, 4742).

Illinois: Clute (see Lloyd, 1909), Harper (¶), Moffatt (1909).

Indiana: Harper (¶). Moffatt (1909).

Iowa: Bessey (1884).

Louisiana: Hedgcock (‖ Nos. 363, 373, 394, 404), Humphrey (‖ Nos. 5328, 5333, 5385, 5687), Langlois (†, 1887).

Maine: Blake (‡, see Ricker, 1902, see Sprague, 1858), Harvey (see Ricker, 1902), Harvey and Knight (1897), Ricker (1902), Von Schrenk (†), Spaulding (‖ Nos. 103, 107, 108), Sprague (1858), Miss White (‡, see Murrill, 1904), White (1902).

Maryland: Graves (‖ No. 3735), Lakin (see Lloyd, 1907), Scribner (†).

Massachusetts: Farlow (1876), Huntington (see Lloyd, 1907), Mackintosh (see Lloyd, 1907), Pierce (see Lloyd, 1907), Smith (see Lloyd, 1906a), Webster (§).

Michigan: Harper (¶), James (see Lloyd, 1902), Longyear (1904), Von Schrenk (‖ No. 1109).

Minnesota: Arthur (†, 1887), Holway (‡), Hedgcock (‖ Nos. 4101, 4121, 4122, 4162).

Mississippi: Earle (‡), Hedgcock (‖ No. 332), Humphrey (‖ No. 5281).

Missouri: Glatfelter (1906), Spaulding (*).

Montana: Anderson (‡, see Murrill, 1904), Blankinship (‡), Mrs. Fitch (‡), Hedgcock (‖ Nos. 955, 966, 4240, 4252, 4299, 4322, 4380, 4408, 4409, 4443, 4528, 4531, 4617, 4645, 4688, 4694), Rydberg and Bessey (‡, see Murrill, 1904).

Nebraska: Bates (see Lloyd, 1909), Webber (1890), Williams (†).

New Hampshire: Jones (see Lloyd, 1906a), Minns (‡), Sargent (see Lloyd, 1908a), Spaulding (‖ Nos. 2221, 2919, 2920, 2950), Warner (see Lloyd, 1906a).

New Jersey: Britton (1881), Ellis (North American Fungi No. 1), Von Schrenk (‖ No. 105), Sterling (see Lloyd, 1908a).
New Mexico: Hedgcock (‖ Nos. 259, 454, 543, 808).
New York: Clinton (†), Clute (see Murrill, 1904), Dobbin (see Lloyd, 1907), Harper (¶), Humphrey (see Lloyd, 1907), Jeliffe (see Murrill, 1904), Peck (** 1869, 1879, 1883, 1884, 1893, 1899, 1901), Smith (†), Spaulding (‖ Nos. 2043, 2051; 2241, 2253), Underwood (see Murrill, 1904), Underwood and Cooke (‡), Weld (see Lloyd, 1906a).
North Carolina: Curtis (§, 1867), Graves (‖ No. 3544), Humphrey (‖ No. 5021), Ravenel (Fung. Amer. No. 208).
North Dakota: Brenckle (see Lloyd, 1907), Waldron (see Lloyd, 1906a).
Ohio: Bubna (see Lloyd, 1908b), James (†), Morgan (1883).
Oregon: Hedgcock (‖ Nos. 36, 1714, 1731, 1732, 1748, 1752, 1825).
Pennsylvania: Dallas (see Lloyd, 1906a), Murrill (‡), Von Schweinitz (1832).
Rhode Island: Bennett (1888).
South Carolina: Curtis (§), Humphrey (‖ No. 5021), Ravenel (Fung. Amer. No. 208).
Tennessee: Murrill (1904).
Texas: Billings (‡), Von Schrenk (1904), Spaulding (‖ No. 444), Wright (§).
Vermont: Frost (††), Pringle (§), Spaulding (‖ Nos. 2090, 2091, 2234, 2235, 2317, 2904, 2905).
Virginia: Humphrey (‖ Nos. 5005, 5007), Murrill (‡).
Washington: Harper (¶), Humphrey (‖ Nos. 5860, 5869, 5934, 5964, 6009, 6048), Piper (see Lloyd, 1902).
West Virginia: Millspaugh (1892), Millspaugh and Nuttall (1896).
Wisconsin: Cheney (‡‡), Harper (¶), Neumann (‡‡, 1905).

The above-cited localities show that *Lenzites sepiaria* is prevalent throughout the United States wherever coniferous forests grow or coniferous species of wood are used.

KINDS OF WOOD ATTACKED BY LENZITES SEPIARIA.

Lenzites sepiaria is generally understood to be limited to species of coniferous wood, while *L. vialis* Peck usually is found only on deciduous species. Like other rules, this one has its exceptions, and *L. sepiaria* is occasionally found on the wood of some deciduous trees. The records available show that it has been found upon the wood of the following species:

Abies sp.—Farlow and Seymour (1888), Saccardo (1898), Waghorne (*).
A. balsamea (Linn.) Mill.—Harper (¶), Spaulding (‖ Nos. 107, 925, 2043).
A. grandis Lindl.—Hedgcock (‖ Nos. 1732, 1931).
A. lasiocarpa (Hook.) Nutt.—Hedgcock (‖ Nos. 619, 621, 921, 4291, 4645, 4696).
Alnus sp.—Rick (1898).
Juniperus pachyphloea Torr.—Burrall (‖ No. 1162).
Larix laricina (Du Roi) Koch—Neumann (1905), Von Schrenk (1904).
L. occidentalis Nuttall—Hedgcock (‖ Nos. 4697, 4725).
Picea sp.—Millspaugh (1892), Peck (**).
P. canadensis (Mill.) B. S. P.—Arthur (1887), Farlow and Seymour (1888).
P. engelmanni Engelm.—Baker, Earle, and Tracy (†), Hartley (‖ Nos. 1606, 1641, 1678), Hedgcock (‖ Nos. 577, 852, 966, 1632, 1635, 4252, 4322, 4617), Hedgcock and Hartley (‖ Nos. 609, 692, 694, 697), Hodson (‖ No. 1177).
P. excelsa Link.—Spaulding (*), Thesleff (1894), Von Thümen (Mycotheca Universalis No. 2202).

P. mariana (Mill.) B. S. P.—Millspaugh and Nuttall (1896), Peck (1893), Spaulding (‖ Nos. 952, 2241, 2266).
P. rubens Sarg.—Spaulding (‖ Nos. 2091, 2205, 2221, 2234, 2235).
Pinus sp.—Ellis (North American Fungi No. 1), Farlow and Seymour (1888), Fries (1863, 1874), Frost (††), Gillet (1874), Gillot and Lucand (1888), Harper (‡‡), Karsten (1876), McAlpine (1895), Neumann (‡‡), Sommerfeldt (1826).
P. divaricata (Ait.) Du Mont de Cours—Hedgcock (‖ Nos. 4162, 4209).
P. echinata Mill.—Hedgcock (‖ Nos. 363, 373), Humphrey (‖ Nos. 5059, 5281, 5333), Von Schrenk (1904).
P. glabra Walt.—Hedgcock (‖ No. 372).
P. lambertiana Dougl.—Hedgcock (‖ No. 1889).
P. monticola Dougl.—Hedgcock (‖ No. 4694).
P. murrayana Oregon Comm.—Hedgcock (‖ Nos. 576, 618, 889, 955, 1930, 4240, 4275, 4408, 4741).
P. palustris Mill.—Bates (1907), Von Schrenk (1904), Spaulding (‖ No. 444).
P. ponderosa Laws.—Anderson (‡), Hedgcock (‖ Nos. 808, 978, 979).
P. rigida Mill.—Peck (1893).
P. sibirica Mayr.—Saccardo (1898).
P. silvestris Linn.—Saccardo (1898), Thesleff (1894).
P. strobus Linn.—Peck (1893), Von Schrenk (‖ No. 1109), Spaulding (‖ Nos. 2919, 2950, 2951).
P. taeda Linn.—Hedgcock (‖ No. 394), Humphrey (‖ Nos. 5117, 5328), Von Schrenk (1904).
P. virginiana Mill.—Graves (‖ No. 3735).
Populus alba Linn.—Farlow and Seymour (1888), Morgan (1883), Saccardo (1898).
P. deltoides Marsh.—Farlow and Seymour (1888), Peck (1884), Saccardo (1898).
P. tremuloides Michx.—Hedgcock (‖ Nos. 620, 1634), Spaulding (‖ No. 2317).
Pseudotsuga taxifolia (Lam.) Britton—Harper (¶, ‡‡), Hartley (‖ No. 1775), Hedgcock (‖ Nos. 36, 651, 877, 1636, 1714, 1731, 1752, 1825, 4299, 4409, 4443, 4452, 4528, 4531, 4688, 4742, 4889), Humphrey (‖ Nos. 5860, 5869, 5934, 5964, 6009).
Salix sp.—Bessey (1884).
S. discolor Muehl.—Farlow and Seymour (1888), Peck (1884), Saccardo (1898).
Tsuga canadensis (Linn.) Carr.—Blake (‡), Dudley (1887, 1889), Farlow and Seymour (1888), Frost (††), Graves (‖ No. 3544), Millspaugh (1892), Millspaugh and Nuttall (1896), Neumann (1905), Peck (1893), Saccardo (1898), Von Schrenk (1904), Spaulding (‖ Nos. 103, 972, 2085, 2090, 2204, 2220, 2253), Underwood and Cook (‡).
T. heterophylla (Raf.) Sarg.—Hedgcock (‖ Nos. 1748, 4380, 4776).

The above reports and collections show that *Lenzites sepiaria* may attack the wood of the species of Abies, Alnus, Juniperus, Larix, Picea, Pinus, Populus, Pseudotsuga, and Tsuga. It may be expected to occur occasionally on the wood of Chamaecyparis, Cupressus, Libocedrus, Sequoia, Thuja, and Taxodium. Whether it may also attack the wood of deciduous species other than those belonging to the genera Alnus, Populus, and Salix is uncertain; specimens of fungi, which are so poorly developed that it is impossible to identify them with certainty, have been collected upon a number of the deciduous species.

This fungus is rather rarely found on the wood of living trees (Hahn, 1908, Hennings, 1903a, 1903b, Von Schrenk ‖ No. 105, Spaulding ‖ No. 2266), and so far as the writer knows has never been

mentioned as occurring parasitically. Hedgcock (‖ No. 1632), however, found an instance where a tree of *Picea engelmanni* about 4 inches in diameter was sharply bent by snow and was unable to straighten up when the weight was removed. The bark became loosened on the top of the bend, and this gave an entrance for the fungus, which worked downward in the injured wood tissues until only a small portion of the lower side of the trunk was alive (fig. 1). There seems to be no doubt in this case that the fungus was a wound parasite. Six inoculations into living trees of *Pinus palustris* made by the writer were wholly without result. For all practical purposes *Lenzites sepiaria* is a saprophyte, attacking timber which is piled for seasoning, or which is in use but exposed to the elements.

FIG. 1.—Cross section of living tree of *Picea engelmanni*, showing parasitic action of *Lenzites sepiaria*. The five annual rings on lower side are alive, but the inner one shows the encroachment of the fungus.

Lenzites sepiaria has been found by the writer upon the heartwood of *Tsuga canadensis* (‖ No. 2085) and *Larix laricina*, and it often attacks the outer layers of the heartwood in many other species. Whether it is able to rot the resinous heartwood of the southern pines seems questionable. The writer has seen no instance where this had taken place, except in the outer layers of heartwood which were not so completely filled with resin as the inner ones.

METHOD OF ENTRANCE AND RATE OF GROWTH OF LENZITES SEPIARIA.

Lenzites sepiaria is undoubtedly able to penetrate wood where it is cut across the grain, and under most conditions can probably enter radially if there is some small break in the fibers so that it can get between them. The evidence seems to show that when it enters upon the side of a timber it does so by means of season cracks.

These afford a very ready access to the interior tissues, since they often extend to the heartwood, or even into it (Pl. II, figs. 1 and 2; and Pl. III, fig. 3). The season cracks are especially favorable for the development of the fungus, as they dry out much more slowly than do the outer layers of wood, and thus give the spores a chance to germinate and to push the germ tube into the adjacent wood cells before the air is too dry for further development.

Lenzites sepiaria grows very rapidly under favorable conditions. Observations on newly cut, green railroad ties have shown that fully developed normal sporophores will form within five months' time upon such timbers. Artificial inoculations made by the writer also resulted in the formation of sporophores within five months (Pl. III, fig. 2). This remarkably short time for the development of a serious wood-rotting fungus is of course possible only under the most favorable conditions.

THE FUNGUS.

ITS NAME.

Lenzites sepiaria has been known in Europe for many years, being easily traced back to 1786, and with less certainty to a considerably earlier date. It has been placed in a number of different genera, according to the ideas of the various authors who have written about it. It was called *Agaricus sepiarius* by Von Wulfen (1786), who first named it; Persoon (1800) called it *Merulius sepiarius;* Fries (1815) changed it to *Daedalea sepiaria,* but later (1838) changed it again to *Lenzites saepiaria;* Karsten (1876) at first used the name *Lenzites saepiaria,* but later (1882) changed to *Gloeophyllum saepiarium,* and still later (1889) changed to *Lenzitina saepiaria;* Murrill (1904) used the name *Sesia hirsuta,* but later (1905, 1908) changed to *Gloeophyllum hirsutum.* The matter has not been investigated thoroughly enough for the writer to venture an opinion as to the merits of the various names. He therefore uses the name *Lenzites sepiaria,* which is used and accepted by most botanists.

THE SPOROPHORES.

The sporophores are rather small for a wood-rotting fungus. They rarely project more than 2 inches from the substratum, and are commonly long, narrow, shelf-like formations, extending horizontally from the surface of the wood. They are frequently compound or are clustered very closely together, and are especially numerous on the ends of affected timbers (Pl. I, fig. 1). When they are on the sides of the timber they are almost certain to be situated in season cracks (Pl. III, fig. 3). Sometimes, on a very badly rotted log, many sporophores situated in a single season crack fuse

laterally and form a single fruiting body, extending the entire length of the log. The usual form of the fruiting surface is that of irregularly branching gills, but cases can be found where it is in the form of more or less regular pores. On the other hand, the gills are sometimes as regular as those of most of the Agaricaceæ. One case was noted where the sporophores grew on the upper horizontal surface of a square timber and had the hymenium in the form of spiny projections. Similarly shaped bodies have been obtained in cultures (Pl. IV). The sporophore is perennial. When the conditions for growth are favorable, a new development takes place on the edge of the fruiting body and its under surface. There is a very marked difference in the color of the sporophore, depending upon its age. The youngest mycelium is snow-white; then, as age increases, the color turns quite rapidly to a yellowish white, then to a deeper yellow, finally to a brown, and in very old specimens it may be almost black. Very often the edges of the sporophores are yellowish white in color, showing that a new growth has taken place very recently. During sporulation the hymenium is yellowish white (Pl. I, fig. 2), and this color is a very good indication that spores are being given off. Sporophores collected in Missouri, in January, when placed in moist chambers gave off spores very abundantly within a few hours, seeming to show that sporulation in northern climates takes place at almost any time when there is enough heat and moisture for the tissues to carry on their functions.

Sporophores of *Lenzites sepiaria* may remain dry and apparently lifeless for a long period and still be able to produce viable spores under favorable conditions. This power to revive after long periods of inactivity is known to be not uncommon with the wood-inhabiting fungi. Buller (1909) found this property to exist to a remarkable degree in certain species: *Daedalea unicolor* (Bull.) Fr. recovered after desiccation for four years. *Lenzites betulina* (L.) after three years, and various others for periods varying from one week to three years. Rumbold (1908) found that specimens of *Lenzites sepiaria* which had been kept dry for 17 months, when moistened were able to produce viable spores. Moreover, they were able to repeat this performance after being dried again and lying a short time inactive. The writer obtained abundant spores in April, 1910, from fruiting bodies which in April, 1908, had been placed in a petri dish and collections of spores made. They soon dried out and had remained thus ever since in a dark drawer. Two years later they were again moistened and spores were produced as above stated. Tests of viability were not made, but Buller (1909) states that the production of spores is an indication that sporophores are alive. This power of reviving after long periods of drought is of considerable

importance, since it means that decayed timbers are a constant source of infection and should be destroyed instead of being left lying upon the ground.

The number of spores produced by an ordinary-sized sporophore of *Lenzites sepiaria* is literally millions. Buller (1909) has shown that a sporophore of *Daedalea confragosa* (Bolt.) Pers., about 2 square inches in area, produced nearly three-fourths of a billion of spores when revived after desiccation. This is much like *Lenzites sepiaria* in the character of its sporophores and may be taken to indicate very roughly the conditions occurring with the latter species. This emphasizes the fact that where there are fruiting bodies of *Lenzites sepiaria* there surely are spores everywhere in the vicinity, and no timber can be expected to remain free from them for any great length of time. Hence, it is doubly wise to destroy all decayed timbers.

DEVELOPMENT OF THE SPOROPHORES.

The first visible sign of the effects of this fungus is a blackening of the ends of the affected timbers over a space of several square inches. This blackening is quite noticeable to a close observer, and is present for some little time before the mycelium appears on the surface. After a few weeks, when there is sufficient moisture in the air, a tiny tuft of white mycelium appears somewhere on the blackened area. This grows larger within a few days if the moist condition continues, until it is about one-fourth inch across; then the tuft thickens until it stands out from the surface of the wood about one-eighth inch. The development of the gills begins early, goes on rapidly, and continues until the sporophore has reached its growth. The gills begin to form while the mycelial mass is still small (one-eighth to one-sixteenth inch), as soon, indeed, as there is room for a gill to be formed beneath. When the gills are well started, and sometimes before, the older parts of the mass turn to a light-brown color, meanwhile passing through the various shades of yellow. In Texas the entire development of the mature fruiting body may take place within 10 days from the very first appearance of the mycelium on the outside of the timber. After the first sporophore has formed it is usually not long before several others are produced immediately adjacent to it.

Some notes made by the writer on the rapidity of the growth of the sporophores in Texas are of interest. On one timber several tiny masses of white mycelium were barely visible on one of the blackened spots at the end of the timber. Seven days later the gills were beginning to form, and the oldest parts had turned brown. On the eleventh day several distinct sporophores which had formed during this time had fused into a single one, three-fourths inch long and

three-sixteenths inch wide, with numerous gills. On another timber tiny masses of white mycelium were visible when the observations were started. Six days later these masses had developed into a single large sporophore nearly 2 inches in length. On still another timber the first traces of gills had formed; four days later there were 16 gills. These observations were made at a time when the weather was very favorable for the growth of the fungus, there being a shower every day, with hot, muggy weather between the showers. Commonly several small pilei form at the same time very closely together, and these then fuse into one or two large ones which afterwards show no signs of their compound nature. The gills first form as very slight ridges on the under side of the mycelial mass, then these ridges grow higher until they form the fully developed anastomosing gills. One very curious case was noted where a railroad tie, with a newly formed sporophore upon it, had been turned with its former upper surface underneath, so that the gills were on the upper instead of the under surface. When found, the gills had just begun to produce a new growth of mycelium. On the sixth day new gills began to form on the former upper surface of the fruiting body; on the eighth day the transformation was complete, and one would never suspect the change which had taken place, the pileus being exactly like a normal one, except for a slight increase in thickness.

THE MYCELIUM.

The hyphæ of this fungus are very plentiful in the rotten wood, but are especially found in the medullary rays and the large cells of the wood. Very often an entire cell cavity is filled with a tangled mass of mycelium. The mycelium consists of two distinct kinds—a larger, dark-colored form, in which no contents can be perceived; and a smaller, colorless form, with a more or less granular content. The former is apparently the older form, and the color of the wood tissues where it is at all plentiful is a dark brown, evidently caused by the presence of so much dark-colored mycelium within, and not by any secretion or infiltration substance. The colorless form is evidently the younger and more active portion, and is much more often found, being very common in badly rotted wood. The hyphæ measure from 2 to 6 microns in diameter.

THE SPORES.

Experience gathered during a number of trips to Texas at different times of the year shows that the spores are produced abundantly there from June to November. The spores were collected by placing wet sporophores in moist chambers upon glass slides. Under these conditions the spores were given off very freely. The spores en

masse are pure white; they are ellipsoid, with more or less variation; many are slightly curved, and they often have a slight remnant of the pedicel attached to them, giving them a pointed appearance at the basal end; they are quite uniform in size and shape, and measure about 3.5 to 4 by 6 to 12 microns. Some are slightly club-shaped, but this is not common. When first set free their contents are finely granular.

GERMINATION OF THE SPORES.

After lying in water or a dilute solution of sugar for some hours, the contents of the spores become coarsely granular and 1 to 3, or in rare cases 4, guttules are formed. The spores did not germinate in very dilute solutions of sodium chlorid, but a solution of cane sugar up to 2 per cent and tap water gave results. In this solution the spore swells and pushes out a germ tube, which branches as it develops. Septa are formed, but they are not frequent. The germ tubes measure about 2 to 3½ microns in diameter, or about the same as that of the spores themselves at this time. A spore commonly produces a single germ tube, but two may be given off, one from either end. More than two germ tubes from a single spore have not been noted. The germ tube soon branches and forms a more or less extensive mycelium. The branches seem to arise from almost any point and are not especially abundant. In cultures the mycelium commonly has coarsely granular contents, which are retracted to the middle of the hyphæ. The germ tubes and hyphæ are quite uniform in size throughout their length.

CULTURES.

On July 24, 1904, while in Texas, the writer was able to collect spores in sufficient quantities for cultural experiments. The first test was made in hanging drop cultures in water. This water was collected from the roof of the house and stored in a galvanized-iron cistern. The cultures resulted in flat failure, although the spores did undergo some changes. After lying for an hour or so in the water their contents became coarsely granular and from 1 to 3 or 4 guttules were formed. No facilities were at hand for weighing small quantities of material, but a dilute solution of cane sugar and one of sodium chlorid were made. These were certainly less than 1 per cent solutions, and were presumably much weaker. Because of enforced absence the next day it is not known how long before germination took place. Judging from the length of the germ tubes, it must have been within 24 hours after the sowing of the spores. The cultures in sugar solution were the only ones that grew, and of these only two showed germination. The cultures were repeated with no results, so the entire study was necessarily made from these two

cultures. Later tests made with spores collected from sporophores brought into the laboratory in January from wood in the vicinity of St. Louis gave better germinations with sugar solutions up to and including 2 per cent of sugar by weight. In these tests the spores showed all of the previously described phenomena. Germination took place in about 30 hours and about 25 per cent of the spores germinated. The ungerminated spores remained apparently unchanged except for a slight swelling. Recently germination in tap water has been observed by the writer.

In the culture work with this and a number of other wood-rotting fungi in 1903 and 1904 the writer (1905) found it much easier to secure cultures from small masses of actively growing mycelium than from the spores themselves. His procedure is to choose actively sporulating, fruiting bodies, cut small pieces from them, pass quickly through the flame of a Bunsen burner, and place in a petri dish containing warm agar or gelatin media. If done skillfully a fair percentage of the plates will produce pure colonies of the fungus by the outgrowth of hyphæ onto the agar from the original mass of mycelium. The same method may be used with tubes of sterilized wood. Another method is to take small pieces of wood which is in the early stages of decay and contains active mycelium and use them in place of the bits of sporophore.

A large number of such cultures have been made upon sterilized wood in test tubes. Many of these cultures, owing to contaminations which it was next to impossible to exclude in the field, have failed, and all have failed to produce normal sporophores, which is the experience of others also (Rumbold, 1908); and a few cultures have developed spinelike fruiting surfaces instead of the usual gill form. (Pl. IV.) This form has been found in natural conditions in the field, as mentioned earlier in this bulletin.

Rumbold (1908) found that *Lenzites sepiaria* is very sensitive to alkaline media when grown in pure cultures. A number of different experiments uniformly gave the same results with this species. It was found that even with one-fourth of 1 per cent of sulphuric acid it grew luxuriantly. This chemical has been recently used successfully as a fungicide in dilute solutions for certain of the fungi (Anonymous, 1907; Kraemer, 1906; Spaulding, 1908b), and formerly was used more or less commonly for the same purpose. (Baierlacher, 1876; Bouchard, 1896; Degrully, 1895a and 1895b; Gellin, 1896; Guillemot, 1893; Von Liebenburg, 1880; Lodeman, 1896; McAlpine, 1898; Oliver, 1881; Zoebl, 1879.)

INOCULATIONS.

Inoculations have been made with living and actively growing mycelium in various ways to test certain points in the life history of

this fungus. The question of the possible parasitism of live trees has been tested by making inoculations into living trees of longleaf pine. These were made by boring holes into the trees with a small bit, then placing in the holes pieces of rotted wood containing active mycelium, and plugging the holes to prevent too rapid drying out. Similar inoculations were made in freshly felled trees to determine the time necessary for the development of sporophores. Absolutely no results could be detected from six inoculations made in the living trees, thus seeming to prove that *Lenzites sepiaria* is a true saprophyte and incapable of attacking living wood. Hedgcock (‖ No. 1632) collected a specimen which seems to show it to be very weakly parasitic. (Fig. 1.) This conclusion is borne out by the results of the inoculations in felled trees. In less than five months from the time of inoculation fruiting bodies were found growing upon the ends of the plugs used to keep the material from drying out. The plugs were about 3 inches in length and the mycelium had grown through the wood for that distance, completely rotting it for a portion of the way, and then forming fruiting bodies on the outside. (Pl. III, fig. 2.) The plugs were made of green wood taken from the tree in which the inoculations were made. The wood of the tree itself was apparently not attacked, this being probably due to the earlier death of the wood of the plug. Moreover, railroad ties, the time of cutting of which was exactly known, had sporophores of this fungus within five months of the time when cut from the green trees. When one considers that some little time must elapse before the wood of the perfectly green tree is dead, he may gain an idea of the rapidity with which this fungus destroys timber under favorable conditions. This is especially true of railroad ties and timbers which are placed under very favorable conditions for the growth of fungi, and which in Texas usually last only about 12 to 24 months in use.

THE DECAYED WOOD.

EXTERNAL APPEARANCE OF TIMBER.

A timber which is affected, but which as yet has no sporophores upon it, has a very characteristic appearance. The ends are generally the parts first to become affected. Here will be seen on dry ties a blackened area of a more or less irregular outline. This may be only an inch or two across, or may be larger, but it is never found extending into the heartwood. To the experienced person it is a sure indication that there is within an affected spot and that sporophores will soon be formed somewhere upon the discolored area. The appearance is as if the wood beneath were water soaked. The wood has been so decomposed that the smallest quantity of water makes it look wet.

The affected wood is also more darkly colored than normal sound wood, and this undoubtedly helps to give the discolored appearance on the exterior. It can not be said that these discolored spots always have a direct relation to the season cracks, but this is very often the case. Whether the spots are a result of the season cracks is uncertain, but in many instances at least they seem to be.

INTERNAL APPEARANCE OF TIMBER.

This fungus attacks coniferous wood wherever the conditions are at all favorable for the growth of the fungus, and it soon reduces the wood to a dry, brown mass, retaining but little resemblance to its normal appearance (Pl. III, fig. 1). The decay has been called a dry rot. It has always been found that when fruiting bodies have been formed at least a small portion of the wood has been completely rotted. At first the tendency is to form small pockets of rotted wood in the interior of the attacked timber, then to spread from these into the adjacent wood, spreading longitudinally faster than radially. The writer found that rot extended longitudinally in the wood from the fruiting bodies at least a foot, and sometimes for twice that distance, but commonly between these limits.

In the early stages of decay the early spring wood of the annual rings sometimes may be completely rotted and reduced to an amorphous powder, while the late summer wood, which is more compact, is almost wholly unaffected. The annual rings may then be very easily separated from each other with the fingers, and it is impossible to cut a block of such wood out of the affected timber, owing to the rings falling apart as soon as cut across. This peculiar action of *Lenzites sepiaria*, the writer believes, is due simply to the structure of the annual ring, which in some species of trees exhibits distinct differentiation between the early, porous portion and the later, more compact portion. Boiling tests made by the writer (1906) showed conclusively that the lignin of the early wood is more easily dissolved than is that of the late wood of the same annual ring, where the two parts are at all distinct. The degree of differentiation in the annual ring seemed to be the controlling factor in this difference in solubility of the lignin. Attention was called to the fact that these tests furnish an explanation for the disintegration of the early wood of the annual ring by certain wood-rotting fungi, while the late wood is but slightly decayed.

The affected wood assumes a shade of light brown, and small cracks run irregularly across the wood fibers, indicating that considerable shrinkage has been caused by the action of the fungus upon the wood (Pl. IV). The infections nearly always take place in season cracks, as is very clearly shown by the position of the fruiting bodies (Pl.

III, fig. 3) and the pockets of the rotted wood within (Pl. II, figs. 1 and 2). More or less extensive sheets or strings of matted mycelium may be found throughout the rotted wood. These mats are of varying shades of brown and yellow. A cross section of a decayed timber shows very plainly that it has been rendered totally unfit for use (Pl. III, fig. 1). In the earlier stages of the disease there are in the sapwood more or less numerous and extensive patches which have turned a dark-brown color, while large fissures run irregularly both radially and between the annual rings, showing that the fungus has caused some very serious changes in the structure of the wood. These patches of rotted wood are generally arranged in pockets with sound wood between them (Pl. II, fig. 2). As these pockets grow larger they extend radially faster than tangentially. This is partly owing to season cracks which frequently open for several inches in depth (Pl. II, fig. 1). In general the heartwood is not attacked (fig. 2), but in the last stages of the decay the outer layers of the heartwood may be more or less affected, owing probably to their not having fully assumed the characters of the older heartwood and also to season cracks opening directly into the heartwood (fig. 2). The outer rings of a peeled log are very commonly not rotted, while those farther in are almost completely disorganized. This difference may be explained by the fact that the sun soon dries out the external layers, so that the fungus has not enough water for its needs. While in the early stages of the rot the annual rings become separated from each other and the fall wood is little affected, in the last stages the fall wood also becomes completely decomposed and crumbles easily between the fingers. A log which is badly rotted at the end shows the fact by the very numerous cracks which are visible (Pl. I, fig. 1). It is noted that tree tops which have the bark left upon them have the sapwood completely rotted where the fruiting bodies show.

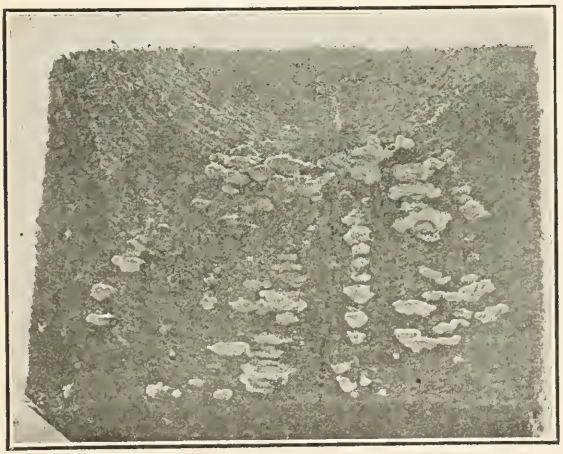

FIG. 2.—End of a new pine railroad tie, showing many sporophores of *Lenzites sepiaria*. Note season crack extending to heartwood; also freedom of heartwood from sporophores.

MICROSCOPIC EXAMINATION OF AFFECTED WOOD.

Radial sections of the rotted wood reveal almost no places where the mycelium has pierced the cell walls as is so common with other wood-rotting fungi. Instead, the hyphæ pass through the pits, this apparently being the rule. Cells and groups of cells, especially of the medullary rays, are often found with masses of the mycelium in their interior. The mycelium often forms an interwoven mass which completely fills the cell lumen.

Cross sections of the pits can be gotten only in tangential and cross sections of the timber. The tangential sections show the cross sections of the rays, and most of them have their component cell walls, especially near the middle, wholly destroyed and the cavity filled with mycelium matted closely together. The rotted wood is so brittle that no free-hand cross sections can be made.

The bordered pits have their closing membrane missing, and, as already stated, the mycelial strands pass freely through them. Very many pits have their borders cracked, with one to several openings running nearly to their periphery. The original opening of the pit is often enlarged, although this is not generally very noticeable. Sections of the wood in the last stages of decay show that the middle lamella is dissolved, thus allowing the cells to fall apart very easily.

The cell walls undergo some change which makes them exceedingly brittle, the razor breaking rather than cutting them. No elasticity is left in the tissues, the thickness of the razor being enough to cause the sections to break into small fragments, which still stick slightly together. The sections were cut free-hand, without embedding the material. Numerous tabular crystals lie directly upon the hyphæ of the fungus, which are apparently formed by the action of the fungus on the wood. These crystals dissolve in hydrochloric acid without effervescing.

MICROCHEMICAL TESTS OF AFFECTED WOOD.

Phloroglucin and hydrochloric acid give a bright red in the rotted tissues. Anilin sulphate and anilin chlorid give a bright yellow in the affected wood. Delafield's hæmatoxylin gives blue throughout. Chloriodid of zinc gives a blue color only in part of the tissues in early stages of the disease, but in later ones it gives blue throughout. This bluing occurs in the early wood of the annual ring, shading off as the late wood begins, then begins abruptly with the next annual ring. Mäule's potassium permanganate test gives a deep red in the healthy wood, but none whatever in the rotted parts. Thallin sulphate gives a yellow in the rotted wood. Resorcin with sulphuric acid gives a violet green not nearly so pronounced in the decayed

tissues as in the healthy ones. Carbazol with hydrochloric acid gives a violet red, deeper in the rotted than in the normal wood.

These tests seem to show that the fungus has extracted or disorganized the coniferin and the hadromal of the lignin, but has left the vanillin.

PROOF THAT LENZITES SEPIARIA CAUSES THE DECAY.

Every indication noted in the field showed that this fungus causes the peculiar form of dry rot which has been attributed to it. The sporophores are located so near the badly decayed places in the wood that there seems to be no doubt of the connection of the two. But this is far from accurate scientific proof. Artificial inoculations made by the writer in freshly felled sound green trees have gone far toward furnishing such proof. A fragment of rotted wood was used for inoculating material, being placed in a small hole bored in the side of the tree; the hole was then plugged with a piece of green wood cut from the same tree, and about 3 inches long. When cut open, five months later, it was found that the plug was badly rotted in the middle through its entire length, while the inoculating material touched it at the inner end, and on the outer end was a small but mature sporophore. (Pl. III, fig. 2.) Besides this, the writer has repeatedly grown pure cultures of *Lenzites sepiaria* upon sterilized wood blocks and has obtained in these cultures the same type of brown dry rot that is constantly associated with the fungus in the open air. (Pl. IV.)

FACTORS GOVERNING THE GROWTH OF WOOD-ROTTING FUNGI.

It is a matter of general knowledge among botanists that there are certain definite factors which control the growth and reproduction of the higher fungi. The more important of these factors may be called food, air, water, and temperature.

It may be said by way of summary that if any one of these factors is unfavorable, the wood-rotting fungi can not live any great length of time and can not grow at all.

Food materials.—Suitable nutritive materials are as essential to the existence of the fungi as they are for any other living organism. The food of the wood-rotting fungi consists of two classes of material, the contents of the wood cells and the wood cell walls themselves. The former consist of a very heterogeneous group of substances, such as starch, oil, protoplasm, tannin, sugar, minerals in soluble form, pitch, resin, crystals, etc. The wood-cell walls consist of a cellulose base or framework, with various laminæ strengthened with lignin, both substances being of a very complex nature. The wood-inhabiting fungi attack these various substances with great variability;

some take only the sugar and starch, and leave the cell walls nearly intact; others dissolve only the cellulose, others the lignin, and still others take all indiscriminately. The amount of stored food material present in the cell cavities of a living tree varies much with the season, at least in the temperate climates. It has been found that trees tend to store food material in large quantities in late summer and fall; in winter these supplies remain practically uniform in quantity; in spring, when the new growth is formed, they are rapidly and practically completely used up, the insoluble starch being changed into the soluble sugars. The sapwood is much richer in stored food matter than is the heartwood, which usually does not contain food materials in large quantities at any time. This fact partly explains the greater resistance of heartwood in general to the attacks of these fungi. The change of the insoluble material into some soluble form in the spring explains the fact that sapwood cut in spring or early summer usually rots very quickly, while the same wood cut in the winter does not rot so quickly, the soluble substances contained in the spring being much more readily attacked by the fungi than the insoluble ones present in winter.

FIG. 3.—Lower end of telephone pole, showing decay at surface of ground while it is practically sound a short distance above.

Air supply.—Wood-rotting fungi are living organisms and need a certain amount of oxygen. Many bacteria are able to live beneath the surface of liquids, and obtain their oxygen from the liquid itself, but the wood-rotting fungi seem to be unable to do this, and must have free access to the atmospheric oxygen in order to exist in a normal manner. Hence, cutting off the air supply stops their growth, and even kills them if continued for a sufficient length of time. This fact explains why wood remains sound for hundreds of years when buried, or when lying on the bottoms of streams and lakes. The rafting of timber is said to have a marked effect in preventing decay, and this effect may probably be partly explained in the same way,

although it is likely that the food substances in the cell cavities are dissolved and partly removed by the solvent action of the water. Undoubtedly the air supply has much to do with the rotting of posts and similar timbers at or near the surface of the soil, while both above and below the surface decay is not so complete (fig. 3).

Water supply.—That a certain degree of moisture is essential for the growth of the wood-rotting fungi is as true of the so-called dry rot fungi as of any other. As soon as a certain piece of timber becomes well seasoned it loses much of its susceptibility to attack by fungi, and as long as it remains relatively free of water it will not rot. Instances are plentiful in Europe where timbers which are now sound have been in place in buildings for hundreds of years. Wood from the royal tombs of Egypt is perfectly sound after a period of over 5,000 years. This fact can be explained in no other way than that it was well seasoned when put in place and has been protected from moisture ever since.

Temperature.—A fourth condition is requisite for the growth of wood-rotting fungi, namely, a favorable temperature. These fungi can grow at ordinary temperatures in most countries, but they make little or no growth at freezing point and below. Many of them appear to have an upper limit even in outdoor temperatures at which they do not thrive. In the usual spring, summer, and autumn weather of this country the wood-rotting fungi thrive, but in winter growth ceases except in the warmer sections, where it probably continues all the time.[a]

METHODS OF PREVENTING THE DECAY CAUSED BY LENZITES SEPIARIA.

The decay of timber caused by *Lenzites sepiaria* is brought about by the action of the vigorously growing mycelium in breaking down the wood tissues and utilizing certain of their constituents in its own life processes. Consequently, anything which influences the growth and vigor of the fungus has a direct influence on the rate and extent of decay which the fungus can cause. It has been already stated that four essential factors govern the growth of *Lenzites sepiaria*, and therefore control the decay caused by it. Of these four factors only temperature may not be more or less regulated in timber which is in service, or while such timber is being prepared for service.

[a] Falck (Die Lenzitesfäule des Coniferenholzes) gives some specific data on the maximum temperature for *Lenzites sepiaria*. He found that the mycelium in dry wood resisted an exposure of two hours to a heat of 97° C., nearly the boiling point of water; but mycelium in agar cultures was killed by 10 hours' exposure to 63°, and by 2 hours at 75°. The optimum temperature for germination of the spores is between 30° and 34° C., while the optimum for the mycelium in cultures is 35° C., and the growth minimum and maximum are 5° and 44° C., respectively.

The food supply can be effectively regulated by cutting the timber at the time when the trees either have their stored food materials in smallest quantity or else have them in the least available form, namely, late summer, autumn, and winter (Zon, 1909); but local conditions may modify the time of cutting to some extent. The supply of air can be regulated to some extent, the floating of timber being one of the most practical methods of such regulation. The water supply is probably the most easily regulated of any factor, the seasoning of timber being the most practical method of regulating it before it is placed in service. While in service, a number of methods are available, according to the location of the timber; for railroad ties, a well-drained roadbed (Dudley, 1887; Fernow, 1890; Von Schrenk, 1902); in other locations the seasoning of timber followed by painting or external coating with preservative substances (Dudley, 1887; Roth, 1895; Von Schrenk, 1902); the use of composite timbers instead of single large ones, leaving beams without boxing them in, and similar expedients are all thoroughly practicable methods of keeping the water content below the danger point.

SEASONING OF TIMBER.

It is a well-known and unquestioned fact that well-seasoned timber is much more durable than green timber of the same kind. The most important result of seasoning is the marked reduction of the water content to a point unfavorable for the rapid growth of wood-rotting fungi. Green coniferous timber contains 40 to 50 per cent of water (calculated on the dry weight of the wood) under ordinary conditions. Air-seasoned coniferous timber contains 10 to 25 per cent of water (Smith, 1908; Eastman, 1908; Sherfesee, 1908c; Hatt, 1907; Tiemann, 1907; Grinnell, 1907; Fernow, 1897). Air seasoning removes one-half to two-thirds of the total water content, lowers the water content especially of the outer layers of wood, and to a large extent prevents the infection of a sound timber; but there is danger of such infection occurring at any time when the timber becomes wet and absorbs enough water to very decidedly raise the water content.[a]

Seasoning is efficient as a method of preventing decay by *Lenzites sepiaria*. It must be done as rapidly as possible, especially in the Gulf States. To this end, open piling (Von Schrenk and Hill, 1903) is far better than the usual close method. It is necessary in eastern Texas to assist seasoning as much as possible, as green timbers will rot in five or six months if piled closely. In the Northern States seasoning progresses more slowly, but with less danger from this fungus.

Kiln drying is here considered as a rapid method of seasoning, the result being identical by either kiln drying or air drying.

[a] Falck, Die Lenzitesfäule des Coniferenholzes, 1909, states that the mycelium of *Lenzites sepiaria* will remain alive in a dry decayed timber for two to three years.

FLOATING OF TIMBER.

The immersion of timber in water has long been held to increase its durability (Dudley, 1887; Fernow, 1890). Such timber seasons quickly after being removed from the water (Von Schrenk and Hill, 1903). It appears that the immersion of timber for several weeks or months will decrease the decay caused by *Lenzites sepiaria*, although no experiments have been made to determine this point.

TREATMENT WITH CHEMICALS.

The treatment of timber with solutions of chemicals which have a deleterious action on the wood-rotting fungi is by far the most efficient method of preventing decay. There is absolutely no question as to the efficiency of this method, as numerous tests show. The following publications of this department may be cited in this connection: Crawford, 1907a, 1907b; Nelson, 1907; Von Schrenk, 1902, 1904; Sherfesee, 1908a, 1908b; Smith, 1908; Weiss, 1907, 1908. Since this fungus will not grow in alkaline media, it is probable that those solutions which are alkaline will prove most efficient, other conditions being alike.

Besides the general experiments of the many who have treated wood with various chemicals, there is an extensive test which has given very definite results as regards *Lenzites sepiaria* and the decay caused by it. In 1902 (Von Schrenk, 1904) a piece of track was laid with experimental ties, both treated and untreated, in eastern Texas. The following coniferous timbers were used: Tamarack (*Larix laricina*) and hemlock (*Tsuga canadensis*) from Wisconsin; longleaf (*Pinus palustris*), loblolly (*P. taeda*), and shortleaf (*P. echinata*) pine from Texas. Eighteen months after the ties were placed in the track the writer assisted in the examination of them. The results are noted herein only for the coniferous species of wood and in connection with *Lenzites sepiaria*.

The untreated hemlock ties were seriously rotted, 90 out of 101 having sporophores of *Lenzites sepiaria* and of *Polystictus versicolor* Fr. The former was present on most of the hemlock ties which bore fruiting bodies. The untreated shortleaf pine had 31 out of 100 which showed *Lenzites sepiaria*. The untreated longleaf pine had 68 out of 93 affected, some being badly rotted. The untreated loblolly had 57 out of 100 bearing fruiting bodies of this fungus. Of the untreated tamarack 37 out of 49 bore sporophores of *Lenzites sepiaria*. Of the methods of treatment tested the Wellhouse, zinc chlorid, and Allardyce processes gave satisfactory protection. The Barschall process did not give good results; treatment with spirittine gave fair results, and so far as *Lenzites sepiaria* is concerned, was satisfactory;

treatment with Beaumont oil was hardly satisfactory, 4 out of 42 loblolly ties having sporophores of *Lenzites sepiaria*. A detailed statement of these results is given by Von Schrenk (1904). The experiment shows that creosote, zinc tannin, zinc creosote, and zinc chlorid are efficient in the order named. The Barschall process, in which a mixture of copper, iron, and aluminum compounds is used, was not satisfactory. The Beaumont oil and spirittine were hardly satisfactory, but were applied in open vats without pressure.

In 1909 further examination of these ties was made (Faulkner, 1910; Winslow, 1910). No detailed statement is given as to the fungi which caused decay, so only the general results are of significance in the present paper; but the result with the best treatments, Allardyce, zinc chlorid, and Wellhouse, are of interest. It was found that a large number of the hemlock and tamarack ties which were treated by these methods are still in service. The following table gives the results:

Percentage of treated ties in service after 7½ years.

Kind of timber.	Method of chemical treatment.		
	Allardyce.	Zinc chlorid.	Wellhouse.
Hemlock	62	69	87
Tamarack	84	98	97

The untreated ties of hemlock averaged 1½ years of service, while the tamarack averaged 2½ years. This increase in service, due to treatment by the methods stated, based upon the service of untreated ties, was 430 per cent for loblolly ties, 370 per cent for hemlock, 280 per cent for tamarack, and 210 per cent for longleaf pine.

Besides the above methods of handling the timber itself, the collection and burning of decayed timber is of importance in reducing the attacks of this fungus. The custom of promptly burning the rotten ties by the American railroads is based on good judgment, and must have an appreciable effect upon the prevalence of wood-rotting fungi upon the ties in their tracks.

SUMMARY.

Practically three-fourths of the timber production of the entire country is furnished by the coniferous species of trees. The wood-rotting fungi are important factors in determining the length of service of this immense quantity of timber, *Lenzites sepiaria* being one of the most important of the fungi which attack coniferous species of wood. With several other species it destroys a large proportion of the coniferous railroad ties and telegraph and telephone poles which are in

service in the country. It alone probably destroys nearly one-fourth of these timbers. The latest statistics show that coniferous ties and poles bought in 1908 cost $32,500,000, making an annual item of more than $8,000,000 worth of timber which has its length of service seriously shortened by this fungus.

Lenzites sepiaria is widely distributed, being prevalent throughout Europe, in Australia, in the East Indies, and in South America. In North America it is undoubtedly present throughout Canada to the northern tree line, everywhere in the United States, and at least in the coniferous forests of Mexico. It occurs on the wood of Populus, Salix, Alnus, Abies, Larix, Picea, Pinus, Tsuga, Pseudotsuga, and Juniperus. It is a saprophyte, but under certain conditions can attack wood that is apparently alive. It usually enters timbers through season cracks and under favorable conditions is able to form mature sporophores within five months' time on newly cut timber. The fungus has been known in Europe for many years, being easily traced back to 1786. The sporophores are rather small, usually occurring in groups or fusing laterally. They may revive after long periods of desiccation, the writer having obtained spores from specimens after two years. The spores are given off by hundreds of millions. Hence, decayed timbers should be destroyed, as they are a prolific source of infection for new timber. Mature sporophores may be produced within 6 to 10 days after the first mycelium shows on the exterior of an affected timber. Many pure cultures have been made by the writer, usually using the living mycelium instead of spores for inoculation. Inoculations into green timber produced sporophores within five months' time in Texas.

The decayed wood is brown in color, irregularly fissured into tiny cubical masses which crumble into dust between the fingers.

Microchemical tests show that the lignin has lost some of its constituents and is disorganized. Pure cultures grown upon sterilized green wood have produced the decay which constantly accompanies the fruiting bodies in the field and forest.

The factors governing the growth of wood-rotting fungi are food, air, water, and temperature. These fungi cause decay by disorganizing the wood tissues in which their mycelium vegetates, and the above factors which govern their growth consequently govern the decay caused by them. Hence, the decay caused by *Lenzites sepiaria* may be prevented or greatly retarded (1) by seasoning, which decreases the water content of the timber to such a point that fungi can not readily grow; (2) by floating, which excludes the air and probably has some effect on the food materials within the timber; and (3) by chemical treatment, which infiltrates the wood with substances deleterious to the fungi.

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PLATES.

DESCRIPTION OF PLATES.

PLATE I. Fig. 1.—End of longleaf pine log with many sporophores of *Lenzites sepiaria*. The small white masses on the left are sporophores of another wood-rotting fungus. Note the season cracks in the wood. Fig. 2.—Several new sporophores of *Lenzites sepiaria* showing their hymenial surface.

II. Fig. 1.—New railroad tie with early stage of decay caused by *Lenzites sepiaria*. The largest rotted area is located at a season crack in the upper surface of the tie. Fig. 2.—New railroad tie with medium stage of decay caused by *Lenzites sepiaria*. The rotted areas are located at season cracks.

III. Fig. 1.—Late stage of decay caused by *Lenzites sepiaria* in a longleaf pine tie which has been cut but a few months and never has been placed in service. Fig. 2.—Plug used in inoculating green timber. Removed in less than five months. A sporophore was formed on the outer end. Fig. 3.—Loblolly pine timber with *Lenzites sepiaria* sporophores in the season cracks.

IV. Longleaf pine block upon which a pure culture of *Lenzites sepiaria* has grown for about six months. This type of rot is the one which accompanies the fruiting bodies of this fungus so universally.

FIG. 1.—SPOROPHORES OF LENZITES SEPIARIA ON THE END OF A LONGLEAF PINE LOG.

FIG. 2.—SPOROPHORES OF LENZITES SEPIARIA, SHOWING UNDER SURFACE.

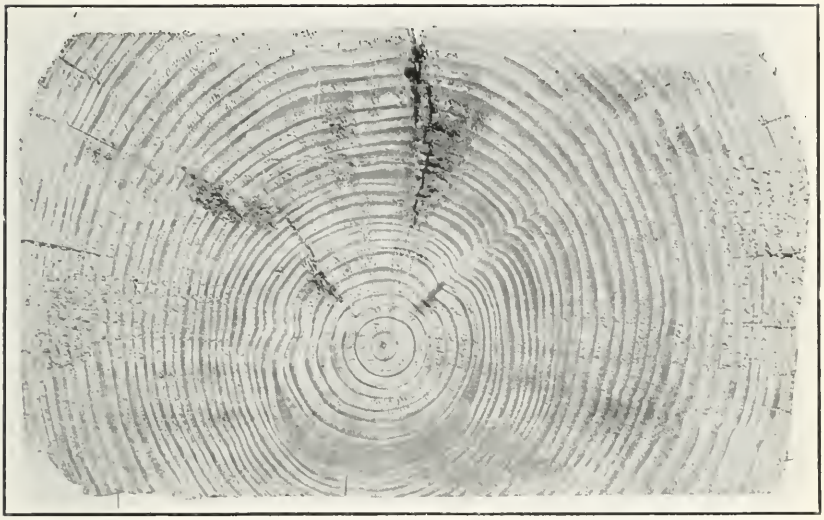


FIG. 1.—EARLY STAGE OF DECAY CAUSED BY LENZITES SEPIARIA.

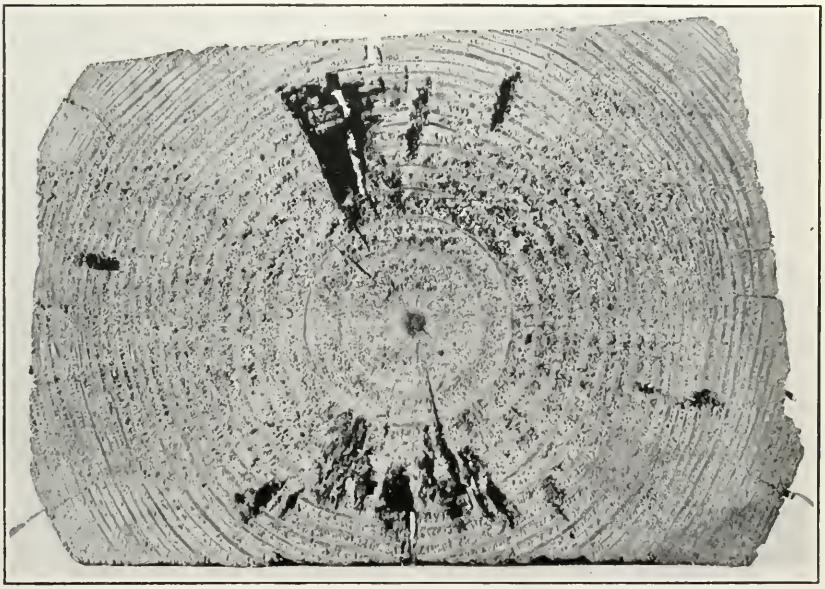


FIG. 2.—MEDIUM STAGE OF DECAY CAUSED BY LENZITES SEPIARIA.

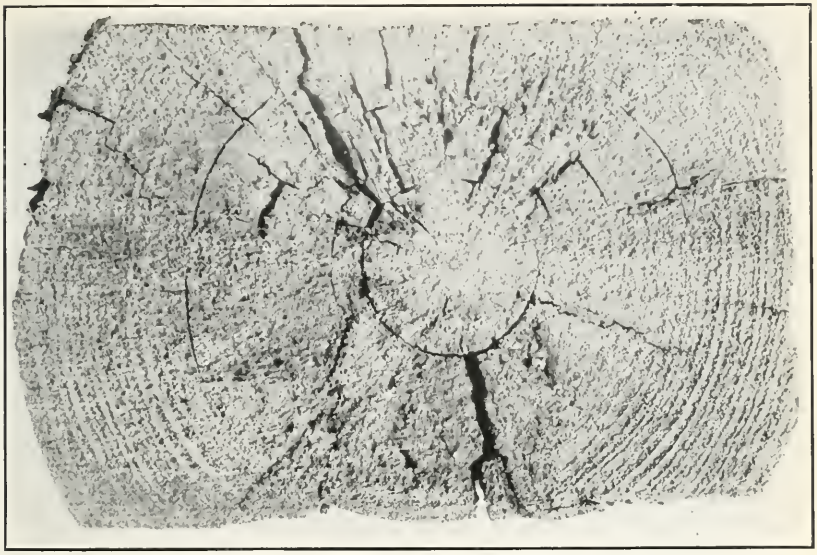


FIG. 1.—LATE STAGE OF DECAY CAUSED BY LENZITES SEPIARIA.

FIG. 2.—PLUG USED IN INOCULATION.

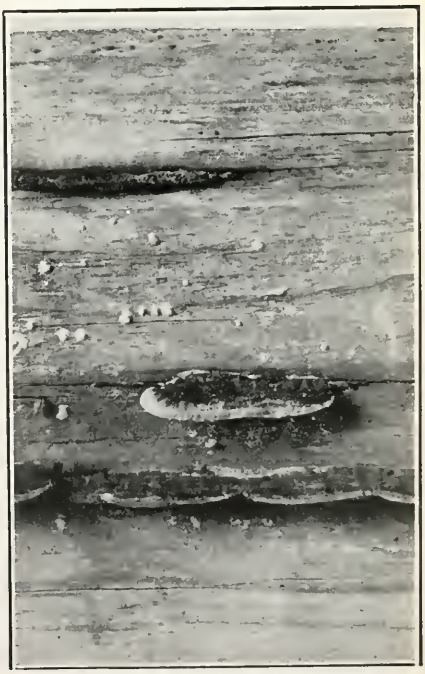
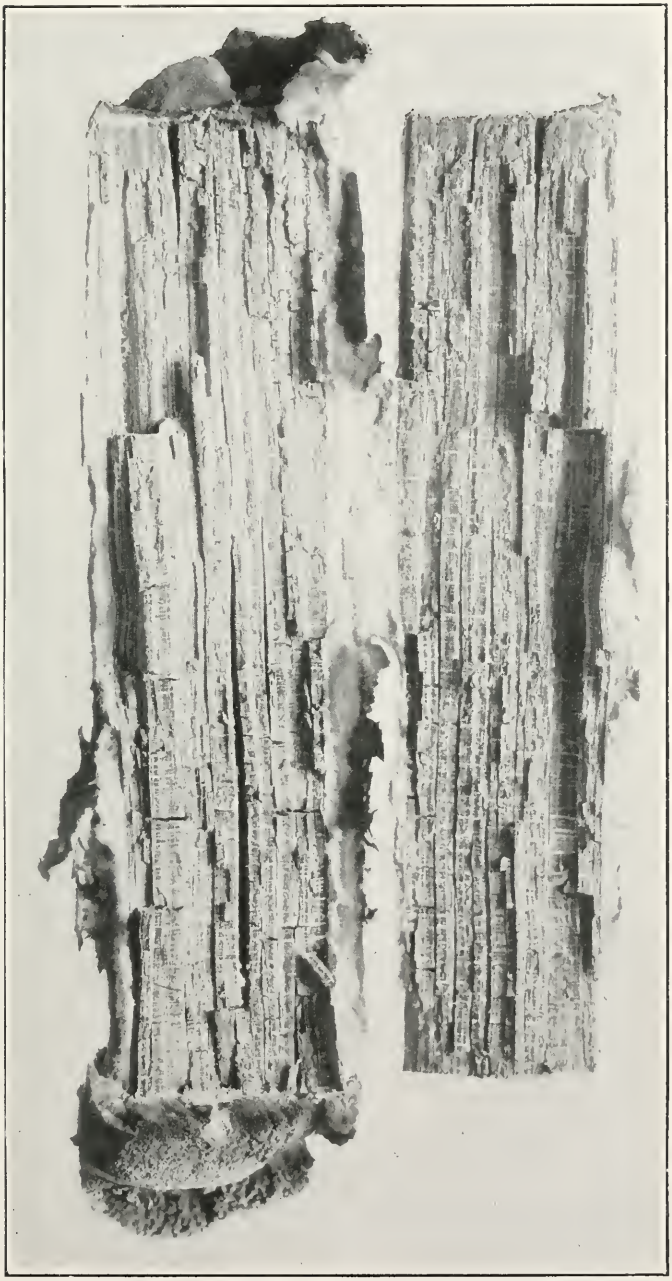


FIG. 3.—SPOROPHORES OF LENZITES SEPIARIA IN SEASON CRACKS.



PURE CULTURE OF LENZITES SEPIARIA ON LONGLEAF PINE BLOCK.

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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 215.

B. T. GALLOWAY, *Chief of Bureau.*

AGRICULTURE IN THE CENTRAL PART OF THE SEMIARID PORTION OF THE GREAT PLAINS.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., March 20, 1911.

Sir: I have the honor to transmit herewith a manuscript entitled "Agriculture in the Central Part of the Semiarid Portion of the Great Plains," and to recommend that it be published as Bulletin No. 215 of the series of this Bureau. This manuscript was prepared by Dr. J. A. Warren, Assistant Agriculturist, under the direction of the Agriculturist in Charge of the Office of Farm Management, of this Bureau, who for a number of years past has been studying the management of "dry farms" and the problems confronting the farmers of the region, besides having had some previous practical experience there. The author wishes to acknowledge his indebtedness to Mr. J. E. Payne, superintendent of the experiment station at Akron, Colo.; Prof. W. P. Snyder, superintendent, and Mr. W. W. Burr, assistant, of the substation at North Platte, Nebr., each of whom has read the manuscript and offered valuable suggestions.

For some time prospective settlers have made a strong demand upon the Department for reliable information concerning this region. There has also been a strong demand from persons already located there for suggestions for the better management of their lands. This manuscript is intended to fill the former want and in a measure also the latter.

Respectfully,

Wm. A. Taylor,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., March 20, 1911.

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CONTENTS.

with its recurring periods of heat and cold, is responsible for our being the busy, hustling nation that we are."[1]

Settlers in new countries, and especially in the dry regions, have often been misled by giving too little attention to climatic conditions. They have found a fertile, easily tilled soil, and without regard to climate have assumed that good crops must be the reward of cultivation.

NATURAL FACTORS OF PLANT GROWTH IN THE GREAT PLAINS FIXED.

Of the climatic factors, rainfall and evaporation are the most important in the semiarid region, because the most faulty. The saying that "rainfall follows the plow" has, in its effect, been one of the worst deceptions ever foisted upon a credulous public. This idea has been the undoing of more plains settlers than has drought itself. If the people had realized that the dry country would always be a dry country many who have settled in the semiarid regions would never have gone there, and those who did go, understanding the hard conditions, might have risen to the emergency and long ago have met the necessity, as did the settlers in Utah and Washington, instead of waiting in the vain hope that Nature would take pity on them and reward their puny efforts by an increase in precipitation. Space does not permit a discussion here of the fixedness of climate, but all students of meteorology now agree that the climate is unchangeable, at least within the limits of a single generation.[2] There are fluctuations from year to year and more or less cyclical changes which give periods of dry years followed by periods of wet years, but the average of a long period of years is practically stable. These fluctuations, although very irregular, lie between fairly well-defined limits as regards total variation.

The main factors affecting evaporation from an open water surface are the relative humidity of the atmosphere, or the proportion of moisture in the air compared to what it can hold, the wind velocity, the temperature of the air and of the water at the surface, and the air pressure. Evaporation from the soil, however, is affected not only by these factors, but also by the character and condition of the soil and by the plant growth thereon. Soil conditions and plant covering are largely under the farmer's control.

The soil in its native state is, like the climate, unchangeable so far as the ordinary limits of time are concerned, but under cultivation very important temporary changes may be brought about.[3]

[1] Ball, Frank Morris, of the department of geology, University of Minnesota, in Monthly Weather Review, May, 1906.

[2] For a discussion of this subject the reader is referred to the Yearbook of the U. S. Dept. of Agriculture for 1908, p. 289; Bulletin D, U. S. Weather Bureau; and Monthly Weather Review, May, 1906.

[3] See Bulletin 55, Bureau of Soils, pp. 61, 71, and 76.

Climate,[1] soil, and topography[2] are the factors determining the native vegetation. As these factors are all fixed and unchangeable to any appreciable extent, the native vegetation is also fixed and unchangeable so far as one lifetime is concerned, except for the limited effects of overgrazing and the effect of increased or diminished burning by fire.

Yet along with the idea of change of climate goes the belief that the plant growth of the native prairies of Nebraska and Kansas has changed decidedly as successful agriculture has pushed its way westward. This notion prevails especially with reference to the long grasses, many believing that even eastern Nebraska and eastern Kansas were covered with buffalo and grama grasses 40 years ago, and that settlement has caused the bluestem to drive the short grasses westward 200 miles. This opinion has, however, no foundation in fact. When the Plains were first settled there were no elements in the flora that had not assumed their proper places. Neither the long grasses nor the short grasses were newcomers. Both had fought the battle for supremacy and each held its chosen ground—the ground which it still holds, except as overgrazing or burning has disturbed the equilibrium. If the stock is removed, the floral covering even on the overgrazed land again assumes its original character, showing conclusively that the character of the plant growth is a fixed resultant of natural causes and is not determined or changed by any obscure and intangible force following in the wake of civilization.

The appearance of the prairies changes noticeably in wet seasons. The wheat-grass and other tall grasses and weeds are much more in evidence, the buffalo and grama grasses grow much taller, and annual plants are more conspicuous; but the real and permanent characters of the flora are unchanged by even half a dozen wet years. The relative sizes of plants, but not the kinds of perennials, change with the season.

The same native flora which existed on the Plains when they were first settled occupies them to-day; the same climatic conditions which caused the ruin of the early settlers must be met by the settlers of to-day; the same soil conditions which the homesteader then found confront the "dry farmer" of the present; the same grass mixture which pastured the first homeseeker's stock and in some cases furnished hay for the winter is still there. As man has not changed the climate, neither has he changed the plant growth on the prairies.

ECONOMIC CONDITIONS IN THE GREAT PLAINS CHANGED.

What has just been stated is not that the farmer on the semiarid Plains to-day has the same combination of conditions to meet that he had 25 years ago when the region was first invaded. It has

[1] See Bulletin 55, Bureau of Soils, pp. 31 and 35. [2] Idem, p. 30.

been pointed out that agricultural factors are of two classes, natural and artificial, and one of these sets of factors is as important as the other. It is just as essential to have a market as to have a crop. While the forces of the first group are fixed, those of the second are constantly changing. Whatever differences there may be between the conditions that surround the settler on the dry lands to-day and those that faced the settler of a generation ago on the same land, these differences are not in soil, climate, or native vegetation. They are economic and industrial differences—differences in the machinery available, the methods of cultivation practiced, the varieties of crops at hand, and the prices of products. The changes in these respects are great, so great that the total combination of all conditions make, as it were, almost another country. The improvement in machinery is so great that Prof. Snyder, of the substation at North Platte, Nebr., has said, "Take away the disk, the press drill, and the corn machinery and western Nebraska would still be a place for the cattleman." A parallel statement with regard to the crops that have been introduced during the last 15 years may be made, but great as is the effect of these changes the advance in prices of products is of still greater importance.

Where success has been attained it has in almost every instance been due to more than normally favorable seasons combined with high prices. There does not appear to have been any great and general revolution in methods of cultivation except what has been brought about by the introduction of new machinery. In spite of the fact that many periodicals have published glowing accounts of a wonderful revolution in methods that has turned the dry region into the most prosperous of farms, there is little foundation for such stories. Otherwise than to use new machinery, the average farmer of the dry country has improved his practices but little. His increased prosperity is due more to unusually favorable seasons and to high prices of grain and stock than to better methods of cultivation or management.

Nevertheless, a very few exceptional farmers, unusually progressive men, who study their work and the conditions to be met, have changed their methods radically and have met with better success.

EXTENT OF THE SEMIARID REGION.

Some writers and experimenters consider the semiarid region as including all the Plains as far east as the ninety-eighth meridian, and thus include a large area of land receiving an average of as much as 27 or 28 inches of rainfall annually, which has supported a prosperous agricultural population for a generation, and in many portions of which farms are readily salable at $70 to $100 an acre. Some of the greatest winter wheat, corn, and hog producing counties in Kansas

and Nebraska lie west of this line. To include this territory seems manifestly unjust and misleading, if it does not make the term "semiarid" actually meaningless. It is impossible to fix a positive and definite line on the one side of which we shall say the country is humid and on the other semiarid, or, as some prefer to say, "subhumid," for there is no sudden dropping off in precipitation, but a fairly uniform decrease from east to west across the two States. As generally used, the term refers to a country receiving an average of between 10 and 20 inches of melted snow and rain annually, but in determining aridity or humidity evaporation is of equal importance with precipitation. In southern Texas much more than 20 inches of precipitation may be required to make a humid country, but 20 inches of rainfall in the Red River region of North Dakota makes a distinctly humid climate. With reference to Kansas and Nebraska the writer prefers to consider the western limit of 20 inches average annual precipitation as the eastern limit of the semiarid region, although in southern Kansas this limit may be too far west and in some other places too far east. So far as the records for Kansas and Nebraska now show, this line in most places lies 20 to 30 miles west of the one hundredth meridian.

The accompanying map (fig. 1) shows the region to which this discussion is intended to apply and the average annual precipitation as shown by records of the Weather Bureau.

CLIMATE.

The climate of the Great Plains region has been thoroughly discussed by several able writers and for that reason it seems unnecessary to give more than a brief summary here. It is a region peculiarly subject to high winds, driving storms, and sudden changes in temperature. The light is intense and the air usually very dry. At least in a large proportion of it hail is of frequent occurrence and does much damage to crops. The native flora and even the soil[1] attest the general dryness. To the careful student of nature these tell a story of perennial dryness over which the myth of changing climate could have no appeal.

PRECIPITATION.

All plants for proper development require a reasonable supply of plant food in available form, favorable temperature, an adequate supply of moisture, and an abundance of sunshine. Given a fertile soil, the yield of the crop depends upon the relative distribution of heat, moisture, and light throughout the season. But a chain is no stronger than its weakest link. Given favorable conditions with respect to all the foregoing except one, that one becomes the limiting factor of success—the all-important question. In most of the Great

[1] Bulletin 55, Bureau of Soils.

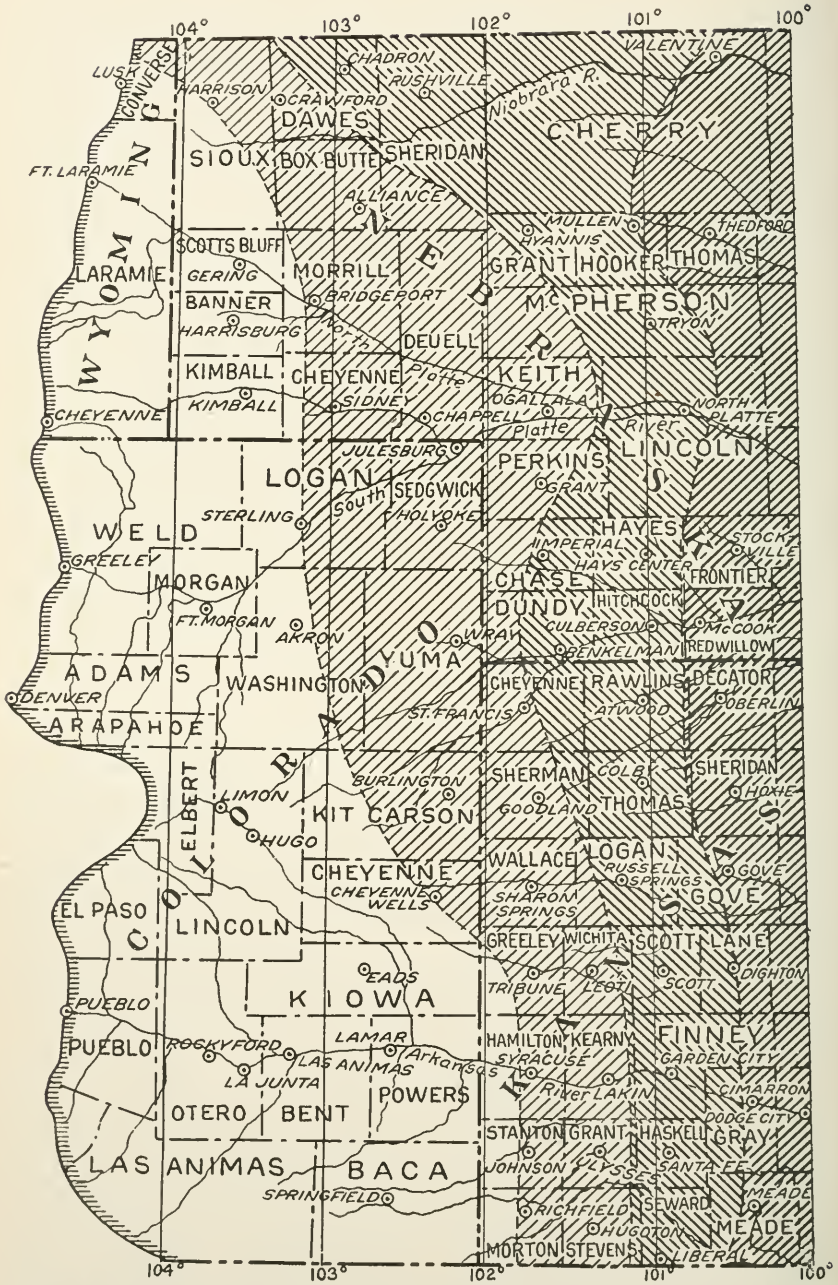

FIG. 1.—Map of the central part of the semiarid portion of the Great Plains, showing the average annual precipitation. In the region shown in the unshaded part of the map there is less than 16 inches of precipitation during the year; the lightest shading shows from 16 to 18 inches; the medium shading, from 18 to 20 inches; the heaviest shading, more than 20 inches.

Plains region all these conditions but one, moisture, are favorable for crop production. Thus it is that the amount and distribution of rainfall become the question preeminent, and moisture conservation becomes the vital problem to all farmers.

As has been said, there is a fairly uniform decrease in precipitation from east to west across the Plains to some distance into Colorado and to about the Wyoming boundary line. (See map, fig. 1.) This decrease is 1 inch to about 17 miles along the south line of Kansas, 1 inch to about 21 miles along the north line, and 1 inch to about 40 miles along the north line of Nebraska from the Missouri River west. Over most of the region 70 per cent or more of the precipitation falls during the growing season. This, it is often argued, makes a much smaller annual precipitation necessary than if much of it came during the winter. The truth of this supposition may at first seem self-evident, but there is grave doubt whether our small-grain crops may not with proper tillage succeed better with a small amount of precipitation which comes in the winter than with the same amount of rain coming in the summer. At least, the regions which are producing satisfactory crops on the least rainfall are regions of winter rain, and there summer rain (after July) is considered a misfortune, except when falling on fallow land.[1]

It seems, however, fairly well established that late-maturing crops, such as corn, must have considerable rain during the middle and latter part of the growing season.[2]

The rainfall of the region is very uneven in distribution, a large part of it falling in the form of local showers which cover but limited areas and are often torrential in character. This makes the rainfall extremely variable, both as to annual precipitation and distribution through the season. Instead of calling the region "semiarid" it would be more properly described as varying from year to year between arid and humid. This variability is the most serious feature of the climate. If dry seasons came with any regularity the settler could be prepared for them, but coming as they do with no regularity and without warning they are the constant dread and often the ruin of the homesteader. If the precipitation were fairly uniform and favorably distributed the conditions might be easily met, but this variability has always been the limiting factor of success. It is this, more than the scarcity of moisture, that must be overcome.

EVAPORATION.

From an agricultural standpoint evaporation is of equal importance with precipitation, although few people appreciate this fact. It is this factor which determines the amount of water needed to

[1] Thatcher, R. W., Director of the Washington Agricultural Experiment Station, in address at Corn Exposition, Omaha, 1909.

[2] For a further discussion of this subject, see Bulletin 85, U. S. Weather Bureau; Yearbook, U. S. Dept. of Agriculture for 1903, p. 215; Annual Report, Nebraska State Board of Agriculture, 1909, p. 312.

produce a crop. The water actually contained in the crop at any time is so small as not to be worth considering. It is the water that passes through the plants into the air and the amount lost from the soil which determine the amount necessary for the welfare of the crop. The amount of water within reach of the roots of plants is of no greater importance than the rate at which it escapes through the leaves and stems. The water used by the plants is that which passes through them and the small amount retained in their bodies. The balance of the precipitation in this region is nearly all lost by evaporation directly from the surface of the soil, very little escaping through seepage.

The amount of water used by plants is far from uniform for all parts of the region, being greatest in the warmest and windiest parts and growing less as temperature and wind velocity decrease. For this reason an inch of water in the Panhandle of Texas is not comparable with an inch of water in North Dakota.[1] The amount of water lost through plants in the semiarid region, or, in other words, the amount of water necessary to produce a crop if all loss from the soil could be prevented, is not very well known. It is, however, known to be far in excess of that required in more humid sections. Experimenters in several States have determined the amount of water lost by various plants in their particular localities and in publishing the results have usually stated that they applied to their particular conditions only; but, in spite of this, results obtained in Wisconsin have frequently been quoted to show what a small quantity of water was needed on the dry Plains. Records indicate that in the drier portion of the Plains the air is about twice as dry as at Madison, Wis. Obviously, results in Wisconsin have no relation to Plains conditions. At the Utah Agricultural Experiment Station it has been found that about 50 tons of water passed through wheat plants for every bushel of grain produced, or the equivalent of 12 inches of water actually passed through the plants to produce 27 bushels of wheat to the acre. To this must be added the water lost from the soil by evaporation and by seepage in order to determine what was required to produce the crop.

The loss of water is controlled mainly by the same factors as the evaporation from an open water surface, namely, the dryness of the air, the temperature of the evaporating surface, the wind velocity, and the lightness of the air. From these facts it is plain that the amount of water necessary for a crop is very variable and is not likely to be the same in the same field in any two consecutive seasons. It must vary from season to season approximately as the dryness of the air, the wind velocity, the temperature of the air, the soil, and the plants vary.

[1] Bulletin 188, Bureau of Plant Industry.

The evaporation of water from an open water surface is not an exact measure of the demands made by the atmosphere upon plants; yet it is a relative measure and the best we have at present. Experiments have shown that the loss of water by plants varies. In southeastern Colorado the evaporation from an open water surface is about 50 inches during the growing season, and diminishes to the northward, on account of the decrease in temperature, to about 35 inches in northwestern Nebraska.

The demands for water during critical periods, which may be only a few hours in duration, are often as important as those for the season; in fact, during dry periods the greater part of the injury to crops is often done within a few extremely trying hours. These demands are frequently excessive and often beyond belief. At Lincoln, Nebr., August 26, 1909, Profs. Montgomery and Kiesselbach found that a single corn plant standing in a field of corn lost 9½ pounds of water in eight and a half hours. August 26 was not nearly so hard a day on the corn as was August 23, when the temperature was higher, the wind more than doubled, and the relative humidity only about two-thirds as high. Judging from the record of August 26, the same plant must have lost about 15 pounds in the same length of time on August 23. Even August 23 was not nearly so trying a day as some that have occurred in southeastern Nebraska during very dry seasons. What the demands upon plants in still drier regions may be at times we can only imagine. In a large part of the region the demands are much greater than at Lincoln.

WINDS.

The semiarid portion of the Great Plains is the windiest extensive area in the United States. There are not many records that fairly represent the wind sweep on the smooth prairies. The following data published by the Weather Bureau are the best available on the subject and are included here as being at least suggestive:

Average wind velocities on the semiarid plains.

Station.	March.	April.	May.	June.	July.	August.	September.
Amarillo, Tex.	19.8	20.2	18.9	18.8	16.1	13.4	16.9
Dodge City, Kans.	12.8	14.2	13.7	13.8	11.8	10.9	11.9
North Platte, Nebr.	10.8	12.6	11.8	10.9	9.0	8.6	9.3
Valentine Nebr.	11.6	13.4	12.2	12.2	10.3	9.5	10.9
Peoria, Ill. (3 years)	11.0	11.0	9.7	8.6	7.1	6.7	7.9

Dodge City, Kans., North Platte, Nebr., and Valentine, Nebr., are near the eastern limit of the semiarid area, and are in valleys which apparently must protect them from the full force of the wind, or at

least prevent as high wind velocities as prevail on the level prairies. Amarillo, Tex., is on a level plain and receives the full sweep of the wind. The conditions at this station may be more representative of the open country under discussion than those at the other stations, yet it seems probable that if there were records on the open prairies farther north they would lie between the figures given. For comparison, the record at Peoria, Ill., is also given.

EFFECT OF WIND ON AGRICULTURE.

High wind velocity has an important bearing on agriculture. It has a positive value as a source of power for pumping water and is occasionally utilized to run feed grinders and other small machinery. It also enables the farmer to cure feed quickly and in excellent condition, but the beneficial results fade into insignificance when compared with the damage done. On many days it is a great hindrance to labor, especially if hay or grain is to be handled; it blows the soil badly, sometimes removing several inches from bare fields in a short time. This drifting absolutely prohibits summer tillage on light soils; the blowing sand cuts off crops and the wind does much damage by whipping and splitting the leaves. All of these facts mentioned, however, are of small importance when compared with the effect of wind on the evaporation of water from the soil and from plants.

The significance of high wind velocity becomes more apparent when its effect upon the rate of evaporation and the consequent drying effect upon soil and plants are considered. Everyone knows that the air takes up water much more rapidly on a windy day than on a calm one, but to get any definite relation between evaporation on a still day and on a windy one is very difficult. Prof. Thomas Russell's experiments with instruments constructed for the purpose gave the following results for evaporation from a water surface: [1]

With the wind at 5 miles an hour evaporation is 2.2 times as rapid as during a calm.
With the wind at 10 miles an hour evaporation is 3.2 times as rapid as during a calm.
With the wind at 15 miles an hour evaporation is 4.9 times as rapid as during a calm.
With the wind at 20 miles an hour evaporation is 5.9 times as rapid as during a calm.
With the wind at 25 miles an hour evaporation is 6.1 times as rapid as during a calm.
With the wind at 30 miles an hour evaporation is 6.3 times as rapid as during a calm.

While the wind can not affect the loss of water from the soil to any great depth at anything like the ratios specified, there is no question that the amount of water required for the best development of plants increases materially as wind velocity increases.

[1] Report of the Chief Signal Officer, War Department, 1888, p. 176; also Monthly Weather Review, U. S. Signal Service, 1888, p. 235.

LIGHT.

The whole semiarid country is a region of intense sunlight. On account of the clearness of the air, the small amount of cloud, and the rarity of the air caused by the high altitude, the sun's rays lose much less energy before striking the earth. Although this is a subject not usually considered it is undoubtedly an important one—how important no one knows. It is known that plants use more water when exposed to strong light. With fairly favorable conditions of heat and moisture the quality and yield of grain depend largely on the intensity and duration of light. It seems comparatively certain that this is one of the main factors responsible for the uniformly high quality of grain produced in the semiarid region and the large yields obtained whenever an adequate supply of moisture is available.[1]

IRRIGATION WATER.[2]

The extent of territory in this region that can ever be irrigated is, indeed, an extremely small proportion of the whole. At best, the water in the streams is sufficient for only small patches in comparison to the whole, or narrow strips along the streams. This water is supplied mainly by the precipitation in the mountains. The amount of water lost by surface run-off in the semiarid region itself is comparatively small and is commonly much exaggerated. It would in reality make only a thin covering over the entire surface. We see water flowing in a draw and think of its volume, but do not stop to think how far apart the watercourses are, and from what a large area the little stream collected the water. Of course, there is considerable movement of water from higher to lower ground, especially during driving storms, so that much more water goes into the ground on one part of a field than on another. Some water also accumulates in low places, where it remains till evaporated, being thus lost to agriculture. Occasionally too, considerable water finds its way into the streams. A considerable but unknown quantity is also lost by seepage.

The Republican River, which rises in the plains of Colorado and has most of its drainage basin in the semiarid region, though its mouth is in a region of much heavier rainfall, has an average annual discharge of only about three-fifths of an inch for its entire basin. In other words, if all the water discharged by this stream during a year were spread out on the land from which it was collected there would be but three-fifths of an inch over the entire area. It must

[1] For a further discussion of this subject, see Bulletin 36, U. S. Weather Bureau.

[2] It would be unnecessary to mention this subject here except to warn persons from accepting statements concerning future irrigation on land where there is no hope for irrigation.

be remembered that this includes not only the storm water but the seepage water also, the only considerable loss not included being the evaporation from the surface of the stream itself.

The North Platte River and its tributaries gather most of their water from mountain areas where the precipitation is generally greater than on the prairies, and in all of which the evaporation is much less and the run-off much greater; yet the amount of water in these streams is sufficient to cover the area from which it has been collected to the depth of only 1.5 inches in a year.[1]

SOILS.

The soils are, in a measure at least, characteristic of the climate. They are strictly dry-climate soils. Little difference in texture between soil and subsoil is found. There is nearly everywhere a high percentage of soluble salts, and in many of the valleys an excess of alkali. This is due to the fact that there is not sufficient rain to leach out the salts. The soil is not often wet to any great depth, and over much of the region there is no seepage whatever, all the water which gets into the soil returning to the air. There is then no means by which these soluble mineral compounds can get away. In nearly all the region a slightly whitish zone is observed at from 1 to 3 feet below the surface. This is due to the accumulation of salts which have been carried down by the rain water and left behind when the water was evaporated to the air. This zone marks the limit below which the soil is not often wet.[2]

The soils are mostly fine sandy loams or silt loams, containing little clay. These soils are locally called "hard land." There are limited areas of dark-colored tillable sands, which, under ordinary tillage withstand drought better than any other soils of the region. Such soils are found north of Haxtum, Colo., north of Oshkosh, Nebr., and in other places. There are large areas of sand hills on which agriculture is out of the question, but within these areas are numerous small valleys where the soil contains some humus and is quite productive. In many of the valleys water is within reach of the plant roots, and here large crops of native hay and some cultivated crops are produced. In many places on the Plains there is more or less gravel, and considerable areas of adobe are found. The adobe is heavy and hard to work, but most of the soils are porous and easy to till when sufficiently moist.

On account of the dryness of the climate there is usually a large store of mineral plant food in the soil, but for the same reason it has

[1] See Bulletin 205, Office of Experiment Stations, U. S. Dept. of Agriculture, entitled "Irrigation in Wyoming," by C. T. Johnson, State Engineer.

[2] This does not refer to the deposits of soft, impure lime rock locally called "magnesia and native lime."

been impossible for any large amount of organic matter to accumulate. The aridity of the climate has not permitted a heavy growth of vegetation and has hastened the burning out of the decomposed matter. No large quantity of organic matter is usually present, much less than is found in soils of humid sections.[1] There is, however, in all the better soils of the region, where the rainfall is 15 inches or more, sufficient humus and nitrogen to produce a number of large crops. As yet the question of fertility has usually not entered into the problem of crop production in the semiarid region. The amount of moisture has not been sufficient to enable the farmers to use the fertility present. Lack of moisture has been the one problem. The writer does not say that if general farming becomes successful and well established fertility will not very soon become a problem or that it might not now be a problem if an abundance of water were available, but that in the past lack of moisture has been the one limiting factor. Under the heavy cropping of irrigation farming, fertility has in many sections become a problem within a very few years after breaking the sod. In fact, in some of the more arid regions more organic matter is needed from the start, as at Wheatland, Wyo. Any system of agriculture to be permanent must provide for the maintenance of the fertility of the soil, but in the territory here discussed the average farmer has not learned how to exhaust this, so its maintenance does not give him any immediate concern. The problem now at hand for the average farmer is to learn how to use profitably the fertility already present and how to produce crops with the limited amount of water received. When this is done, when he has learned how to utilize the native fertility of the soil under the prevailing climatic conditions, then attention may well be given to soil maintenance and improvement. It is altogether probable, however, that the addition of humus would so change the water-holding properties of the soil as to enable a crop to be produced with less rainfall.

The large crops produced in wet seasons and the large crops grown under irrigation all attest the value of the soil. The size of these crops is probably due in no small measure to the very dryness of the climate, contradictory as this may seem.

> A severe and long-continued drought * * * usually leaves the soil in excellent shape for a crop the following season, indicating that a complete drying out of the soil for a prolonged period brings about beneficial changes in the soil. Indeed, in keeping soils of poor or average fertility in an air-dry condition in the laboratory for several months they are usually found more productive when tested with plants again.[2]

[1] Bulletin 55, Bureau of Soils, pp. 27 and 28.

[2] Bulletin 55, Bureau of Soils, p. 63.

HISTORY OF THE SETTLEMENT OF THE REGION.

For 40 years, at least, the history of the settlement of the Plains has been one of periodic advance and retrogression. Periods in which settlement was rapid, energetic, and general have alternated with periods when abandonment, desertion, and return were almost as rapid and often prosecuted with as little judgment. But each wave of settlement pushed permanent agriculture farther west. The recoil never forced it back to its former limits, nor were the desertions ever complete. After each exodus, scattered settlers remained all over the territory that had been occupied.

The first wave that really populated the semiarid region was at its height in 1886. This wave carried settlement across the western counties of Kansas and Nebraska and well into Colorado. There was, however, a wide strip of public land still vacant east of the foothills across Colorado and farther north, in Wyoming, and in some of the extreme western counties of Nebraska. Not only did the settlers fail to appreciate the difficulties before them but many were wholly unprepared to face any hardships. They came, not only without any knowledge of the country, but without money with which to establish themselves—without means of maintenance till crops could be grown, to say nothing about stock and machinery. They had little or no working capital. They believed that if they could only get a "claim" they would succeed some way.

A few good crops came, then poor seasons, and the return commenced. Dry seasons and the panic of the nineties struck together with disastrous results. Lands which had been priced at from $5 to $20 or more per acre were offered for taxes, and often without a bidder. Under these conditions much of the land naturally fell into the hands of loan companies and far-seeing speculators. In one county several thousand quarter sections were allowed to revert to the county for taxes. These were finally all sold to a single company at $30 per 160 acres.

The abandonment was so complete in places that towns once of several hundred inhabitants were marked only by the empty school buildings, the cellars, and the hydrants remaining from the city water systems. Even within the last few months newspapers have reported the moving of one of these towns during a single night to escape the payment of bonds for over $30,000 voted during boom days to provide a water system.

At the time these lands were first taken little or nothing was known by the average settler concerning the climate. If there was a suspicion that rainfall was deficient it was entirely lost sight of in the delusion that rainfall followed the plow. The homesteaders confidently expected that in a few years the short-grass country would

prove itself the equal of eastern Nebraska and Iowa, and that the same methods of farming would be equally successful. They finally awoke to their mistake and, not knowing any way to meet the hard conditions, returned, generally to the region from which they had come. In many cases they carried with them an opinion of the dry country which was as much worse than the truth as their expectations had been too high. For these reasons the man who left the semiarid regions 15 or 20 years ago is likely to undervalue the possibilities which they possess.

As has been said, this desertion took place during the period of the lowest prices which a generation has known and during the most severe series of dry seasons experienced in 40 years, if not in the entire history of the country; years when farmers in the best agricultural sections of the country were obliged to sell horses, cattle, and hogs for anything they would bring, for lack of feed to keep them. Economic factors were as potent in bringing about these conditions as natural ones.

CONDITIONS THAT HAVE BROUGHT ABOUT RESETTLEMENT.

With the return of normal financial conditions and the increase in demand for agricultural products, prices began to rise and continued to rise till now they are at a point scarcely dreamed of 15 years ago. Favorable seasons returned large crops and the result has been the greatest period of prosperity that farmers have ever known. Farming became a very profitable business. In consequence, land values rose enormously, and of necessity rents also. Men who had failed to secure a foothold for themselves and those who thought their farms too valuable for what they produced, began to seek cheaper lands. New crops and new machinery had been introduced into the dry country and the few settlers who had remained produced good crops at a good profit. If any other influence was needed to bring about the settlement of the dry lands it was furnished by the land speculators and other promoters who took advantage of the opportunity and made every effort to give impetus to the movement, many of them using the most unscrupulous methods. Magazine writers, speculators, and enthusiasts heralded what was said to be the discovery of new methods of tillage which were certain to produce enormous crops every year. It has been commonly stated that such methods were in general practice on the Plains and that the good crops of recent years were entirely due to them, when as a matter of fact these crops have in most cases been due to more than normal rainfall. There is no marked improvement in the methods of tillage practiced on the majority of farms. This does not mean that nothing better can be done. Reference is here made only to what has been

done and is now being done on the overwhelming majority of farms in the region under discussion. There are a very few farms on which improved methods have been followed, and these farms indicate that much better crops are possible than the average farmer has secured.

Seeing the movement to the dry lands gaining momentum, speculators bought large ranches and employed agents in all parts of the country to parcel out the lands at a large profit. The result was an organized campaign for settlers. This could not have been condemned if the advertisers had been content with describing the semiarid region as it really is, but much of the advertising has been misleading and much of it positively untrue.

Railroads have been important factors in promoting settlement in all the western country. It was a good business proposition for them to increase the population of the country through which they had built, and, furthermore, many of them had large tracts of land which they were anxious to sell.

Strange as it may seem, the establishment of experiment stations in the region has had a strong influence in bringing in settlers. Somehow people seem to take the location of an experiment station as a guaranty that farming will be successful in the vicinity. The location of a station has almost always immediately increased the demand for and enhanced the price of land in the neighborhood. It should not be forgotten that most of the experiment stations in the region have been established only a few years, during which more than the usual number of favorable seasons have occurred. Many of the heavy yields produced at these stations have been largely due to abundant rainfall, as has sometimes been stated in their bulletins, but people frequently lose sight of the climatic conditions and attribute the results entirely to the methods and the seed used.

FARM PRACTICES IN THE REGION.

A very common method of putting in grain has been to go into a field which has received no preparation whatever since the last crop was harvested and with a seeding attachment on a disk cultivator, go over the ground once, and perhaps give one harrowing with a spike-tooth harrow afterwards. The writer has seen thousands of acres treated in this way, till so much perennial grass had gained a footing that it was often difficult to tell just where the field ended and the virgin prairie began.

Most of the land has seldom been plowed. Corn and sorghum have generally been listed in without any previous preparation of the soil and have been cultivated one to three times, the ground being treated the same way year after year or alternated with small grain disked in on the stalk ground. Since disk drills came into use it has

been common to drill grain right into the stubble without any soil preparation.

Shiftless as these methods may seem, it is hardly safe to so characterize them. These old settlers are not, as a rule, shiftless, but are energetic, practical, and optimistic. Many of them before going to the semiarid country were good farmers in more humid sections; the methods which they use have been reluctantly resorted to after long experience and are not without some merit. Their methods are to be considered as adaptations to the existing conditions. In reply to questions concerning these practices a common response is, "If the season is good anything will produce a crop, and if it is bad nothing will do any good. If I do good work I lose it either way." So far as the methods of cultivation common in humid sections are concerned, this statement is not without at least a coloring of truth. The principle has been to cover the largest possible acreage with the least possible work and expense. Some failures, many light crops, and a few large crops have been obtained; yet the evidence is that where the rainfall is from 18 to 20 inches corn and wheat have been produced at about the same cost per bushel as in eastern Nebraska and eastern Kansas. It must not, however, be concluded from this that farming has been as profitable on the average as farther east. There are many disadvantages connected with crop failure besides the loss of the crop itself. It is a great disadvantage to have to tide over one or two seasons at any time without a crop. There are also many social disadvantages connected with living in a sparsely settled country, often at long distances from markets, schools, and churches.

These conditions and practices make large areas necessary for the support of a family; but large areas have usually been available. Grazing land has been free or obtainable at a nominal rental, and very little feed has been used, even during the winter. Yet on the whole the condition of the settlers has been far from satisfactory, especially when the rainfall is less than 18 inches. If these men had been confined to the use of their own lands existence would hardly have been possible.

Within the last few years a number of important changes have taken place. Larger and better machinery has come into use; the hand separator and the centralized creamery have made a market for cream at every station; new crops have been introduced. Durum wheat, which gives a better average yield than other spring wheats and a much better yield in dry seasons, has become a common crop from Kansas north. Turkey Red winter wheat has advanced into the dry country and by the use of the press drill and better methods of cultivation is made, in many counties, a much more productive crop than spring varieties ever were. This is especially true of a

number of counties in Kansas near the eastern limit of the region. Emmer and new varieties of oats have helped. Sorghum and, in Kansas and southeastern Colorado, kafir and milo have become important crops. A very few farmers are using what are generally considered good dry-land methods. Most of the region has had an unusual number of wet seasons during the last 10 or 12 years, especially that portion north of the north line of Kansas, most of which has now had five or six unusually favorable seasons in succession.

But the most potent factor in bringing about more prosperous conditions has been the great advance in prices of products, while there has been but slight advance in the farmer's necessary expenses. A few years ago wheat sold at 30 to 40 cents a bushel, where it now brings 80 cents to $1, while the cost of production, aside from rent, has remained almost the same, if it has not actually decreased. Without considering rent, 8 bushels of wheat to the acre is a profitable crop at present prices. There is more than a living in it. But what was such a crop at 30 cents? Then, 25 bushels to the acre was not as good as 8 bushels now. During several years, when there was a surplus, corn was worth more to burn than to sell. It was cheaper fuel than coal. In fact, there were times when, if the grower were obliged to stay overnight on the trip to market, his load would have scarcely more than paid his expenses if he stayed at a hotel and put his team in a barn, as he does now. Cattle were correspondingly low; hogs were $2 to $3 a hundredweight; and eggs and butter were scarcely salable at all.

Interest is another factor of great importance to the man short of money. Fifteen years ago 2 to 3 per cent a month in advance were common rates of interest on chattel loans. The writer once saw a banker attempt to lend a farmer $64 in return for a note for $100 due in one year. This amounts to over 56 per cent interest. Now, very reasonable rates can be secured, though not as low as farther east.

THE AGRICULTURAL FUTURE OF THE REGION.

The hopes for better results in the future than have been secured in the past lie in (1) the continuance of high prices of agricultural products, (2) the general adoption of better methods of cultivation especially adapted to the conservation of moisture, (3) the introduction and development of more drought-resistant varieties of grains, forage crops, grasses, and vegetables, (4) the more careful and systematic management of the farm as a whole, (5) a change of attitude among the people from that of sojourners and speculators to that of permanent home builders, and (6) the fact that there is now a considerable population of "drought-resistant" settlers.

FUTURE PRICES OF PRODUCTS.

In the light of the history of agricultural development throughout the country it would seem comparatively certain that prices of farm products must average higher in the future than they have during the last 25 years. All prominent industrialists and political economists appear to be agreed upon this point. Therefore, it seems comparatively safe to assume that smaller yields of grain than have been required in the past will be sufficient to produce a living profit.

IMPROVED METHODS OF TILLAGE.

In the Great Plains region by far the largest portion of the precipitation comes during the warm months, and it is probably impossible to conserve as large a proportion of the rainfall as can be saved in the regions where the heavy precipitation occurs during the cooler weather; but the work at North Platte, Nebr., and other stations in the Plains region shows that on summer-tilled fields probably 40 or 50 per cent of the summer rainfall can be gotten into the first 6 feet of soil and held there for the use of the next season's crop.

It has been frequently asserted that all the rainfall of one year may be imprisoned in the soil and retained there for the use of the following crop. This, however, is a serious mistake. It requires about 3 or 4 inches of dry surface mulch to prevent serious loss of water from the soil below. All the water which does not get through this mulch into the lower layers of soil will be lost to the air by evaporation and not be available for storage. It is evident that, in a region where a large part of the rainfall comes in light showers during the warm weather, a very large proportion of the precipitation serves only to wet the surface mulch and is evaporated from it directly into the air. Ordinarily, showers of one-third of an inch or less coming in the warm part of the year are utterly useless as far as storing water in summer-tilled land is concerned and not infrequently are a source of positive loss, as, being only sufficient to wet the surface mulch and cause a crust to form, they make cultivation necessary for no other purpose than to break the crust thus produced, in order to prevent the loss of water already stored in the lower layers of soil and to prevent the growth of weeds that would immediately spring up. These statements must not be understood as applying to growing crops. Light showers may be of great value to a growing crop, but for the storing of water by summer tillage light showers are often not only of no value, but are a positive damage.

In the Great Plains region, then, it seems fair to assume that not more than 40 to 60 per cent of the rainfall can be gotten deep enough into the soil of a summer-tilled field to be retained there. Most of

the soils are capable of holding between 10 and 17 inches of water in the first 6 feet, but it is not always possible to get them filled to their full capacity, nor can plant roots draw all the water out of the soil. There will always be a considerable amount of water remaining in the soil when plants cease to grow, and even when they die on account of drought. The more rapid the evaporation, the greater will be the quantity of water in the soil when plants begin to suffer, because plants can not draw water as rapidly from a comparatively dry soil as from a wetter one. The amount of water which soil still contains when plants have ceased to grow normally varies with the character of the soil, being greatest in clay and least in sand. In most of the Plains soils it is from 4 to 7 per cent of the dry weight of the soil, or approximately that number of inches of water is distributed through the first 6 feet of soil. On the other hand, plants will live, though they will not grow much, till they have reduced the water content of the soil nearly, though not quite, as low as the dry air will be able to reduce it. Hence, it may be assumed that one-half to three-fourths of the water which is stored in the soil is actually available for normal plant growth. In a season, then, of 16 inches of rainfall, if one-half of it is stored in the first 6 feet of soil there will be 8 inches of water conserved. Probably 4 to 7 inches will be actually available for the use of plants; that is, a reserve of 4 to 7 inches of water is carried over to supplement the rainfall of the succeeding season or to start winter grain and keep it growing till spring rains come. This stored water, however, is much more valuable to growing crops than an equal amount of rainfall, because it is down so far in the soil that a much smaller percentage of it is lost by evaporation from the surface than of the rain which falls upon the crop. A small amount of water is often invaluable in enabling a crop to pass successfully through a dry spell which it would not otherwise withstand. In this way even a very small reserve may determine the fate of the crop.

From this it will readily be seen that there are many places on the Great Plains where it would not seem probable that summer tillage would conserve sufficient moisture, together with the rainfall of the succeeding season, to produce a profitable crop.

How much rainfall is absolutely necessary to produce one crop in two years is largely a matter of speculation. At present, however, it does not seem that in the region under discussion profitable crops can be expected without a precipitation of at least 15 or 16 inches in one of the two years; that is, either while the ground is being summer-tilled or while it is growing the crop. When a season with only 8 or 10 inches of rainfall is followed by one equally dry it does not seem possible that even summer tillage will produce a paying crop. But

if the summer tillage is conducted through a season of considerable rainfall followed by a dry season, it may be altogether possible to produce a profitable crop. When a dry season is followed by a dry season, the prospects for success seem small indeed.

In all the region under discussion, even in the eastern part, seasons of much less than 16 inches of rainfall are likely to occur. Where the average is only 15 or 16 inches, fully half the seasons will have less than this amount, and presumably, even with the best known methods for the conservation of moisture, many light crops and a considerable number of failures must be expected.

Statements have frequently been published by uninformed or unscrupulous persons which leave the impression, if they do not actu-

FIG. 2. A field of wheat on summer-tilled land, Phillips County, Colo., 1909.

ally say, that 40 to 60 bushels of wheat per acre can be produced every other year by summer tillage wherever the average precipitation is 10 inches. Such statements must be considered as purely visionary and without any foundation in fact.[1] So far as the writer is aware, the best yields of wheat obtained on summer-tilled land anywhere in the Great Plains region for a period of years have been secured by one farmer in Logan County, Colo., and one in Phillips County, Colo. (See fig. 2.) The first reports an average of 28 bushels to the acre for five years and the second 35½ bushels to the acre for

[1] In the State of Washington, where the conditions are especially favorable for wheat growing, and where summer tillage has reached a high development, the yield of wheat in those regions where there is an annual precipitation of 10 to 12 inches seldom exceeds 20 bushels to the acre. The yields usually obtained with that amount of rainfall will run from 7 to 15 bushels, depending on conditions.

seven years. These crops were all produced on land that had been thoroughly summer-tilled and during a period of seasons more favorable than the average. It must be remembered that each of these crops required two years for its production.

In the matter of summer tillage for the conservation of moisture there is considerable variation in the practices of the best dry-land farmers. The best method appears to be to double-disk the land in the summer as soon as possible after the grain is cut (if a small-grain crop was grown), and again in the spring as early as the ground can be worked, and then disk or harrow as often as is necessary to keep down the weeds and to keep the crust broken till about June; then plow as deeply as the available horsepower will permit, disking or harrowing each half-day's plowing before leaving the field, or, better, using a revolving pulverizer attachment on the plow. After this the ground must be double-disked, harrowed, or worked with some other surface cultivator as often as is necessary to keep the crust broken, maintain a good surface mulch, and keep the weeds down till time for seeding winter wheat. A field tilled in this way is shown in figure 3, while the crop grown on an adjoining field similarly tilled the preceding year is shown in figure 2.

The depth of surface mulch required will vary somewhat with different soils and other varying conditions, but will generally need to be 3 or 4 inches. This should not be a dust mulch but a mulch of granular soil or small clods. A dust mulch is not only less effective in conserving moisture than a mulch of small clods but is a very uncertain thing to hold in a region of high winds. A dust mulch of 3 or 4 inches might be blown off the entire field in a single day. In maintaining the mulch it is best to vary the depth of cultivation so as to prevent the formation of a crust below the mulch.

Before time for seeding, that part of the soil between the surface mulch and the bottom of the plowing should be well firmed in order to reestablish connection between the furrow slice and the soil below, enabling the water to rise by capillarity to within easy reach of the young plants and form a firm seed bed, which is an absolute necessity for the best development of the small grains and grasses. This condition may be secured by subsurface packing immediately after the plow, but the subsequent working will usually give the required condition. When considerable rain falls between the time of plowing and the time of seeding this will serve to firm the lower part of the furrow and connect it with the soil below. Packing will insure the filling of the air spaces left in the soil as it falls from the moldboard of the plow and will bring the moist soil in contact with whatever stubble or trash may be turned under and hasten its decay—an important point. Packing will show the greatest benefits on light soils in dry

seasons and on late plowing. It may be of no benefit in a wet season and may be harmful on heavy soil.

There are various types of corrugated rollers and special subsurface packers made for this purpose, and the work may be done with the common disk by setting it straight and weighting it to make it run deep. Subsurface packing is important not only on summer-tilled but also on spring-plowed land that is to raise a crop the same season.

In western Kansas and eastern Colorado the lister is very much in favor and the farmers use it for every possible purpose. Most of the few who have done any summer-tilling do not plow the ground but list the land that is to be summer-tilled just after they get through

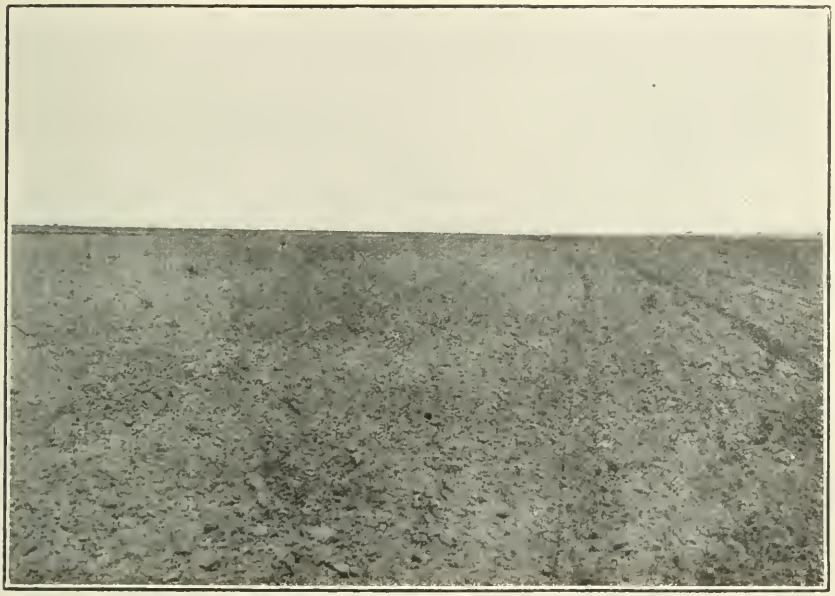

FIG. 3.—A summer-tilled field where winter wheat will be grown, adjacent to the field shown in figure 2.

planting corn (fig. 4), then throw down the ridges. About the last of June or the first of July they list again, splitting the middles left by the first listing. They then throw down the ridges and do whatever additional cultivation is necessary to keep down the weeds and maintain the surface mulch. This is a cheaper way of doing the work than plowing, because less cultivation is required. It has an advantage also in the fact that if the weeds attain any considerable size (which should never be allowed), the ridges enable the farmer to kill the weeds without plowing the ground. It does not seem to the writer, however, that this method can, on the average, produce as favorable results as that previously described, although there are no data at hand to show the comparative values of the two methods.

Conservation of moisture is not the only benefit derived from summer tillage, although it is one of the most important reasons for the good results following. Such tillage puts the ground in very much better physical condition for plant growth, aside from the more favorable moisture content. There is abundant evidence also that there is more available plant food in the upper layers of soil and within easy reach of the plant roots. This, however, must not be interpreted to mean that fertility has or has not been added to the soil. The temperature and moisture conditions secured by the clean and thorough cultivation given are favorable for changing the condition of the plant food already in the soil so that plants may use it, while it was previously in unusable form.

FIG. 4.—A field summer-tilled by listing instead of plowing, Rawlins County, Kans., 1909.

One of the very important effects of summer tillage on winter wheat is that it enables the wheat to start at once with a vigorous growth and so enter the winter in good condition. It thus comes through the winter strong, well rooted, and ready to take advantage of any opportunities for growth. In this condition it is able to withstand considerable hardship, when wheat on land less thoroughly prepared suffers in the winter and comes through weak or dies. It will often happen that the field which was summer-tilled the preceding season will contain little, if any, more moisture in the spring in the first 3 feet of soil than a field which grew a crop and was plowed and seeded to wheat, but the wheat on the summer-tilled land will have so much better start that it will go on and make a crop under conditions that would cause the other to fail. It appears also that grain on summer-tilled land, either by virtue of better root systems or because

of the better capillary condition of the soil, is able to draw water from a greater depth.[1] Summer tillage is not practicable on all soils nor for all crops. Soils which are likely to blow, especially very sandy soils, can not be bare tilled because they will blow away.

Summer tillage has proved a success for winter wheat and by this method of cultivation winter wheat becomes the surest crop in the region; but without summer tillage winter wheat is as uncertain in much of the region as spring grain. Summer tillage considerably increases the yield of spring grains also. It is still uncertain whether it is profitable to summer-till for spring grain or whether all the summer-tilled land should be utilized for winter wheat and potatoes.

There are now sufficient experimental data at hand to show conclusively that summer tillage is not profitable for corn, all results indicating that corn on spring-plowed land will outyield that on summer-tilled land. With all cultivated crops frequent, thorough, and shallow cultivation is of the utmost importance. Unless the season is unusually favorable the harrow should be used frequently on corn, potatoes, and small grain until the plants are so large as to be damaged by this implement.

Up to the present time the methods employed in the semiarid plains have, for the most part, been merely a makeshift. That these methods must and will be changed for the better is certain. General agriculture can never have a substantial foundation in this region until tillage for the conservation of moisture is generally practiced. In the face of the fact that so much has been done in the way of tillage for the conservation of moisture in parts of Utah, Oregon, Washington, and Canada, it is a wonder that so little has been done on our semiarid plains. The work of the agricultural experiment stations and of the progressive farmers in the region has now gone far enough to prove that by the use of methods for moisture conservation which have accomplished so much in the far northwest a considerable amount of moisture can be conserved and used for crop production in all portions of the semiarid region.

INTRODUCTION AND DEVELOPMENT OF DROUGHT-RESISTANT CROPS.

As regards the introduction and development of drought-resistant crops, much is to be expected. The Department of Agriculture has experts scouring all parts of the world in search of plants which may prove valuable in the various sections of the United States. There are many regions in the Old World where the climatic conditions are very similar to those of our semiarid Plains and upon which civilized men have maintained themselves for thousands of years. Many varieties of drought-resistant plants adapted to our semiarid

[1] See "Some Soil Studies in Dry-Land Regions," by Dr. F. J. Alway, in Bulletin 130, Bureau of Plant Industry, 1908, p. 38.

regions have already been discovered in such places and introduced to the decided advantage of the Plains farmers. Among these may be mentioned durum wheat, brome-grass, alfalfa, milo, kafir, and sorghum. It is to be expected that many other useful varieties will yet be discovered and introduced.

The development of new varieties requires in most cases a considerable period of years, and yet since the semiarid region was first settled marked improvements have already been made in many of our crops. Some of the varieties of corn, for example, which have been developed on the dry lands by selection are much more capable of producing crops under the severe conditions existing than was the original stock from which they have descended. Milo a few years ago was a tall plant of irregular height and produced drooping heads, but careful breeding has developed a quite uniform dwarf strain with erect heads. Hardy varieties of winter emmer, barley, and oats may be expected in the near future, each of which would be of great value to the dry country as well as to many other sections.

As nearly all our common grains have been developed from plants which in their early history were adapted only to much more humid climates than those in which we now grow them, there is no reason for thinking that varieties and strains of these plants may not yet be produced which will succeed with far less water than is required by the present varieties. With the rapid advancement which has been made in agricultural science and plant breeding within the last few years it would seem only reasonable to expect results to be obtained in much less time than has been required in the past. There is work now going on at some of the agricultural experiment stations which indicates that it may be possible within a few years to breed a variety of corn which will produce an equal amount of grain with perhaps only two-thirds as much water as is now required. To what extent drought-resistant varieties may be developed and how much may be accomplished in reducing the water requirements of plants is purely a matter of speculation, but the work has already gone far enough to give assurance of considerable success in this line. A word of caution to the individual may be necessary here. Plant breeding, while it will surely play an important part in the future development of the region, is too slow a process for the individual to wait for or to depend upon. It is a regional rather than an individual proposition.

Over all the region in question the only winter wheats that have proved themselves sufficiently hardy are of the Crimean type, such as Turkey Red and Kharkof. Common spring wheat is largely grown on the table-lands south of Wray, Colo., and also in eastern Wyoming and the adjacent parts of Nebraska. It seems comparatively certain, however, that with good tillage, winter wheat will far outyield the common spring varieties, even in these sections.

Durum, or macaroni, wheat is giving better yields than the common spring varieties in nearly all sections north of the Kansas-Nebraska line, but south of this line it has not been so satisfactory. The yields of durum wheat commonly approach those of winter wheat when given similar advantages of cultivation. Durum wheat, however, is at a great disadvantage, because it is commonly discriminated against on the market to the extent of 5 to 20 cents a bushel. The lower price frequently offsets the higher yield and makes the crop no more profitable than other spring varieties. Experiments at North Platte, Nebr., indicate that durum wheat will produce more feed to the acre than either oats or emmer. There would seem, then, no good reason why it should not be grown in preference to either of these crops unless the straw of oats and emmer is enough better feed than the wheat straw to overcome the difference in favor of the wheat.

Early varieties of oats are much more certain than late varieties in all the southern part of the area under discussion, and are at least as productive in the northern portion. The late varieties, however, succeed much better north of the South Platte River than they do farther south.

Barley is a valuable feed crop throughout the region, but in most places is not so popular as other grains. It has generally been more satisfactory in the northern than in the southern sections. California feed barley appears to be one of the best varieties for feed and the common six-rowed the best for market.

Emmer, commonly called spelt, is quite generally grown as a substitute for oats. While this is one of the most drought resistant of our spring grains it does not appear to be able to produce any more, if as much, feed to the acre than oats, barley, or durum wheat.

South of the Rock Island Railroad milo is one of the surest and most productive grain crops, and at the same time it makes considerable fodder, though for fodder it is inferior to sorghum and kafir. Kafir is also grown in the same territory, but it requires a longer season and produces less grain to the acre than milo, though it is a much better fodder plant.

Sorghum stands without a rival as the most important fodder crop of the semiarid region as far north as the South Platte River, and may be used to advantage throughout the limits of the territory under discussion in these pages.

Millets are of more or less importance throughout the region, but are much less productive and less drought resistant than the sorghums. They have the advantage, however, of being able to mature in much less time.

In a large portion of the region, especially that receiving the heaviest rainfall, alfalfa can be produced with more or less success, but

in the drier parts its production on uplands by the use of ordinary methods is doubtful. Moderately successful small fields have been maintained for a number of years in many localities, as at Santa Fe, Kans.; Vernon, Colo.; Colby, Kans.; and Sextorp, Harrison, and Alliance, Nebr. Most of the large yields of alfalfa reported from the region have been grown on subirrigated valley land, but the public has commonly credited them to the upland. There are still many subirrigated patches growing buffalo grass that ought to be seeded to alfalfa. A number of fields of alfalfa seeded in rows 30 to 36 inches apart and thoroughly cultivated, very much as corn and potatoes are cultivated, have produced very profitable crops for several years. The promise for the production of alfalfa seed by this method is very bright for the entire region.

PROMISING SYSTEMS OF FARM MANAGEMENT.

It does not seem advisable for anyone to attempt to do exclusive grain farming in this region and expect to make it a permanent success. In the past this has proved inadvisable here, as it has nearly everywhere else. On the other hand, it has also been proved by the majority of old settlers that for the man with limited means it is precarious to depend on stock alone. At least, the most certain means of securing a more or less constant income is to give attention to a number of different products. This also enables one to accomplish much more with the same number of laborers, because it furnishes more constant employment.

As has been mentioned previously, the growth of grass is comparatively small in this dry country. For this reason a large area is required for pasturage. In most places somewhere from 8 to 20 acres of native grass, together with 2 tons of rough feed, though often this amount of rough feed is not used, are required to carry one grown horse or cow the year round, or 1 square mile will commonly pasture from 30 head on the drier and sandier lands to 80 head on the best lands. This, together with the frequent light crops, makes it essential that settlers own or control larger areas of land than are required to maintain a family in more humid regions.

It is impossible to say definitely what the farm unit should be or on how small a tract a family can live. The acreage required must necessarily vary much with the local conditions, but it will vary even more with the man. There is much truth in the old saying "Thar's more in the man than thar is in the lan'." However, some general statement on this subject may serve to give those unfamiliar with the conditions a better idea. It is the writer's opinion that on the better lands near the eastern limit of the territory 320 acres should be sufficient to support a family, but near the western limit for general agriculture two to four sections will be needed. In most cases only

150 to 200 acres should be broken and the rest used for pasture. These figures must be taken as only suggestive. It will appear to many that the area here allowed for the pasturing of one animal is excessive, but it is none too much. It will also be suggested that cultivated grasses can be sown which will very much reduce this area; but experience has not yet proved it advisable to make a general practice of plowing up the native grass with the expectation of making a better pasture by sowing something else. It must be remembered that the higher rate of evaporation in the southern portion of the territory makes the conditions there more severe and the acreage required larger than is necessary with the same rainfall farther north.

On the heavier lands it now seems that the most promising system of management is about as follows: Leave a large portion of the farm, probably three-fourths, or all but 100 to 200 acres, in native pasture, and keep all the dual-purpose cows the pasture will carry, along with the young cattle, horses, and colts. Butter or cream is one of the surest sources of income and profit. There should be pasture enough to feed one animal for every 1 to 2 acres of land under cultivation; in the best portions of the region, however, the farmers have not always found it most profitable to keep so much stock. There should always be a large flock of poultry. Hens will lay in dry seasons as well as in wet. One of the first objects on the farm land, then, must be to raise feed for the stock. In seasons of good crops the farmer must stack feed to carry over and to tide him through dry years. He must reverse the old adage learned in his youth, "Lay by something for a rainy day," and in this country must learn, both with regard to himself and his stock, to lay by something for the dry day. Of the farm land one-fifth to one-third should be summer-tilled each year for winter wheat and potatoes for money crops, these to be followed with corn or some fodder crop, and the third year with spring grain or summer fallow.

Assuming that the farm contains 640 acres, one-fourth of which is under cultivation, the foregoing plans would call for one of the following rotations on each field:

Rotation for farm of 640 acres, one-fourth under cultivation.

THREE-YEAR ROTATION.	FOUR-YEAR ROTATION.	FIVE-YEAR ROTATION.
First year, summer-tilled.	Summer-tilled.	Summer-tilled.
Second year, winter wheat and potatoes.	Winter wheat and potatoes.	Winter wheat and potatoes.
Third year, corn and rough feed.	Corn.	Corn.
Fourth year, summer-tilled.	Spring grain and rough feed.	Spring grain.
Fifth year, same as second.	Summer-tilled.	Rough feed and corn.
Sixth year, same as third.	Winter wheat and potatoes.	Summer-tilled.

These rotations would give the following acreages of each crop on the farm each year:

Acreages in different crops for farm of 640 acres, one-fourth under cultivation.

THREE-YEAR ROTATION.	FOUR-YEAR ROTATION.	FIVE-YEAR ROTATION.
53 acres summer-tilled.	40 acres summer-tilled.	32 acres summer-tilled.
53 acres in winter wheat and potatoes.	40 acres in winter wheat and potatoes.	32 acres in winter wheat and potatoes.
53 acres in corn and rough feed.	40 acres in corn.	32 acres in corn.
	40 acres in spring grain and rough feed.	32 acres in spring grain.
		32 acres in corn and rough feed.

In the southern part of the territory winter barley and in the northern part winter rye may replace a part of the winter wheat. No one of these systems, of course, could be followed on all farms, but some one of them can easily be varied to meet almost any of the local conditions. On the sandier lands summer tillage can not be practiced and winter wheat does not do well. On such lands corn, sorghum, emmer, and rye must be the main crops.

In the best part of the area the five-year rotation will probably give the largest net returns, while in the western part the three-year rotation will best fit the conditions. It should be noted that winter wheat and potatoes follow summer tillage in all rotations. That is because summer tillage has proved more profitable for these crops than for any others. Summer tillage has not proved profitable for corn, and therefore this crop follows winter wheat. The thorough cultivation which corn requires leaves the soil in good condition for spring grain; in fact, many tests have given as good yields of spring grain following corn as on summer-tilled land. Sorghum is the most vigorous feeder and the most drought-resistant crop of all, and for that reason is placed last in the series. Sorghum also dries out the ground so completely that the following crop is entirely dependent upon the rainfall of the current season, there being little or no available water left in the soil by the time the sorghum is mature. For this reason any crop following sorghum is almost sure to give a low yield unless timely and abundant rains occur. Following the sorghum with summer tillage gives an entire season in which to replenish the soil moisture before another crop is planted.

If the manure is cared for there will be enough to give each field a light dressing at least once in the rotation. The writer is well aware that farmers in the dry country are generally afraid of manure, but he is convinced that the trouble is mostly due to too heavy applications. Manure should be spread as evenly as possible and great care should be taken that no large bunches are left. The thinner

it is spread the better. It should always be disked or otherwise worked into the soil before the ground is plowed. If these precautions are observed, only good results should be expected. The best place in the rotation to apply the manure is probably just preceding the sorghum. The sorghum will generally be listed, and so the roots will be below the manure. What manure is not incorporated with the soil is near the surface and will help conserve moisture instead of burning out the crop, as it is very likely to do if a heavy dressing is plowed under before it has time to rot. Summer tillage following the sorghum gives another full year for the manure to rot before a small-grain crop occupies the land. If the land is poor manure may be spread very thinly on the winter wheat to good advantage, but if the land is rich and a wet season follows the wheat is almost sure to lodge. Manure may safely be applied on ground that is to grow corn, or it may be spread lightly on the stalk ground before it is disked for spring grain. When used in this way it is often of great benefit in preventing the soil from blowing.

None of these rotations provides for a protein feed, but no satisfactory high-protein feed crop is available. Any land that will grow alfalfa should be seeded to it, and probably with proper care this crop can be grown to some extent on every farm. The experiments with alfalfa in rows 3 or 3½ feet apart and cultivated as regularly as corn are giving flattering results, and where it is too dry for the ordinary seeding it seems almost certain that this method will produce valuable seed crops and at the same time some feed.

Most of the soils of the dry region are short of nitrogen and humus, and if alfalfa can be grown for a few years the land will produce better crops of other kinds. The writer is personally familiar with a small field of alfalfa in northeastern Colorado which died in 1894. In the summer of 1908 the native grasses which had taken possession were more than twice as thick and tall as on the remainder of the field. In irrigated fields on the plains of Colorado yields of grain are frequently doubled where alfalfa has been grown. Alfalfa, however, leaves the ground very dry, and for that reason the first crop following it often suffers severely from drought and makes a light yield unless the season is very favorable.

In much of the sand-hill country and on some of the rough land nothing but stock production seems possible; but even here, where in reach of a market, the small stockman will find it almost necessary to sell cream. In the sand hills hay is usually plentiful, and where the settler has valleys that will grow alfalfa or peas milking should be profitable. In many of the better valleys potatoes may be produced, but in some places the difficulty in getting them to market makes them impossible as a market crop.

To make the homestead more attractive and to furnish shade and windbreaks, everyone wants some trees around the house. Besides the comfort secured from trees nothing adds more to the appearance of a place, and in the whole region nothing is more conspicuous by its absence from the settler's home. This is unnecessary. There are hardy trees that can be kept growing anywhere in the region where crops can be grown if they are given proper care. Windbreaks are valuable for the protection they afford to growing crops and stock as well as about the house, and may also be made to yield material for fuel, fencing, and farm timbers. They should be kept thoroughly cultivated, at least for several years, and fenced from stock at all times. The honey locust is one of the best varieties to use, and has generally succeeded in the drier portions of the region. The green ash is very hardy and may be kept growing, but should be planted in the moister situations. In places it may be attacked by borers sooner or later. The white elm is also very hardy, and while not equal to the honey locust, is in general a more desirable and more satisfactory tree. The black locust is quite hardy and a rapid grower, but it is almost sure to be destroyed by borers. In some portions of the Central Plains region it might be advisable to plant Russian mulberry, Russian olive, and Osage orange. The Forest Service also recommends the western yellow pine, the jack pine, and the Austrian pine for this region. So far as present knowledge goes this about exhausts the list of forest trees adapted for planting in this section.

Fruit growing on a commercial scale is not to be recommended, but every farmer wants some fruit even if it costs him more in labor than it would to buy it. Small fruits, including Early Richmond cherries, plums, and currants, can be grown if a little special care is given them. Strawberries may also be produced if a little water can be secured. Frequently enough water can be spared from the well to help a great deal in the garden or on the small fruit. On most farms there are slopes where a deep furrow would collect considerable storm water and run it to the garden, even when there was only a light shower. Advantage should be taken of every such opportunity offered. Without a garden and some fruit it is hard to call a place home. Where ground water is available a windmill may supply water for a garden.

Two of the most prosperous and painstaking farmers of northeastern Colorado have worked out on their farms almost exactly such systems as here outlined and have followed them for a number of years with marked success, while a considerable number of progressive farmers scattered over the region are partially following such systems.

OPPORTUNITY FOR FARMERS IN THE GREAT PLAINS.

The Great Plains is not a region where a farmer should expect to make large profits with a small investment if he is to confine his operations to his own lands. Large profits have been made, but in most cases they have been the result either of speculating in lands or of running cattle on free grass. More capital is needed to start to advantage than in a more humid section, because there is more danger of failure. One must often wait till the second year before he has any certainty of a profitable crop, for it takes a full season to get land in shape for a good crop of wheat. If the season is unusually favorable the spring crops may be very profitable, but the risk in depending on them is great. Spring or early summer breaking is almost equal to summer-tilled land for small grain if the land has not grown many weeds.

No man should go empty handed into this country, but many men with limited means who are willing to endure some privations will be able to secure a foothold and establish homes. We are often asked how much capital is necessary and whether the land is too high priced. Obviously, these are questions which the individual must answer for himself. In the Great Plains, as anywhere else, it is not necessary that one have sufficient capital to enable him to start free from debt. In general, we may say that in our opinion from $6,000 to $8,000 should buy enough land to support a family of average size, and that where it is mainly a stock proposition $50 should buy enough land to pasture one cow. It is evident that one can not afford to pay $10 an acre for land where 4 square miles are required to give one family a moderate support. By comparing these statements with those concerning the necessary size of the farm, the reader may draw his own conclusions.

No man should think of "dry farming" by what are generally considered improved methods as an indifferent or lazy man's job. Dry farming, to be successful and permanent, is necessarily good farming. The indifferent farmer will get a few good crops, many poor ones, and many almost complete failures. The man who has failed in a more humid region should not expect to succeed in the Great Plains. In a humid region any kind of cultivation is almost sure to bring some kind of crop, but not so in the dry country. It is only the best and most systematic farming that can be expected to give even moderate returns in unfavorable seasons, and in some seasons even this will fail.

That there will be many failures among the settlers now locating in the drier parts of the region goes without saying. In many places inexperienced men are crowding in too thickly and are expecting to make a living on far too small an area. They are trying to farm by

the same methods used in more humid sections or by more careless methods, and are depending on timely rains to bring results. Then, too, among the immigrants to any undeveloped country there is a relatively large proportion of individuals who do not go with a fixed purpose to establish a home but expect to sell at the first opportunity. In fact, in many sections it is difficult to keep from gaining the impression that land speculation is receiving more attention than crop production. Far too many, whether their holdings are deeded lands or merely homestead entries, are hoping to sell at a profit rather than to establish homes. To such this discussion makes no appeal. It is written not as a guide to speculators but as an aid to home seekers. The writer does not wish to be understood as condemning land speculation, but land speculation does not develop the agricultural possibilities of a region or support a stable population. What is needed in the semiarid region is not speculators but home builders—not a shifting but a stable, producing population. There probably are many people both in this country and in Europe who could be happier, freer, more healthy, and more prosperous on the semiarid Plains than in their present situations.

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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 216.

B. T. GALLOWAY, *Chief of Bureau.*

THE RUSTS OF GRAINS IN THE UNITED STATES.

BY

E. M. FREEMAN, *Collaborator,*

AND

EDWARD C. JOHNSON,

Pathologist in Charge of Cereal Disease Work, Office of Grain Investigations.
In Cooperation with the Minnesota Agricultural Experiment Station.

ISSUED AUGUST 15, 1911.

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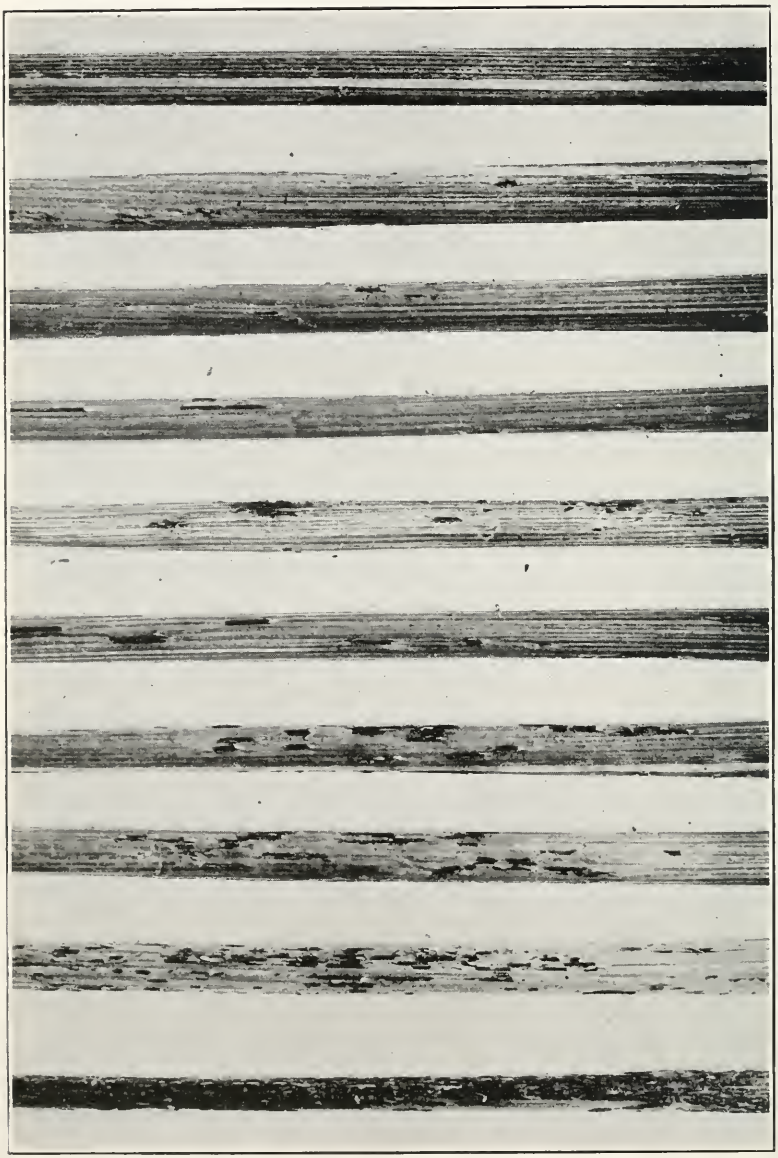

STEM RUST OF WHEAT ON WHEAT CULMS, SHOWING VARIOUS DEGREES OF ATTACK.

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BUREAU OF PLANT INDUSTRY.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., March 25, 1911.

Sir: I have the honor to transmit herewith a technical paper entitled "The Rusts of Grains in the United States," by E. M. Freeman, Collaborator, and Edward C. Johnson, Pathologist in Charge of Cereal Disease Work. This paper embodies the results of recent research by the Office of Grain Investigations in cooperation with the Minnesota Agricultural Experiment Station into the distribution, relationships, physiology, and life history of the important grain rusts, and gives much new information on the "biologic forms" of rusts, vitality of successive uredo generations, wintering of the uredo generation, and climatology in relation to rust epidemics. Former experiments on rust prevention are summarized and methods of selection and breeding of grains for rust resistance indicated.

The grain rusts continue to be of large economic importance, and as this paper is another step advancing our knowledge concerning them I recommend that it be published as Bulletin No. 216 of the series of this Bureau.

Respectfully,

Wm. A. Taylor,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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THE RUSTS OF GRAINS IN THE UNITED STATES.

INTRODUCTION.

That rusts are among the most serious diseases of grains in the United States is generally granted. As they are always present in humid grain-growing districts to a greater or less extent, it is almost impossible to make accurate estimates of the damage caused by them. Estimates are, perhaps, more often too low than too high, so that the losses of fifteen to twenty million dollars annually, estimated by Bolley (28,[1] p. 615) for the United States, certainly seem within reason. Numerous references to losses from rust epidemics in different countries may be found.

The most severe epidemic in the last decade occurred in the United States in 1904. It was particularly prevalent in the spring-wheat belt of the northern Mississippi Valley, where the three States, Minnesota, South Dakota, and North Dakota, in which the bulk of the hard spring wheat of the United States is raised, suffered perhaps more than any other section of the country. Table I shows a comparison of the wheat crop in these three States for the years 1903, 1904, and 1905, affording a basis for an estimate of the losses sustained in this epidemic.

TABLE I.—*Wheat crop in Minnesota, South Dakota, and North Dakota in 1903, 1904, and 1905.**

Year.	Acreage.	Yield per acre.	Total yield.
		Bushels.	*Bushels.*
1903	13,167,110	13.15	173,146,171
1904	13,193,695	11.65	153,793,233
1905	14,069,251	13.66	192,190,759

* Compiled from U. S. Crop Report.

Average yield per acre for 1903 and 1905=13.4 bushels; for 1904=11.65 bushels. Reduction in yield per acre in 1904 below the average for 1903 and 1905=1.75 bushels. Total reduction in yield in 1904=13,193,695 (acres)×1.75=23,088,966 (bushels). Average price for the three States for 1903 and 1905=66.8 cents. Reduction in value in 1904 below the average for 1903 and 1905=23,088,966×66.8 cents=$15,423,429.28.

[1] The serial numbers in parentheses throughout this bulletin refer to the titles in the "Bibliography" on pages 79–82.

An average reduction in yield of 1.75 bushels per acre in 1904 as compared with the preceding and following years gives a total reduction of over 23,000,000 bushels, valued at more than $15,000,000.[1] The greater part of this reduction in yield and consequent loss was undoubtedly due to rust. It is exceedingly conservative to put the loss in these three States in 1904 as high as $10,000,000; and when we consider the additional losses in the other wheat-growing districts of the United States the aggregate is enormous.

KINDS OF RUSTS IN THE UNITED STATES.

This paper deals only with the rusts of the small-grain crops, wheat, barley, rye, and oats, and includes the following forms:[2]

Puccinia graminis Pers. on wheat, rye, oats, and barley, commonly known by the misleading term "black rust," but more appropriately known as "stem rust," as it generally is confined more or less closely to the stem and sheath (Pl. I).

P. rubigo-vera tritici[3] on wheat, known as "orange leaf rust," or "leaf rust of wheat."

P. rubigo-vera secalis[3] on rye, known as "orange leaf rust," or "leaf rust of rye."

P. coronata Corda. on oats, known as "leaf or crown rust of oats."

P. simplex (Körn.) Erikss. and Henn. on barley, known as "leaf rust of barley."

These rusts, in common parlance, are classed as stem or leaf rusts, a convenient grouping which directs attention to the chief though not exclusive location of the rust on the host plant.

DISTRIBUTION OF RUSTS IN THE UNITED STATES.

GENERAL STATEMENT.

All of the grain rusts, with the possible exception of stem rust of rye and the leaf rust of barley, are found throughout the United States wherever their host cereals are grown. As to the distributon of the barley leaf rust, less is known, because it may have been but recently introduced into this country and appears, as a rule, late in the season. It has been reported from California, Virginia, Minnesota, and Iowa and is probably of wide distribution.

Although the rusts are for the most part practically coextensive with the hosts, they are not serious in all localities. Epidemics may occur in almost any grain-growing region, but they occur less frequently in some sections than in others. In general, the area most affected is the valley of the Mississippi and its tributaries, comprising the region west of the Alleghanies and east of the ninety-eighth

[1] Even comparing the yield, 11.65 bushels per acre, with the 10-year average (12.2 bushels per acre) for the three States from 1896 to 1905 (obtained by computing the averages in the three States), there was a reduction in yield in 1904 of more than 0.5 bushel to the acre.

[2] A few other rusts have been reported, some perhaps by mistake and some of such rare occurrence as to be of no economic importance. *Puccinia glumarum* (Schm.) Erikss. and Henn., the yellow rust of wheat, which is a very common and serious rust in Europe and India, has not yet appeared in this country.

[3] The trinomial terminology for these two rusts was first used by Carleton (30, p. 10).

meridian, which marks the eastern border of the semiarid lands of the central United States. It thus includes a large portion of the great grain-growing districts of this country. The rusts are also very severe and of annual occurrence in the small, isolated districts on the west side of the Coast Range in California, in eastern and southern Texas, and in parts of the Atlantic Coast States. They are an important factor in the grain-growing regions of eastern Washington and Oregon. In general, where the annual rainfall is 20 inches or more, rust may be a serious menace to crops. In areas where the annual rainfall is less than 20 inches rusts are generally of little importance. Such dry areas occur in the United States just east of the Rocky Mountains, extending eastward to the ninety-eighth meridian and in the intermountain areas, including Wyoming, much of Montana, Idaho, Utah, Colorado, New Mexico, southeastern Oregon, and the interior valleys of California. Here in most years rusts are comparatively rare, though in the great rust epidemic of 1904 some of these areas, including California, were affected.

AREAS MOST LIABLE TO RUST.

The area where rust is particularly a menace is the hard spring-wheat belt of Minnesota, North Dakota, and South Dakota. The States bordering the Ohio River Valley, including Kentucky, Illinois, Indiana, and Ohio likewise are frequently attacked by rust. In the Southern States of the eastern half of the United States—that is, south of and including Tennessee and North Carolina—rust of certain cereals has been so serious as almost to prohibit the growing of them in those regions. It is very difficult, for instance, to grow spring oats profitably in portions of the southernmost tier of States east of the Mississippi River, one of the chief difficulties being rust. Almost nowhere in this southeastern part of the United States are the small grains, with the exception of winter oats, grown at all extensively.

DISTRIBUTION OF THE DIFFERENT RUST SPECIES.

Stem rust of wheat.—The stem rust of wheat (*Puccinia graminis tritici* Erikss. and Henn.) is of great importance in the hard winter and hard spring wheat belts of the Great Plains area and in the States bordering the Ohio River. In Maryland, Virginia, and other Eastern States it has been almost entirely absent for many years, but is by no means unknown. In Washington and Oregon it is frequent and virulent. In the interior mountain valleys, between the Rocky Mountains and the Sierra Nevada Mountains, and in the nonirrigated western area of the Great Plains, it is only occasionally found and is seldom serious. In the interior valleys of California it is occasionally epidemic, though usually of slight importance. On the coast of California

it is always present and almost always virulent. Little grain is grown in this region. In the Southern States only a small quantity of wheat is grown, and here this rust is often severe. In the southern half of Texas it makes wheat growing a hazardous undertaking. Even in northern Texas it is a factor of great importance. The greatest rust epidemic of the last decade, which was due to the stem rust of wheat, occurred in 1904 and extended over the entire Mississippi Valley and up into the wheat fields of the Canadian Northwest, being particularly severe in the spring-wheat belt. It invaded the dry lands west of the Rocky Mountains and was severe in the interior valleys of California. A serious attack of stem rust of wheat was also experienced in the spring-wheat belt in 1902 and in 1905.

Leaf rust of wheat.—The occurrence of leaf rust (*Puccinia rubigo-vera tritici* Carleton) is also coextensive with wheat culture. It is more common in many districts than stem rust. In the whole eastern half of the United States it is present every year, usually to a considerable extent. Visitations amounting to epidemics are not infrequent, but the losses caused are not comparable to those of the stem-rust epidemic and are disregarded by the ordinary farmer, who accepts them as inevitable. In the Atlantic States the leaf rust is the chief rust of wheat and is very severe in some seasons. Like the stem rust, it follows more or less closely the rainfall lines, being of little importance in the arid sections of the country. In the Palouse district of Washington, Idaho, and Oregon, however, it is usually abundant.

Stem and leaf (or crown) rusts of oats.—The presence of stem and leaf rusts of oats (*Puccinia graminis avenae* Erikss. and Henn., and *Puccinia coronata* Corda) is coextensive with the culture of that grain. The stem rust of oats, if not more harmful, is fully as destructive as the stem rust of wheat, and its distribution is somewhat similar. It is almost invariably accompanied by the leaf rust (*Puccinia coronata*), which is probably the most destructive of the leaf-rust group.

Attention should be called to the fact that the stem rust of oats is not nearly so closely confined to the stem as is that of wheat, but is very frequently found on the leaf blades. The leaf and stem rusts of oats are usually commingled, and it is difficult to determine how much of the resulting damage is due to each. The leaf rust, however, is seldom found on the spikelets or the spikelet stems. It is here that much of the real damage is done by the stem rust. These rusts are found extensively only east of the dry belt of the Great Plains region, with the possible exception of eastern Oregon and Washington. In the Gulf Coast States, except northern Texas, and in Georgia and South Carolina they are paramount in importance and almost prohibitive of spring-oat growing, though winter oats are quite extensively grown. Proceeding northward, the rusts continue to be of great importance.

Even as far north as Wisconsin regions are known where oat growing has been discontinued on account of rust, and epidemics have been known to extend to the Canadian line and even beyond. Two features of an oat-rust epidemic explain instances of successful crops which often occur in the midst of an epidemic. They are (1) the great variation in time of ripening of different varieties of oats, amounting to as much as three weeks or a month in some latitudes, and (2) the apparent suddenness of the appearance of the epidemic. Frequently a variety one week later than another will be ruined by rust, while the earlier variety will escape entirely. This results in the presence every year of considerable rust, amounting to a severe attack in some localities and on some varieties, while other localities and varieties escape.

Stem rust of barley.—The occurrence of stem rust of barley (*Puccinia graminis hordei*)[1] is practically coextensive with the culture of that grain, but its presence is not often a serious menace. In general, the early date of maturing of barley seems to assist this crop in avoiding injury. Barleys planted very late—for instance, those planted for fodder—are sometimes seriously attacked, while the grain barleys usually escape damage. It may be noticed, however, that this rust, like the stem rust of oats, is not so nearly confined to the stem as the wheat stem rust, but is often abundantly present on the leaves. The rust assumes more serious proportions in the Southern States. In the Great Plains area and in the dry intermountain districts it is comparatively rare.

Leaf rust of barley.—Leaf rust of barley (*Puccinia simplex* (Körn.) Erikss. and Henn.) seems to be of recent introduction. It was reported from Iowa in 1896, from California in 1905, from Minnesota in 1905, 1906, 1907, and 1908, and occurred in Virginia in 1906 in a considerable degree. In 1910 it was abundant at Laurel, Md., and also occurred in Virginia. The most abundant outbreak was in Virginia, in 1906, where the plants were well covered with rust. In Minnesota it seems to appear late in the season and has had no injurious effect on the crop. It may be classed as one of the least conspicuous of the grain rusts in point of economic importance.

Stem rust of rye.—The stem rust of rye (*Puccinia graminis secalis* Erikss. and Henn.) is fairly common, but causes little injury. The explanation of this probably lies in the fact that winter rye is grown almost exclusively in the United States, and the stem rust appears at so late a date as to cause no appreciable damage. It was fairly common in Minnesota in 1906–1908 in experimental plats on spring rye, and in 1909 was abundant. As these were light rust years as

[1] As shown later, the physiological specialization of this rust in the United States is sufficiently different from that of the stem rust on wheat to make a distinction in the name desirable, and the trinomial terminology, as here applied, is used throughout this paper.

regards stem rust, no conclusions are possible from them as to the possibility of epidemic visitation on spring rye. Bolley, at Fargo, N. Dak., during the summer of 1907, had experimental plats of winter rye in the vicinity of barberry bushes which were infected with stem rust, and these winter-rye plats were badly rusted. The rust also appeared spontaneously on greenhouse material at Washington, D. C., in 1906. The exact limits of the stem rust are not determinable on account of the meager reports, but it is safe to say that the rust at present is of very little economic importance. On the other hand, it seems probable that it is widely distributed in small quantities.

Leaf rust of rye.—The occurrence of the leaf rust of rye (*Puccinia rubigo-vera secalis* Carleton) is coextensive with the culture of that grain, and is often very abundant. It is found everywhere, appearing usually in abundance on the young plants in the fall; in the spring it is ordinarily the first rust to appear on cereal crops. No damage is usually attributed to it, and probably little or none is actually suffered, for the rye matures so early and the rust is so closely confined to the leaves that appreciable injury is almost always avoided. As with stem rust of rye, nothing can be predicted concerning the possibilities of leaf rust on spring rye, because comparatively little spring rye has been grown in this country up to the present time.

BOTANICAL CHARACTERISTICS, LIFE HISTORIES, AND PHYSIOLOGICAL SPECIALIZATIONS OF RUSTS.

GENERAL STATEMENT.

Investigations of recent years have shown conclusively that botanical characteristics, life histories, and physiological specialization of parasitic fungi vary to such an extent with the geographical distribution that a sequence of forms for one locality is not necessarily the sequence for any other. This brings us face to face with the problem of rust life histories in the United States. Although the European and American forms may be apparently identical morphologically, they are not necessarily identical in their life histories or physiological specialization. Investigations on the rusts in this country have shown that while the work of European botanists may be suggestive it can not be accepted as conclusive or final for the rusts of the United States without confirmatory experimental evidence. Some information has been gained in recent years on the specialization of the rusts growing on the different cereals, but much still remains to be done.

This bulletin represents an attempt to show briefly our present knowledge of these rusts in the United States in comparison with our knowledge of rusts in Europe. For detailed descriptions of the

European forms the reader is referred to the works of Eriksson and Henning, Klebahn, Ward, and others, cited later in this paper.

RELATIONSHIPS BETWEEN AMERICAN AND EUROPEAN FORMS.

Stem rust of wheat, rye, oats, and barley.—It has been known for more than 40 years that the stem rust (*Puccinia graminis* Pers.) of wheat, rye, oats, and barley in Europe may pass on to the barberry, producing æcidia, the cluster-cup stage, on this plant. The same has been likewise conclusively proved for the forms in this country. That the stem rust always does pass through the barberry stage in each season's sequence of forms, or that it can not live for more than one season without passing on to the barberry, is not only not implied but, as will be shown later, is absolutely disproved by field experience and experiment. We know that rust can live for more than one season without the intervention of the barberry, but we also know, on the other hand, that the barberry stage is not uncommon in many rust-infected districts, so that it may still be an important factor. This feature will be further discussed.

Leaf rust of wheat.—The æcidial stage of leaf rust (*Puccinia rubigovera tritici* Carleton) of wheat is not known either in Europe or in this country. Arthur (5) has shown that a similar rust on *Elymus virginicus* L. has a very common æcidium on the jewel weed (*Impatiens fulva* Nutt.). It can not be stated at present, therefore, whether this rust has an æcidium in this country, or whether it has entirely lost this stage, as seems to be the case with *Puccinia graminis* in Australia. It is a fact easily observed in almost any wheat area of the United States, at least as far north as St. Paul, Minn., and Fargo, N. Dak., that the uredo stage exists through the winter months in the severest winters and the rust may thus live independent of an æcidial stage.

Leaf (or crown) rust of oats.—In Europe the crown rust (*Puccinia coronata* Corda.) of oats has its æcidial stage on species of Rhamnus. The exact identity of the European and American forms may perhaps be open to doubt, though without question they are very closely related. It has been shown that in Europe two species of crown rust exist (62), one (*Puccinia coronata* Corda.) with æcidia on *Rhamnus frangula* L. and the other (*Puccinia coronifera* Kleb.)[1] with its æcidia on *Rhamnus cathartica* L. Neither of these æcidial host species are indigenous to this country, although they have been introduced and grown quite extensively as ornamental shrubs in different localities. The æcidia of our own rust on oats is found on *R. lanceolata* Pursh.,

[1] Eriksson on the basis of careful inoculation experiments has since separated the crown rusts into a large number of physiological species, dividing *Puccinia coronifera* Kleb. with its æcidium on *Rhamnus cathartica* into 8 physiological species and the *Puccinia coronata* (Corda.) Kleb. with its æcidium on *Rhamnus frangula* into 3 physiological species (49).

R. caroliniana Walt., and *R. cathartica* L. The exact relationship of the American and European crown rusts can be determined only by parallel inoculation experiments with European and American forms. These have not yet been performed.

Leaf rust of rye.—In Europe the leaf rust of rye (*Puccinia dispersa* Erikss.) forms its æcidium on *Anchusa officinalis* L. and *Lycopsis arvensis* L. Arthur (11, pp. 236, 237) succeeded once in growing the spermogonia of the American form (*Puccinia rubigo-vera secalis* Carleton) on *L. arvensis* L. in this country. It is believed, therefore, that the American and European forms are identical, but further experimental evidence should be obtained.

Leaf rust of barley.—Leaf rust (*Puccinia simplex* (Körn.) Erikss. and Henn.) of barley was not reported in the last bulletin on rust issued by this Bureau and seems, in fact, not to have been previously reported. The American form agrees in all morphological characteristics with the European form. It is chiefly characterized by the predominance of the one-celled teleutospores. The teleuto stage is often somewhat scarce. The earliest collection of this rust available for examination was obtained in Iowa in 1896. It was collected in California in 1905. It has been noticed in abundance, especially toward fall, chiefly on volunteer or very late barley, in Minnesota during the seasons of 1905 to 1908, in Maryland in 1910, and was reported in the spring of 1906 from Virginia, where it occurred in great abundance, but, like the leaf rust of wheat, it caused little appreciable damage.

BIOLOGIC FORMS.

General Discussion.

Rust fungi exhibit great variety in regard to complexity of life histories. Some are confined to single-host species, others range over two or more species of one host genus, while still others range over two or more genera and often on different tribes of the same family. This comprehensive range may obtain in addition to the alternation of host plants, as in the stem rust of cereals. For instance, the stem rust of oats passes its æcidial stage on barberry, while the uredo and teleuto stages may be found on practically all species and varieties of oats and on several grasses, some of which are not at all nearly related to oats, but are, in fact, genera of tribes somewhat removed from that of the oat (30, pp. 61–63). Attention must be called to the fact that the ranging to other species occurs most abundantly in the uredo and teleuto stages, though it is not unknown in the æcidial stage. Further complexity arises in the following way: What may appear to the the eye, and often under the highest power of the microscope, as one and the same species of rust on a number of species, or even varieties,

may really not be identical, since they are not interchangeable from one host to the other. For instance, the leaf rusts of wheat and rye can not be distinguished from each other under the best microscope lenses of the present day; yet the leaf rust of rye can not ordinarily be transferred by inoculation from rye to wheat and probably is not so transferred in nature. In other words, one finds here two fungi exactly similar in morphological characteristics, but physiologically different. These have been variously designated,[1] probably the most convenient and expressive term being "biologic forms." It is seen at a glance that the biologic forms may complicate very greatly the rust life history. They offer great difficulties to the investigator of rusts and, at the same time, are the basis for a most promising field of work of much importance, viz, the study of rust-resistant varieties.

A further complication arises from the facts obtained through experiments in various countries, which have shown that what is apparently the same species (the host being morphologically the same) may consist of a large number of strains or varieties which may behave differently in different geographic areas. The stem rusts of wheat and barley, for instance, are very similar, interchanging hosts easily and being capable of transfer to various grasses in this country. (See pp. 17–21; also Carleton, 30, pp. 54–56.) The researches of Eriksson (41, p. 70; 40, p. 294; 42, p. 500; 46, p. 198) show that in Sweden the stem rust of wheat goes with difficulty to barley and rye, while the stem rusts of barley and rye interchange hosts very easily. The chief factors in the complexity of the life history of cereal rusts may be summarized thus: (1) Alternation of hosts for different spore forms; that is, between the barberries and grasses. (2) The restrictions of different biologic forms of a single species of rust to various definite groups of host plants; as, for instance, *Puccinia graminis avenae* on oats; also found on Dactylis, etc., but not on wheat. (3) The variation of the biologic forms in different geographic areas.

The biologic forms of cereal rusts have been somewhat fully worked out by Eriksson and Henning, Klebahn, and others for various localities in Europe. The reader is referred to these authors for more complete details (39 and 63).

The forms in this country have received some attention, though scarcely as much as those in Europe. Practically the only work done in this line has been that of Hitchcock and Carleton (58) and of Carleton (30 and 31). The results of the latter agree in the main with the results recorded in this paper, but differ considerably

[1] Some of the terms used are Species sorores, Schröter (94, p. 69); biologische Spezies, Klebahn (62, pp. 232, 258); biologiske arter, Rostrup (88, p. 40); physiological species, Hitchcock and Carleton (58, p. 4); formae speciales, Eriksson (40, p. 292); Gewohnheitsrassen, Magnus (69, p. 82); and biologiske rassen, Rostrup (89, p. 116).

in some details. The tendency in recent years has been to consider the biologic forms of our rusts as somewhat closely limited to their host species. Hitchcock and Carleton (58) and Carleton (30) find the stem rust to contain the following forms:

(a) *Puccinia graminis tritici* Erikss. and Henn. on wheat, barley, *Koeleria cristata* (L.) Pers., *Festuca gigantea* (L.) Vill., *Agropyron richardsoni* Schrad., *Elymus canadensis glaucifolius* Muhl., *Elymus canadensis* L., *Hordeum jubatum* L., *Hordeum murinum* L., *Dactylis glomerata* L., *Agrostis alba* L., and *Agropyron tenerum* Vasey.

(b) *P. graminis avenae* Erikss. and Henn. on oats, several species, *Arrhenatherum elatius* (L.) Beauv., *Hordeum murinum* L., *Ammophila arenaria* (L.) Link., *Trisetum subspicatum* Beauv., *Dactylis glomerata* L., *Koeleria cristata* (L.) Pers., *Alopecurus alpestris*, *Holcus mollis*, *Agrostis scabra* Willd., *Polypogon monspeliensis* (L.) Desf., *Festuca* sp. indet., *Phleum asperum* Vill., *Bromus ciliatus* L., and *Eatonia obtusata* (Michx.) Gray.

Puccinia graminis secalis Erikss. and Henn. was not mentioned. Eriksson (41, p. 70; 40, p. 294; 42, p. 500; 46, p. 198; 44 and 47) finds them as follows:

(a) *P. graminis tritici* Erikss. and Henn. on wheat, sparingly on barley and rye.

(b) *P. graminis avenae* Erikss. and Henn. on oats, *Avena sterilis* L., *Avena brevis* Roth., *Arrhenatherum elatius* Mert. and Koch., *Dactylis glomerata* L., *Alopecurus pratensis* L., *Milium effusum* L., *Lamarckia aurea* Mch., *Bromus arvensis* L., *Trisetum distichophyllum* Beauv., *Bromus brachystachys* Horn., *Bromus madritensis* L., *Koeleria setacea* DC., *Festuca myurus* Ehrh., *Festuca tenuiflora* Sibth., *Festuca scinroides* Roth., *Phalaris canariensis* L., *Phleum asperum* Vill., and *Briza maxima* L.

(c) *P. graminis secalis* Erikss. and Henn. on rye, barley, *Hordeum jubatum* L., *Hordeum comosum* J. and Presl., *Bromus secalinus* L., *Elymus sibiricus* L., *Elymus arenarius* L., *Agropyron repens* Beauv., *Agropyron caninum* R. and Sch., and *Agropyron desertorum* Fisch.

The differences in results obtained by these European and American investigators have led the writers to examine further into the possibility of breaking down the barriers between the so-called biologic forms. This object, as will be seen below, has been accomplished without much difficulty, and at the same time considerable light has been shed on the true nature of the parasitism of cereal rusts.

Experiments on Biologic Forms.

Description of methods.

Rusts were collected in Minnesota and were transferred to their own host plants by artificial inoculations in the greenhouses at Washington, D. C. These constituted the stock rusts. In all the experiments the uredo stage was the spore form used. The cereal host plants were raised in small pots, about 10 plants to a pot, and inoculated in the seedling stage, either on the first or on the second leaf. The spores were placed on the leaf dry, or they were slightly moistened to enable them to adhere to the leaf surface. The plants were then sprayed with water by means of an atomizer until the leaf

surfaces were covered with very fine drops and then placed under large bell jars for two days. They were then removed from the bell jars to the greenhouse bench.

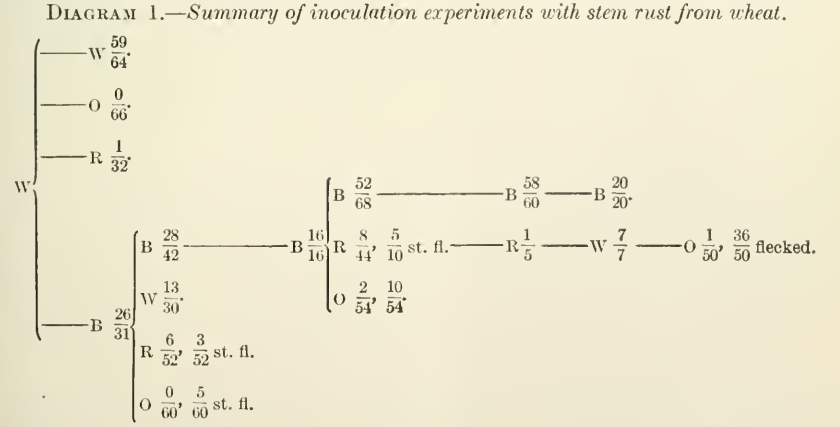
In the accompanying diagrams, W, B, O, and R represent wheat, barley, oats, and rye, respectively.[1] The succession of inoculations reads from left to right, the original host plant being on the extreme left. The figures in the form of a common fraction following each host plant are used as follows: (1) The numerator shows the number of leaves successfully infected; that is, leaves showing rust pustules. (2) The denominator shows the number of inoculated leaves. The fraction $\frac{7}{33}$, therefore, indicates 7 pustuled leaves out of a total of 33 inoculated. Again, the fraction $\frac{1}{3}$ followed by the word "flecked" indicates that 1 leaf out of 3 was flecked. The term flecked indicates a more or less close approach to the successful parasitism. The abbreviation "st. fl." means strongly flecked.

These diagrams show the results of various sets of inoculation experiments with the different grain rusts, on their own and other hosts, which have been carried on at different times.

EXPERIMENTS WITH BIOLOGIC FORMS OF STEM RUST.

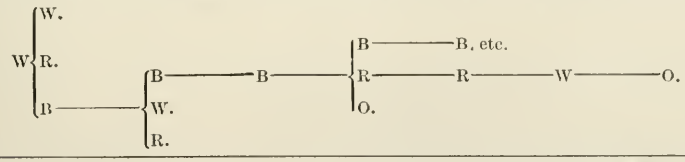
Diagrams 1, 2, 3, and 4 present summaries of inoculation experiments with *Puccinia graminis tritici* (stem rust) from wheat.

DIAGRAM 1.—*Summary of inoculation experiments with stem rust from wheat.*



The results indicated in diagram 1 are further summarized in diagram 2, which shows only the successful infections:

DIAGRAM 2.—*Summary of the successful inoculations shown in diagram 1.*



[1] Except in a few instances the grains used in these experiments were Preston wheat, Manchuria barley, Early Gothland oats, and spring rye, grown in Minnesota.

The wheat stem rust was carried directly to wheat, rye, and barley, but not to oats.[1] The figures show plainly that it goes with great ease to barley and wheat and very rarely (1 out of 32 inoculations) to rye. This rust can infect barley with about the same ease with which it goes to its own host. Although this may be interpreted as indicating the identity of barley and wheat stem rusts, it is not conclusive, since the barley rust behaves differently from the wheat rust toward the same cereals. When the wheat rust is taken to barley and then transferred to the other cereal hosts, it is seen that the barley has a decided influence on the rust. Diagram 3 summarized from diagram 1 shows its effect.

DIAGRAM 3.—*Summary of successful transfers of wheat rust through barley.*

$$\mathrm{W}\text{———}\mathrm{B}\,\frac{26}{31}\text{———}\begin{cases}\mathrm{B}\,\frac{28}{42}\text{———}\mathrm{B}\,\frac{16}{16}\text{———}\mathrm{O}\,\frac{2}{54}.\\ \mathrm{W}\,\frac{13}{30}.\\ \mathrm{R}\,\frac{6}{52}.\end{cases}$$

The wheat rust from barley infects the wheat and barley again with great ease, and the rye with greater ease than the direct infection from wheat. Finally, from the second barley host the wheat rust may even infect oats, a result rarely obtained directly from wheat. In brief, the wheat rust, after passing on to barley, is capable of infecting all of the four cereals, but when transferred from the wheat without passing to barley, only barley and wheat are usually infected, rye being rarely infected and oats very rarely, indeed. Among the cereals, therefore, the stem rust of wheat in this country is not confined to wheat as closely as Eriksson has found it to be in Sweden, nor is it confined to barley and wheat, as found by Carleton.

Diagram 4, summarized from diagram 1, shows the actual course of infection of wheat rust taken, in succession, on all of the small grains.

DIAGRAM 4.—*Summary of successful inoculations of diagram 1, showing succession.*

$$\mathrm{W}\text{——}\mathrm{B}\,\frac{26}{31}\text{——}\mathrm{B}\,\frac{28}{42}\text{——}\mathrm{B}\,\frac{16}{16}\text{——}\mathrm{R}\,\frac{8}{44}\text{——}\mathrm{R}\,\frac{1}{5}\text{——}\mathrm{W}\,\frac{7}{7}\text{——}\mathrm{O}\,\frac{1}{50}.$$

The effect on the wheat-rust parasite when barley is taken as a host is clearly shown to be that of enabling a wider range of infection. An interesting feature of this diagram is seen in the fact that the final successful inoculation of oats was directly from the wheat, but the rust had previously passed on to three barley plants and two rye plants.

Diagrams 5 to 10, inclusive, present summaries of inoculation experiments with *Puccinia graminis hordei* (stem rust) from barley.

[1] Mr. H. B. Derr, of the United States Department of Agriculture, reports having obtained in the laboratory the following successful inoculations: W——>O——>B——>O——>O. The number of successful infections in each case was not recorded.

DIAGRAM 5.—*Summary of inoculation experiments with stem rust from barley.*

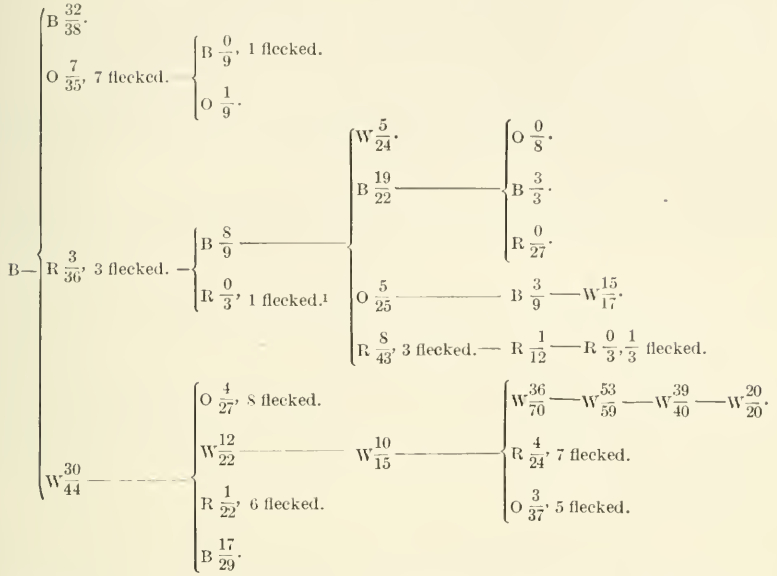
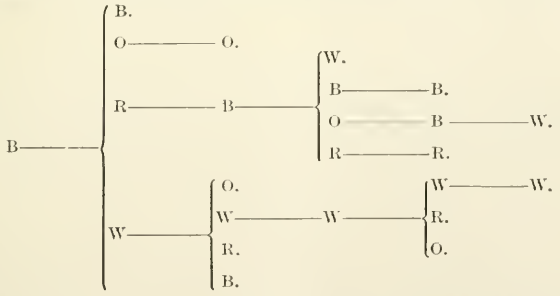


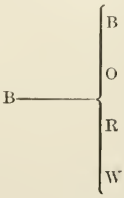
Diagram 6 summarizes the successful infections shown in diagram 5.

DIAGRAM 6.—*Summary of successful inoculations shown in diagram 5.*



The barley rust is seen at a glance to be more versatile than wheat rust. All four cereals are directly infected by this rust, as shown by diagram 7.

DIAGRAM 7.—*Summary of successful direct inoculations of stem rust of barley.*



[1] Eaten by slug.

The stem rust of barley, like that of wheat, goes with equal ease on barley and wheat. Rye is more easily infected by barley rust than by the wheat rust. Oats are capable of direct infection by barley rust. The oat pustules were very small and weak, and thus precluded the possibility of very numerous experiments with the barley rust from oats; but diagram 5 shows that successful infections were obtained as follows:

DIAGRAM 8.—*Summary of successful inoculations of oats with stem rust of barley.*

B——O $\frac{7}{35}$——O $\frac{1}{9}$.

The barley rust, after being transferred to rye, was carried to barley and then to all of the four cereals; it was likewise transferred to wheat and then to the other cereals. The rye and wheat rusts, as shown by other diagrams, are usually incapable of direct transfer in this manner. That the barley rust is carried through wheat and then transferred to the other cereals is shown in diagram 9 summarized from diagram 5:

DIAGRAM 9.—*Summary of transfer of stem rust of barley through wheat to other cereals.*

B——W——B $\frac{17}{29}$.
W $\frac{12}{22}$.
O $\frac{4}{27}$.
R $\frac{1}{22}$.

The barley rust, then, after passing through rye and wheat, is still able to infect all four cereals.

Diagram 10, summarized from diagram 5, shows that barley rust was successfully transferred to all of the four cereals.

DIAGRAM 10.—*Summary of transfers of stem rust of barley directly to other cereals.*

B——R $\frac{3}{36}$——B $\frac{8}{9}$——O $\frac{5}{25}$——B $\frac{3}{9}$——W $\frac{15}{17}$.

The comparatively large percentages of infection obtained are probably accounted for by the fact that in each case barley intervened as a host between rye and oats and between oats and wheat.

The barley stem rust enjoys the widest range of any of the biologic forms of the cereal rusts. On the other hand, a transfer of any of the other stem rusts to barley widens the range of that rust. We have here, then, a decided reaction of host upon parasite, enabling the latter to adapt itself to hosts not ordinarily congenial; for instance, W——>B——>O.

As shown under wheat rust, the barley rust and wheat rust are seen to be not necessarily identical, though the fact that they are

but slightly changed forms of the same species can not be doubted. Although they infect barley and wheat plants with almost equal ease, they behave differently in the other inoculations. That this difference may be attributed to the influence of host on the parasite is clear from the fact that wheat rust after passing to barley behaves in a similar manner to the barley rust, although the latter retains a more versatile character even after passing to the other host plants. Table II (p. 26) throws further light on the relationships of wheat and barley rusts. Diagrams 11 and 12 summarize the inoculation experiments with *Puccinia graminis secalis* (stem rust) from rye.

DIAGRAM 11.—*Summary of inoculation experiments with stem rust from rye.*

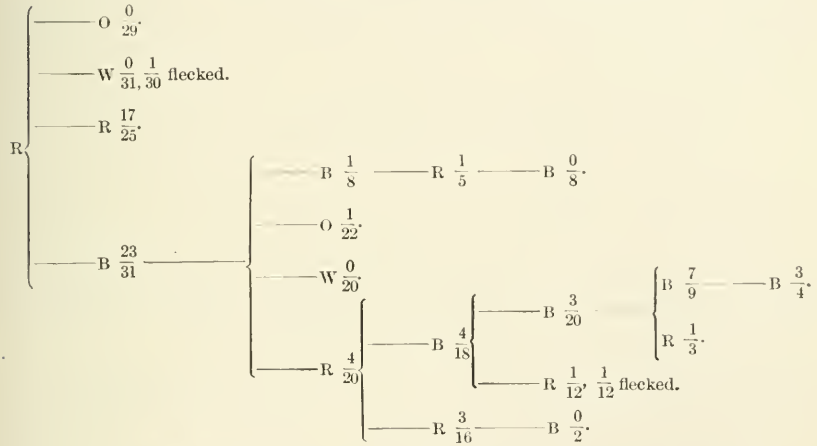
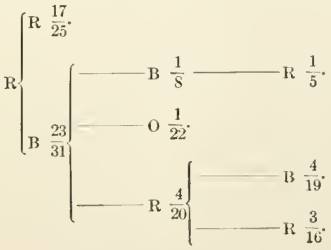


DIAGRAM 12.—*Summary of the important results shown in diagram 11.*



The rye rust infects rye and barley with about equal ease; in fact, the proportion of successful inoculations indicates even greater preference for barley than for rye, though a much larger number of inoculations would be necessary to decide this point conclusively. Wheat is rarely directly infected. Derr reports having obtained the successful infection of rye rust to wheat in a few instances. These infections were not obtained directly, but 1 out of 22 inoculations was successful after the rye rust had passed to barley. In our own

experiments no infections to wheat after barley and only one to oats after barley were obtained with the rye rust, but only a small number of attempts on wheat and oats were made from the rust on barley. There is little doubt that rye rust can be made to go to wheat after passing to barley, as has been shown by Derr's experiments previously cited. Diagram 13, summarized from diagram 11, shows the direct infections obtained with stem rust from rye.

DIAGRAM 13.—*Summary of the direct inoculations of barley and rye with stem rust from rye.*

Diagram 14 shows the possibility of infection of all four cereals by passing to barley.

DIAGRAM 14.—*Summary of inoculations by stem rust of rye directly to two cereals and through barley to wheat and oats.*

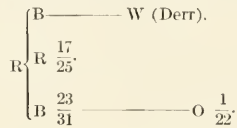


Diagram 15 presents a summary of inoculation experiments with *Puccinia graminis avenae* (stem rust) from oats:

DIAGRAM 15.—*Summary of inoculation experiments with stem rust from oats.*

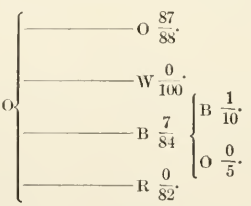
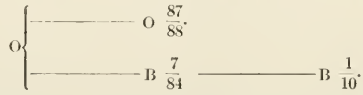
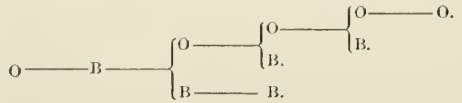


Diagram 16 presents a summary of the successful infections[1] shown in diagram 15.

DIAGRAM 16.—*Summary of successful inoculations shown in diagram 15.*



[1] Derr reports having obtained a direct infection of oats to wheat and one of oats to rye. In the case of the wheat the rust was further transferred as follows: O——>W——>B——>W. He also further carried the oats to barley and transferred infection as follows:



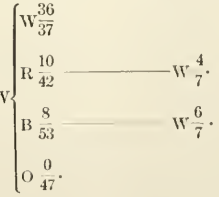
The number of successful inoculations of stem rust of oats to wheat and rye has been insufficient to make absolute statements concerning them, but there is little doubt that under highly favorable conditions they can be made. On the other hand, there is no doubt that the oat rust can be carried to barley and from barley to either oats or barley, as a large number of successful trials by Derr have shown. In all cases the pustules obtained in the course of the inoculations were small and weak and the rust was very evidently not on a congenial host. The oat rust is thus seen to be the most closely specialized of the biologic forms of *Puccinia graminis* on the small grains, but in its ability to infect the other species under rarely occurring conditions still shows its close affinity to the other rusts. Of all the stem rusts on the small grains that of oats is the most distinctive and individualistic in appearance, having larger pustules of uredo spores which are formed very commonly both on stems and leaves (as in barley), in sharp contrast with the more restricted location of the pustules in the rusts of wheat and rye. As a biologic form, the stem rust of oats may be said to be generally confined to oats. It can at times be carried to barley, but never produces large or vigorous pustules. It is only rarely that the transfers to wheat and rye can be made.

EXPERIMENTS WITH BIOLOGIC FORMS OF LEAF RUST.

Fewer experiments have been made with the biologic forms of leaf rusts than with the stem rusts, but these experiments indicate that, as a rule, the leaf rusts are not as versatile as the stem rusts, being confined more closely to the original hosts.

Diagram 17 presents a summary of inoculation experiments with *Puccinia rubigo-vera tritici* (leaf rust) from wheat.

DIAGRAM 17.—*Summary of inoculation experiments with leaf rust from wheat.*



The leaf rust of wheat was carried directly to wheat, rye, and barley, but in 47 inoculations it would not transfer to oats. It is similar to *Puccinia graminis tritici,* which can easily be transferred to the first two cereals, to rye rarely, and to oats only in very exceptional instances. But the leaf rust of wheat will not infect barley nearly as readily as the stem rust of wheat, but seems to transfer to rye more easily than the stem rust. No experiments were made

to determine whether or not this rust goes more easily to the other cereals after having been grown on barley, as is the case with the stem rust of wheat. Carleton (30, p. 20) reports negative results with *Puccinia rubigo-vera tritici* in inoculations on oats, barley, and rye. This indicates either that the strain of rust which he used for his inoculations may have been slightly different from the strains used in our inoculations, or that the conditions were not as favorable for infection. Such a difference in strains, perhaps, may exist in the same species of rust gathered from different localities even in the same country.

Diagram 18 presents a summary of inoculation experiments with *Puccinia simplex* (leaf rust) from barley.

DIAGRAM 18.—*Summary of inoculation experiments with leaf rust from barley.*

B { W $\frac{0}{49}$. R $\frac{0}{37}$. B $\frac{44}{46}$. O $\frac{0}{50}$.

These experiments indicate that the leaf rust of barley is closely confined to the one host, barley, as no infection took place on either wheat, rye, or oats in a large number of inoculations on each. In this particular it is very different from the stem rust of barley, which may be transferred to the other three cereals.

Diagram 19 presents a summary of inoculation experiments with *Puccinia rubigo-vera secalis* (leaf rust) from rye.

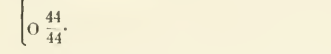
DIAGRAM 19.—*Summary of inoculation experiments with leaf rust from rye.*

R { W $\frac{0}{48}$ (most leaves flecked). R $\frac{33}{35}$. B $\frac{0}{50}$ (many leaves flecked O $\frac{0}{46}$.

The leaf rust of rye is also highly specialized and in numerous inoculations did not transfer to the other cereals. Carleton's results (30, p. 43) in numerous trials are identical. The flecking of the wheat and barley showed that they were infected with the rust, but that extensive development of the rust mycelium did not take place. The rye stem rust, on the other hand, easily transfers from rye to barley.

Diagram 20 presents a summary of inoculation experiments with *Puccinia coronata* (leaf rust) from oats.

DIAGRAM 20.—*Summary of inoculation experiments with leaf rust from oats.*



Although highly specialized, the leaf rust of oats can be transferred to barley, but it did not transfer to either wheat or rye. The effect of barley on it was not determined, except to show that from barley to oats the rust in a few trials transferred as easily as from oats to oats. In Carleton's experiments (30, p. 46) inoculation with this rust on barley gave negative results.

In many of the experiments on biologic forms previously cited, it was noticeable that the same rust species would not give the same percentage of infection on various hosts if the inoculations were made from rusts gathered in different localities. This may account for the diverse results of different investigators and leads to the belief that there may be a large number of strains of a rust species, none of which will act exactly like another toward the same hosts. Undoubtedly by variation and adaptation to varying conditions a certain rust species, widely distributed, may form a large number of strains or types which, when this process has been continued for a considerable time, differ widely in their physiological reactions. These may become the physiological or "biologic" species.

EFFECT OF CHANGE OF HOST ON THE MORPHOLOGY OF THE UREDOSPORE.

In experimental cultivation of *Puccinia graminis tritici* from wheat on barley and *Puccinia graminis hordei* from barley on wheat it was found that there existed a slight morphological difference between the uredospores of the stem rust of barley and the stem rust of wheat. Upon closer examination this difference seemed to be measurable—that is, the uredospores of barley measured (on a basis of measurement of 50 spores, widely selected) considerably shorter and very slightly narrower than those on wheat. An experiment was therefore inaugurated to determine what the effect would be on the size of the spores of the barley rust when grown on wheat and of the wheat rust when grown on barley. Transfers were accordingly made of the barley rust to two pots of wheat and of the wheat rust to two pots of barley. The barley rust on wheat was then transferred to wheat plants continuously for about a year, and the wheat rust in a similar manner was grown on barley. The rust in each pair of pots was transferred to two other pots, so that two separate strains were

kept continuously for check purposes. All precautions possible to ordinary greenhouse work were taken to avoid accidental infection and the mixing of cultures. The results given in the table below show the measurements of (1) the original material—that is, of barley rust on barley and wheat rust on wheat; (2) the same rusts after 6 inoculations had been made on new hosts; and (3) after 17 inoculations, covering a period of almost one year. The spore measurements were carefully made from typical spores. Where 50 spores were measured, they were taken from at least 5 different pustules and from 2 or 3 leaves. Where 10 were used they were selected from normal, mature pustules. The difference in size between spores from mature and immature pustules is quite marked, the immature being smaller than the mature. As the variation in width of the spores from different hosts is very slight, measurements of 10 may show a small difference from the measurement of 10 original spores toward either plus or minus.

TABLE II.—*Change in morphology of the uredospores by cultivation of stem rust from wheat on barley and from barley on wheat.*

Description of culture.	Date of collection of spores.	Number of spores measured.	Spore measurements, original material (μ).		Number of intervening inoculations.	Date of collection.	Number of spores measured.	Spore measurements (μ).			
								Cultivated material.		Difference (compared with original material).	
			Width.	Length.				Width.	Length.	Width.	Length.
	1906.					1907.					
Wheat rust transferred to and grown continuously on barley	Nov. 13 and Nov. 14	50	18.15	31.33	6	Feb. 28	10	18.17	30.13	+0.02	−1.20
					17	Sept. 26	50	17.52	29.01	− .63	−2.32
Barley rust transferred to and grown continuously on wheat	Nov. 14 and Nov. 22	50	17.46	28.51	6	Feb. 28	10	17.53	30.22	+ .07	+1.71
					17	Sept. 26	50	17.67	31.12	+ .21	+2.61

This table shows that in the original material the wheat-rust uredospores are 0.69 μ wider and 2.82 μ longer than the corresponding barley-rust spores. As stated above, the difference in width is too small (a little more than 0.5 μ) to allow of safe conclusions as to its variations. After 6 successive infections of each rust on the other host, the wheat rust had lost an average of 1.2 μ in length, the width remaining practically the same (+0.02 μ). The barley rust, on the other hand, had gained in length 1.71 μ, the width running practically the same (+0.07 μ). The two rusts at this time gave almost identical measurements, viz, wheat rust on barley 18.17 μ by 30.13 μ and barley rust on wheat 17.53 μ by 30.22 μ. After 17 successive intervening inoculations (almost a year from the time of the collection

of the original material) the wheat rust on barley had lost from the original material 0.63 μ in width (practically negligible) and 2.32 μ in length, while the barley rust on wheat had gained 0.21 μ in width (again practically negligible) and 2.61 μ in length. If these measurements are compared with those of the original material it will be seen that the wheat rust on barley has decreased in spore size to almost exactly that of the original barley rust and the barley rust on wheat has increased in spore size to nearly that of the original rust on wheat, as follows:

Original wheat rust	18.15 μ by 31.33 μ.
Barley rust after 10 months on wheat	17.67 μ by 31.12 μ.
Original barley rust	17.46 μ by 28.51 μ.
Wheat rust after 10 months on barley	17.52 μ by 29.01 μ.

Although these differences are not great, they seem sufficient to indicate that the host plant exercises not only a decided physiological and biological reaction upon the parasite but that it may, even in such a short period as one year, exert a measureable effect on the morphology.[1] It has already been shown (p. 17) that wheat rust if first transferred to barley may be transferred to oats with considerable ease, thus showing the physiologic change going hand in hand with the morphologic change.

GENERAL SUMMARY OF CONCLUSIONS DERIVED FROM EXPERIMENTS ON BIOLOGIC FORMS.

In summarizing, the following points in regard to biologic forms of rusts of cereals may be emphasized:

(1) *Puccinia graminis tritici* Erikss. and Henn. (stem rust of wheat), *P. graminis hordei* F. and J. (stem rust of barley), *P. graminis secalis* Erikss. and Henn. (stem rust of rye), and *P. graminis avenae* Erikss. and Henn. (stem rust of oats) are undoubtedly biologic forms of the same species *Puccinia graminis* Pers.

(2) These forms are not entirely confined to their respective hosts, but vary in range in part according to the host plants they have been recently inhabiting.

(3) *P. rubigo-vera tritici* Carleton (leaf rust of wheat) and *P. rubigo-vera secalis* Carleton (leaf rust of rye) are more highly specialized than the corresponding stem rusts.

(4) *P. graminis hordei* (stem rust of barley) has ordinarily the widest range, while *Puccinia simplex* Erikss. and Henn. (leaf rust of barley) and *P. rubigo-vera secalis* (leaf rust of rye) have more restricted ranges.

(5) Under very favorable conditions, particularly after first transferring to barley, all the stem rusts can be carried successfully to the other cereals.

(6) When the rusts are transferred to uncongenial hosts and produce pustules on these, the pustules are almost invariably minute and weak, producing comparatively few spores. Some pustules apparently never open. The congenial hosts of each rust may be summarized as follows:

P. graminis tritici (stem rust of wheat) on wheat and barley.

[1] Evans (50, p. 461) has shown previously that many of the biologic forms of the genus Puccinia can be distinguished by slight differences in morphology of the early uredo mycelium, particularly in the formation of the substomatal vesicle.

P. graminis hordei (stem rust of barley) on barley, wheat, and rye.
P. graminis secalis (stem rust of rye) on rye and barley.
P. graminis avenae (stem rust of oats) on oats.
P. rubigo-vera tritici (leaf rust of wheat) on wheat.
P. simplex (leaf rust of barley) on barley.
P. rubigo-vera secalis (leaf rust of rye) on rye.
P. coronata (leaf rust of oats) on oats.

(7) Two biologic forms may inhabit the same cereal or cereals (for instance, wheat and barley rusts on wheat and barley) without being identical.

(8) By gradual variation and adaptation to varying conditions a certain rust species, widely distributed, may form a number of strains or types, differing in physiological reactions.

(9) The host plants exercise a strong influence, not only on the physiological and biological relationships, but in some cases even on the morphology of the uredospores.

In regard to the relationships of the cereal rust forms to the numerous grass rusts of the United States there is much to be done. A beginning has been made, and experiments have been performed confirming Carleton's results (30, pp. 55, 61–63) in regard to the infection of *Hordeum jubatum* with the stem rusts of wheat and barley and orchard grass with the stem rust of oats. That *Agropyron repens* also acts as host for the stem rust of wheat has been proved. The relationship of *Puccinia phlei-pratensis* to other rusts has been investigated and a summary of results published (59, p. 791). The importance of this phase of the biologic forms of cereal rusts is very great and demands early attention. The most extensive results obtained up to the present time are those of Carleton with the American and Eriksson with the European rusts.[1]

THE ÆCIDIAL STAGE OF RUSTS.

HISTORY OF BARBERRIES IN RELATION TO RUST.

Up to 1864–65, when De Bary demonstrated the heteroecism of *Puccinia graminis* Pers., rust life histories were very incompletely

[1] During the course of preparation of this bulletin several important papers have appeared throwing further light on biologic forms of rust. J. C. Arthur ("Cultures of Uredineæ in 1909," Mycologia, vol. 2, no. 5, 1910, pp. 227, 228) cites experiments of his own showing that *Puccinia poculiformis* (Jacq.) Wettst. (*P. graminis* Pers.) has been grown on *Triticum vulgare* from æcidiospores derived from inoculations on *Berberis vulgaris* with teleutospores from *Agropyron repens, A. tenerum, A. pseudorepens, Agrostis alba, Cinna arundinacea, Elymus canandensis,* and *Sitanion longifolium,* respectively. He concludes that "although in the uredinial stage this rust shows racial strains that inhibit the ready transfer from one species of host to another * * * yet in the æcial stage racial strains play no part, and the barberry acts as a bridging host between each and every other gramineous host."

Jaczewski, on the other hand, in a recent article (Zeitschrift für Pflanzenkrankheiten, vol. 20, no. 6, 1910, pp. 356, 357) cites comprehensive inoculation experiments to show that the stem rusts of grains and grasses in Russia as a rule are not interchangeable even with the barberry as a bridging host, but retain distinct physiological specialization in the æcidial as well as in the uredo stage. He also shows that the æcidia produced from inoculations with the teleutospores from the stem rusts of wheat and barley, respectively, behave differently when used for inoculation on the same series of grains and grasses, and he believes it easily possible that the stem rust on barley is a distinct physiological species, a conclusion independently derived in another way by the writers of this bulletin (pp. 17–20 and 25–27). Although it is evident from the experiments cited by Arthur that the barberry may act as a bridging host for rusts between some gramineous hosts, in the light of the work of Jaczewski and others it seems that further experimentation on a large number of rusts is necessary before the sweeping statement that "in the æcial stage racial strains play no part" can be generally accepted.

understood. That the proximity of barberries to grainfields was injurious to the crops had long been believed, although no one could say just what caused the damage. In many cases stringent laws making the destruction of barberries compulsory had been enacted. Klebahn (63, p. 205) finds the first mention of such legislation by De Magneville (68, p. 18), who says that laws against the growing of barberries were extant in Rouen, France, in 1660. The citations of Loverdo (67, p. 199) and Prillieux (86, p. 221) undoubtedly are taken from this reference. Klebahn, however, was unable to locate the original law, even though M. Ch. de Beaurepaire, Archiviste Paleographe de la Prefecture de la Seine Inferieure in Rouen, looked through the record of laws for 1660 and also for 1760. There is, thus, some doubt about this report.

In the eighteenth century rust literature became much more extensive. According to Klebahn (63, pp. 205, 206), Erhart (38, p. 59) says that in 1720 an English farmer destroyed his neighbor's barberries with hot water because they hurt his wheat. This instance is also cited by Hornemann (56, p. 8). De Bary (12, p. 35) found the noxiousness (Schädlichkeit) of barberry to wheat mentioned by Krünitz (65, p. 198), who says:

> Man hat sie ohne Grund beschuldigt, dass sie in den nahe dabei stehenden Korn den Brand verursachten, weswegen dieselben sogar aus den Zaünen um die Landgüter verbannt werden.

Withering (105, p. 199) in 1776 wrote:

> This shrub should never be permitted to grow in corn lands, for the ears of wheat that grow near it never fill, and its influence in this respect has been known to extend as far as 300 or 400 yards across a field.

According to Davis (37, p. 82) the oldest legislation in the United States concerning barberries was enacted in Connecticut in 1726, when towns were empowered to pass regulations at their town meetings for the destruction of barberries within their respective townships, "it being by plentiful experience found that where they are in large quantities they do occasion, or at least increase, the blast on all sorts of English grain." In Massachusetts an act was passed in 1755 making the destruction of barberries in that Colony before June 13, 1760, compulsory (73, pp. 797, 798) because "it has been found by experience that the blasting of wheat and other English grain is often occasioned by barberry bushes to the great loss and damage of the inhabitants of this province." Similar laws, but much less stringent than those of Massachusetts, were passed in Rhode Island in 1766 and 1772, and again in Connecticut in 1779.

In 1781–1784 Marshall (71, pp. 19, 359; 72, p. 11), by reason of the strong existing prejudice against barberries in England, undertook actual experiments to determine whether or not barberries were the

cause of rust in grain. In February, 1782, he planted a barberry bush in the middle of a wheat field. He states that a little before harvest:

> The wheat was changing and the rest of the piece (about 20 acres) had acquired a considerable degree of whiteness (white wheat), while about the barberry bush there appeared a long but somewhat oval-shaped stripe of a dark, livid color, obvious to a person riding on the roadside at a considerable distance.

Marshall continues as follows:

> The part affected resembled the tail of a comet, the bush itself representing the nucleus, on one side of which the sensible effect reached about 20 yards, the tail pointing toward the southwest, so that probably the effect took place during a northeast wind.
>
> At harvest, the ears near the bush stood erect, handling soft and chaffy; the grains slender, shriveled, and light. As the distance from the bush increased the effect was less discernible, until it vanished imperceptibly.
>
> The rest of the piece was a tolerable crop and the straw clean, except on a part which was lodged, where the straw nearly resembled that about the barberry; but the grain on that part, though lodged, was much heavier than it was on this, where the crop stood erect.
>
> The grain of the crop, in general, was thin bodied; nevertheless, 10 grains, chosen impartially out of the ordinary corn of the piece, took 24 of the barberried grains, chosen equally impartially, to balance them.

This experiment was repeated by Marshall in Staffordshire with similar results, and he became more firmly than ever of the opinion that barberry was injurious to wheat.

In 1787 Withering (106, p. 366) in speaking of *Berberis vulgaris* repeated the statement which he made in 1776, already quoted (p. 29).

According to Windt (104), Schöpf (93, p. 56) in 1788 said that in America the barberries in proximity to fields were blamed for injuring grains and other field crops. Just how the injury was caused no one could say.

A severe epidemic of "mildew" took place in England in 1804 (84, p. 51). Arthur Young, secretary of the board of agriculture at that time, issued a circular asking for information as to the cause of "mildew" in wheat. In answer to the question "Have you made any observations on the barberry as locally affecting wheat?" numerous correspondents reported that injury resulted wherever barberries occurred near a wheat field. According to the same authority, Sir Joseph Banks (14, p. 521) in 1805 said: "Is it not more than possible that the parasitic fungus of the barberry and that of wheat are one and the same species, and that the seed is transferred from the barberry to the corn?"

In 1806 Windt (104, p. 8), from his own observations and experiments, came to the conclusion that the barberry was the cause of rust in wheat and that it acted as a center of infection.

Thomas A. Knight, president of the Royal Horticultural Society of London, in 1813 recognized the importance both of the uredo stage and of the barberry. He says (64, p. 85):

A single acre of mildewed wheat would probably afford seeds sufficient to communicate disease to every acre of wheat in the British Empire, under circumstances favorable to the growth of the fungus.

Knight adds:

There is also reason to believe that the barberry tree communicates this disease to wheat, and I have also often noticed a similar apparent parasitical fungus upon the straws of the couch-grass in the hedges of cornfields.

In 1818 a paper was published by the Royal Agricultural Society of Denmark, which was the result of investigations by Schoeler (92, p. 289) in Denmark from 1807 to 1816. He had planted grains around barberries and found that rye and oats were liable to be destroyed every year by rust; that when large and small barberry bushes were planted in his field—

The larger bushes did not give rise to rust when they lost their foliage in the process of transplanting, but, on the contrary, the smaller bushes, which did not lose their leaves so readily, did give rise to the rust in rye to a very marked degree.

In 1816 Schoeler actually cut freshly rusted barberry leaves, carried them in a box into a rye field, and rubbed them on rye plants moist with dew. The plants were carefully marked and in five days were found to be severely affected with rust, "while at the same time not one rusty plant could be found anywhere else in the field."

A German farmer performed similar experiments in 1818 (77, p. 280; 52, p. 408). He gathered the dust (Staube) which fell from the cup (Kapsel) on barberry leaves as he shook them and placed it on rye plants far from the rye fields and where there were no barberries in the neighborhood. After five or six days the plants thus treated were attacked by rust, while there was nothing similar on any other plants. He concluded that the dust from barberries blown by the wind to grains causes the rust.

While many botanists still believed that the rusts on barberry and wheat belonged to different genera, some were sufficiently good observers to believe that the Puccinia and Uredo were in some way connected. In 1852 Tulasne (97, pp. 79–113) proved the genetic relation between the summer rust (Uredo) and the autumn rust (Puccinia) and also showed (97, p. 141) that the autumn spores of many of the rust species, among which are *Puccinia graminis* and *P. coronata*, go through a resting period from autumn until spring before they will germinate.

It remained for De Bary, in 1864–65, to publish the results of his experiments, which actually proved heterœcism of *Puccinia graminis* (12, pp. 15–49). He demonstrated in 1864 that the sporidia from

teleutospores from *Agropyron repens* Beauv. and *Poa pratensis* L. would give rise to the æcidia on Berberis and, in 1865, that æcidiospores from Berberis sown on rye would produce uredospores and later teleutospores. He did not stop here, but kept at work on other rusts, and in 1865 showed that *Puccinia coronata* has its æcidium on *Rhamnus frangula* and *P. rubigo-vera* its æcidium on *Lycopsis arvensis*. From this time on, life-history work on the Uredineæ has made rapid strides and the connection of one æcidial form after the other has been discovered by such men as Oersted, Fuckel, Magnus, Schröter, Wolff, Rostrup, Winter, Nielsen, Reichardt, Hartig, Rathway, Cornu, Plowright, Farlow, Barclay, Thaxter, Eriksson, Klebahn, Arthur, Holway, Kellerman, and others.[1]

In 1884 Plowright produced successful infection on Berberis with rust from teleutospore material from *Agropyron repens* sent by Arthur from the United States, and Bolley produced successful infection on barberry with teleutospore material from wheat in 1889 (1, p. 395). Carleton (30, p. 54) produced successful infection on barley from æcidiospores from Berberis. These experiments have been repeated by Arthur time and again and have been performed by the authors. They prove that the *Puccinia graminis* in America is the same species as the *P. graminis* in Europe, although the physiological specializations and consequent biologic forms are different in the two countries.

More work has been done on *Puccinia graminis* than on any other rust fungus, and its relationship to the æcidium on several species of Berberis has been proved repeatedly by Eriksson. Species of Mahonia have also been proved to be æcidial hosts of this rust (85, p. 234; 13, p. 96).

The relationships of *Puccinia graminis* on oats and rye to the æcidium on species of Berberis and the relations of *P. coronata* to the æcidium on species of Rhamnus have also been demonstrated, while the æcidial forms of the other leaf rusts are not known. This has led again to the mooted question whether or not the æcidial stage is necessary in the life history of rusts, and, if not absolutely necessary, what function the æcidial stage fills.

FUNCTIONS OF THE ÆCIDIUM.

General Discussion.

As early as 1882 Plowright (85, p. 234) questioned whether the æcidium is an essential stage in the life history of rusts and grasses, and gave as his principal reason for raising this question the apparent

[1] For citations of literature see Plowright (84), 1889; Eriksson and Henning (39), 1894; Klebahn (63), 1904; McAlpine (76), 1906; Arthur (2, 4–11), 1899–1909.

"disproportion which exists in England between the amount of of mildew (rust) and the number of barberries." He further states that there is a wonderful difference between the extent of injury caused by "mildew" when derived directly from the barberry and when derived from a uredo that has reproduced itself through several generations, the former being much greater than the latter. He adds:

> This is only what one would expect when the fact is taken into consideration that the æcidium spore is a sexual product, whereas the uredospore is not.

Bolley (21, p. 12) holds a similar view and says:

> The services rendered by it [the barberry] should probably be considered as that of reinvigoration, much the same as that which is rendered by reproduction in ordinary plants.

Arthur (3, pp. 67–69) similarly believes the æcidium is a device to restore vigor to the rust fungus, the æcidiospore giving rise "to a much more vigorous state of the fungus than the uredospores do," and, as a consequence, the prevention of the production of the æcidiospore by the removal of the æcidial host would reduce very largely the injury which the rust is capable of producing.

This view has been greatly strengthened since Blackman's (18) discoveries of cell fusions and the origin of the binucleated condition in the æcidium of *Phragmidium violaceum* on *Rubus fruticosus* and *Gymnosporangium clavariaeforme* on Crataegus and in the further studies of Christman (33 and 34), Blackman and Fraser (19), and Olive (79 and 80), all of whom have shown that in various rust species a cell fusion takes place and the consequent binucleated condition arises at the base of the æcidium. The authors differ in certain instances as to the details of this fusion, and in the species studied, but generally agree that this fusion is sexual. If it is functionally a sexual union the final step of which is the nuclear fusion in the teleutospore,[1] the reinvigoration of the rust as claimed by Plowright, Bolley, and Arthur is to be expected as a natural consequence.

Experiments to Determine the Vitality of Successive Uredo Generations of Various Grain Rusts.

Material used and methods employed.

To test this invigoration theory in part and to determine, if possible, whether or not the æcidial stage is necessary in the life history of rusts, continuous cultural experiments from the uredospore of the various cereal rusts were undertaken by the authors in 1907 and

[1] Dangeard and Sapin-Trouffy demonstrated the nuclear fusion in the teleutospore of rusts as early as 1893 (Comptes Rendus 116, pp. 267–269 and 1304–1306) and regarded this a "pseudofecundation." These studies led to further investigations on the sexuality of the Uredineæ and consequent discovery of cell fusion in the æcidium.

were carried without a break until August, 1909. In these experiments 52 generations of uredospores were grown without the intervention of any other spore form. These generations consisted of *Puccinia graminis* on wheat, barley, and oats; *P. rubigo-vera* on wheat and rye; *P. simplex* on barley; *P. graminis*, originally from barley, on wheat; and *P. graminis*, originally from wheat, on barley. At the end of these experiments cultures were as easily made and the rusts grew as luxuriantly as at the first inoculation with material obtained directly from the field.

In these experiments care was taken to avoid accidental infection from outside sources. Plants showing indications of such infection were destroyed. As far as possible series of 10 plants were used and each inoculation was made with material from separate leaves of the stock plants. The source plants were always maintained until evidence of successful infection appeared. If infection did not take place by reason of unfavorable conditions at the time of inoculation, inoculations were again made from the source plants. For instance, if A was used to inoculate B, A was not destroyed until B showed fresh pustules. If B gave no evidence of the presence of rust, another B was inoculated from A. The following rusts were used: *Puccinia graminis* on wheat, *P. graminis* on oats, *P. graminis* on barley, *P. graminis* on rye, *P. rubigo-vera* on wheat, *P. simplex* on barley, *P. coronata* on oats, and *P. rubigo-vera* on rye.

The original source material was brought from the Minnesota Agricultural Experiment Station, October 5, 1906. Between that date and February 6, 1907, at least four transfers were made, probably as many as six or eight. During a part of the time the series were run at Minnesota and the remainder of the time at Washington, D. C. When transfers were to be made, heavily pustuled leaves were picked, inclosed in envelopes, and sent by mail. Inoculations were made on their arrival at their destination. Infection almost invariably took place readily. The transfers were necessary by reason of change of location of the men in charge of the experiments.

SUMMARIES OF THE EXPERIMENTS.

The following tables give the dates when all inoculations were made as well as the number of successful infections from each inoculation:

TABLE III.—*Summary of experiments to determine the vitality of successive uredo generations of various grain rusts.*

PUCCINIA GRAMINIS TRITICI ON WHEAT.

Capital letter series.					Lower-case letter series.				
Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
	1907.		1907.			1907.		1907.	
A	Feb. 6	10	Feb. 19	[1] 10	a	Feb. 6	10	Feb. 19	[1] 7
B	Feb. 19	10	Mar. 5	[1] 10	b	Feb. 19	10	Mar. 5	[1] 8
C	Mar. 5	10	Mar. 21	[1] 10	c	Mar. 5	10	Mar. 21	[1] 10
D[2]	Mar. 26	10	Apr. 8	10	d[2]	Mar. 26	10	Apr. 8	10
E	Apr. 17	[3] 10	May 7	5	e	Apr. 19	[4] 10	May 7	9
F	May 8	10	May 27	[5] +5	f	May 8	10	May 27	[5] +5
G	May 28	10	June 13	[5] +5	g	May 28	10	June 13	[5] +5
H	June 14	10	June 25	[5] +7	h	June 14	10	June 25	[5] +7
I	June 25	10	July 8	10	i	June 25	8	July 8	8
J	July 8	10	July 23	6	j	July 8	10	July 23	4
K	July 24	10	Aug. 6	10	k	July 24	10	Aug. 6	10
L	Aug. 7	10	Aug. 21	10	l	Aug. 7	10	Aug. 21	10
M	Aug. 22	10	Sept. 8	10	m	Aug. 22	10	Sept. 8	10
N	Sept. 9	10	Sept. 25	10	n	Sept. 9	10	Sept. 25	10
O[6]	Sept. 26	10	Oct. 8	10	o[6]	Sept. 26	10	Oct. 9	10
P	Oct. 16	7	Oct. 29	6	p	Oct. 22	9	Nov. 8	8
Q	Oct. 31	9	Nov. 18	9	q	Nov. 9	10	Dec. 2	9
R	Nov. 20	10	Dec. 10	10	r	Nov. 20	8	Dec. 10	8
			1908.					1908.	
S	Dec. 12	10	Jan. 7	10	s	Dec. 12	10	Jan. 7	10
	1908.					1908.			
T	Jan. 7	10	Jan. 25	10	t	Jan. 7	10	Jan. 25	10
U	Jan. 25	10	Feb. 15	4	u	Jan. 25	10	Feb. 15	9
V	Feb. 15	5	Mar. 3	5	v	Feb. 15	10	Mar. 3	7
W	Mar. 3	10	Mar. 19	10	w	Mar. 3	10	Mar. 19	10
X	Mar. 28	10	Apr. 13	5	x	Mar. 28	10	Apr. 13	6
Y	Apr. 13	10	Apr. 28	10	y	Apr. 13	10	Apr. 28	10
Z	Apr. 28	10	May 13	10	z	Apr. 28	10	May 13	10
AA	May 13	10	May 26	10	aa	May 13	10	May 26	10
BB	May 26	10	June 12	7	bb	May 26	7	June 12	7
CC	June 12	10	June 25	10	cc	June 12	10	June 25	10
DD	June 25	10	July 10	9	dd	June 25	10	July 10	10
EE	July 10	10	July 28	10	ee	July 10	10	July 28	7
FF	July 28	10	Aug. 12	[7] 5	ff	July 28	7	.....	.....
GG	Aug. 12	10	Sept. 1	6	.....	.....	.....	.....	.....
HH	Sept. 1	10	Sept. 17	([8])	.....	.....	.....	.....	.....
II	Sept. 17	10	Oct. 2	10	ii[9]	Sept. 17	10	Oct. 2	10
JJ	Oct. 2	10	Oct. 22	10	jj	Oct. 2	10	Oct. 22	10
KK	Oct. 22	10	Nov. 6	10	kk	Oct. 22	10	Nov. 6	10
LL	Nov. 6	10	Nov. 20	6	ll	Nov. 6	10	Nov. 20	10
MM	Nov. 20	10	Dec. 12	9	mm	Nov. 20	10	Dec. 12	10
			1909.					1909.	
NN	Dec. 12	10	Jan. 10	9	nn	Dec. 12	10	Jan. 10	10
	1909.					1909.			
OO	Jan. 10	10	Feb. 7	2	oo	Jan. 10	10	Feb. 7	5
PP	Feb. 7	8	Feb. 23	8	pp	Feb. 7	10	Feb. 23	10
QQ	Feb. 23	9	Mar. 14	9	qq	Feb. 23	10	Mar. 14	10
RR	Mar. 14	10	Mar. 30	10	rr	Mar. 14	10	Mar. 30	10
SS	Mar. 30	10	Apr. 12	7	ss	Mar. 30	10	Apr. 12	6
TT	Apr. 14	10	Apr. 27	10	tt	Apr. 14	10	Apr. 27	10
UU	Apr. 27	4	May 20	4	uu	Apr. 27	5	May 20	5
VV	May 20	10	June 14	10	vv	May 20	10	June 14	10
WW	June 14	10	June 26	10	ww	June 14	10	June 26	10
XX	June 26	10	July 7	9	xx	June 26	10	July 7	9
YY	July 7	10	July 21	10	yy	July 7	10	July 21	10
ZZ	July 21	10	Aug. 2	10	zz	July 21	10	Aug. 2	10
AAA	Aug. 2	10	([10])	([10])	aaa	Aug 2	10	([10])	([10])

[1] Pustules vigorous.
[2] Series sent from Washington, D. C., to Minneapolis, Minn.
[3] Inoculations made from material from dried leaves.
[4] Three leaves inoculated from dried material, two from fresh.
[5] Plus sign signifies "more than"; i. e., exact number of leaves pustuled not noted.
[6] Series sent from Minneapolis, Minn., to Washington, D. C.
[7] Greenhouse excessively hot.
[8] Several.
[9] Inoculated from HH.
[10] Experiment discontinued.

TABLE III.—*Summary of experiments to determine the vitality of successive uredo generations of various grain rusts*—Continued.

PUCCINIA GRAMINIS AVENAE ON OATS.

Capital letter series.					Lower-case letter series.				
Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
	1907.		1907.			1907.		1907.	
A	Feb. 6	10	Feb. 19	[1] 9	a	Feb. 6	10	Feb. 19	[1] 4
B	Feb. 19	9	Mar. 5	[2] 0	b	Feb. 19	10	Mar. 5	[1] 2
C	Mar. 5	10	Mar. 25	[3] 9	c	Mar. 5	10	Mar. 25	[4] 9
D[5]	Mar. 25	10	Apr. 4	9	d[5]	Mar. 25	8	Apr. 4	7
E	Apr. 17	10	May 7	[6] 10	e	Apr. 17	10	May 7	[6] 9
F	May 8	10	May 26	10	f	May 8	10	May 26	10
G	May 27	10	June 10	10	g	May 27	10	June 10	10
H	June 11	10	June 23	10	h	June 11	10	June 23	10
I	June 24	10	July 8	10	i	June 24	10	July 8	10
J	July 8	10	July 21	[7] 4	j	July 8	10	July 21	10
K	July 22	10	Aug. 6	[8] 10	k	July 22	10	Aug. 6	10
L	Aug. 7	10	Aug. 21	10	l	Aug. 7	10	Aug. 21	10
M	Aug. 22	10	Sept. 8	10	m	Aug. 22	10	Sept. 8	10
N	Sept. 9	10	Sept. 25	10	n	Sept. 9	10	Sept. 25	10
O[9]	Sept. 26	10	Oct. 8	([10])	o[9]	Sept. 26	10	Oct. 8	([10])
P	Oct. 17	10	Oct. 29	10	p	Oct. 17	10	Oct. 29	10
Q	Oct. 31	10	Nov. 18	10	q	Oct. 31	10	Nov. 18	10
R	Nov. 20	10	Dec. 10	9	r	Nov. 20	8	Dec. 10	8
			1908.					1908.	
S	Dec. 12	10	Jan. 7	10	s	Dec. 12	10	Jan. 7	10
	1908.					1908.			
T	Jan. 7	10	Jan. 25	10	t	Jan. 7	10	Jan. 25	10
U	Jan. 25	10	Feb. 15	10	u	Jan. 25	10	Feb. 15	10
V	Feb. 15	10	Mar. 3	10	v	Feb. 15	10	Mar. 3	10
W[11]	Mar. 3	10	Mar. 19	10	w[11]	Mar. 3	10	Mar. 19	10
X	Mar. 30	10	Apr. 12	10	x	Mar. 30	10	Apr. 12	10
Y	Apr. 12	10	Apr. 28	10	y	Apr. 12	10	Apr. 28	10
Z	Apr. 28	10	May 13	10	z	Apr. 28	10	May 13	10
AA	May 13	10	May 26	10	aa	May 13	10	May 26	10
BB	May 26	10	June 12	10	bb	May 26	10	June 12	10
CC	June 12	10	June 25	10	cc	June 12	10	June 25	10
DD	June 25	10	July 10	10	dd	June 25	10	July 10	10
EE	July 10	10	July 28	10	ee	July 10	10	July 28	10
FF	July 28	9	Aug. 12	[12] 0	ff	July 28	10	Aug. 12	[13] 0
GG	Aug. 13	[14] 4			gg	Aug. 12	4	Sept. 1	3
					gg	Sept. 1	8	Sept. 17	([15])
HH	Sept. 17	10	Oct. 2	[16] 10	hh	Sept. 17	10	Oct. 2	10
II	Oct. 2	10	Oct. 22	10	ii	Oct. 2	10	Oct. 22	10
JJ	Oct. 22	10	Nov. 6	10	jj	Oct. 22	10	Nov. 6	9
KK	Nov. 6	10	Nov. 20	9	kk	Nov. 6	10	Nov. 20	9
LL	Nov. 20	10	Dec. 12	9	ll	Nov. 20	10	Dec. 12	9
			1909.					1909.	
MM	Dec. 12	10	Jan. 10	9	mm	Dec. 12	10	Jan. 10	8
	1909.					1909.			
NN	Jan. 10	9	Feb. 7	5	nn	Jan. 10	10	Feb. 7	4
OO	Feb. 7	10	Feb. 23	10	oo	Feb. 7	10	Feb. 23	10
PP	Feb. 23	10	Mar. 14	10	pp	Feb. 23	10	Mar. 14	10
QQ	Mar. 14	10	Mar. 30	10	q	Mar. 14	10	Mar. 30	10
RR	Mar. 30	10	Apr. 12	8	rr	Mar. 30	10	Apr. 12	5
SS	Apr. 12	10	Apr. 27	10	ss	Apr. 12	10	Apr. 27	10
TT	Apr. 27	9	May 20	9	tt	Apr. 27	9	May 20	9
UU	May 20	10	June 14	10	uu	May 20	10	June 14	10
VV	June 14	10	June 26	10	vv	June 14	10	June 26	10
WW	June 26	10	July 7	10	ww	June 26	10	July 7	10
XX	July 7	10	July 21	10	xx	July 7	10	July 21	10
YY	July 21	10	([17])	([17])	yy	July 21	10	([17])	([17])

[1] Pustules vigorous.
[2] Accidental infection by *Puccinia coronata;* plants discarded.
[3] Slightly mixed with *Puccini coronata.*
[4] Mixed with *Puccinia coronata.*
[5] Series sent from Washington, D. C., to Minneapolis, Minn., April 8, 1907.
[6] Inoculations made from material from dried leaves.
[7] Badly mixed with *Puccinia coronata.*
[8] Inoculations made from j.
[9] Series sent from Minneapolis, Minn., to Washington, D. C., October 8, 1907.
[10] Several.
[11] Series sent from Washington, D. C., to Minneapolis, Minn., March 19, 1908.
[12] Failure due to extreme heat in greenhouse.
[13] Five leaves pustuled with *Puccinia coronata,* accidental infection. Failure of *Puccinia graminis* due to extreme heat in greenhouse.
[14] Inoculated from EE.
[15] Not recorded.
[16] Inoculated from gg.
[17] Experiment discontinued.

TABLE III.—*Summary of experiments to determine the vitality of successive uredo generations of various grain rusts*—Continued.

PUCCINIA GRAMINIS HORDEI ON BARLEY.

Capital letter series.					Lower-case letter series.				
Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
	1907.		1907.			1907.		1907.	
A	Feb. 6	10	Feb. 19	[1] 7	a	Feb. 6	10	Feb. 19	8
B	Feb. 19	10	Mar. 5	10	b	Feb. 19	10	Mar. 5	8
C	Mar. 5	10	Mar. 25	10	c	Mar. 5	10	Mar. 25	10
D[2]	Mar. 25	7	Apr. 4	6	d[2]	Mar. 25	10	Apr. 4	10
E	Apr. 17	[3] 10	May 7	7	e	Apr. 17	7	May 7	8
F	May 7	10	May 25	10	f	May 7	10	May 25	10
G	May 26	8	June 13	[4]+5	g	May 26	8	June 13	[4]+5
H	June 14	10	June 25	[4]+5	h	June 14	10	June 25	[4]+5
I	June 26	10	July 9	[5] 2	i	June 26	9	July 9	[6] 8
J	July 10	5	July 21	3	j	July 10	9	July 21	7
K	July 22	10	Aug. 6	10	k	July 22	10	Aug. 6	10
L	Aug. 7	10	Aug. 21	10	l	Aug. 7	10	Aug. 21	10
M	Aug. 22	10	Sept. 8	10	m	Aug. 22	10	Sept. 8	10
N	Sept. 9	10	Sept. 25	10	n	Sept. 9	10	Sept. 25	10
O[7]	Sept. 26	10	Oct. 8	([8])	o[7]	Sept. 26	10	Oct. 8	([8])
P	Oct. 22	11	Nov. 8	11	p	Oct. 16	9	Oct. 29	9
Q	Nov. 9	10	Nov. 20	10	q	Oct. 31	7	Nov. 18	7
R	Nov. 20	9	Dec. 10	5	r	Nov. 20	9	Dec. 10	8
			1908.					1908.	
S	Dec. 12	10	Jan. 7	10	s	Dec. 12	10	Jan. 7	10
	1908.					1908.			
T	Jan. 7	10	Jan. 25	[9] 10	t	Jan. 7	10	Jan. 25	[10] 10
U	Jan. 25	10	Feb. 15	[10] 8	u	Jan. 25	10	Feb. 15	9
V	Feb. 15	10	Mar. 3	10	v	Feb. 15	10	Mar. 3	10
W[11]	Mar. 3	10	Mar. 19	10	w[11]	Mar. 3	10	Mar. 19	10
X	Mar. 27	10	Apr. 13	7	x	Mar. 27	10	Apr. 13	6
Y	Apr. 13	10	Apr. 28	4	y	Apr. 13	10	Apr. 28	3
Z	Apr. 28	10	May 13	10	z	Apr. 28	10	May 13	5
AA	May 13	10	May 26	8	aa	May 13	6	May 26	5
BB	May 26	10	June 12	8	bb	May 26	10	June 12	5
CC	June 18	10	July 10	[12] 0	cc	June 18	10	July 10	1
					dd	July 10	7	July 28	3
					ee	July 28	7	Aug. 12	1
					ff	Aug. 12	10	Sept. 1	8
					gg	Sept. 1	10	Sept. 17	([8])
HH	Sept. 17	10	Oct. 2	[13] 10	hh	Sept. 17	10	Oct. 2	8
II	Oct. 2	10	Oct. 22	10	ii	Oct. 2	10	Oct. 22	10
JJ	Oct. 22	10	Nov. 6	10	jj	Oct. 22	10	Nov. 6	10
KK	Nov. 6	10	Nov. 20	10	kk	Nov. 6	10	Nov. 20	10
LL	Nov. 20	10	Dec. 12	8	ll	Nov. 20	10	Dec. 12	8
			1909.					1909.	
MM	Dec. 12	10	Jan. 10	9	mm	Dec. 12	8	Jan. 10	5
	1909.					1909.			
NN	Jan. 10	5	Feb. 7	1	nn	Jan. 10	10	Feb. 7	3
OO	Feb. 22	5	Mar. 14	3	oo	Feb. 22	6	Mar. 14	2
PP	Mar. 14	10	Mar. 30	10	pp	Mar. 14	10	Mar. 30	10
QQ	Mar. 30	10	Apr. 12	9	qq	Mar. 30	10	Apr. 14	6
RR	Apr. 12	10	Apr. 27	10	rr	Apr. 12	10	Apr. 27	10
SS	Apr. 27	10	May 20	7	ss	Apr. 27	4	May 20	4
TT	May 20	8	June 14	7	tt	May 20	10	June 14	9
UU	June 14	10	June 26	8	uu	June 14	10	June 26	8
VV	June 26	10	July 7	10	vv	June 26	10	July 7	10
WW	July 7	10	July 21	7	ww	July 7	10	July 21	10
XX	July 21	10	Aug. 2	10	xx	July 21	10	Aug. 2	10
YY	Aug. 2	10	([14])	([14])	yy	Aug. 2	10	([14])	([14])

[1] Pustules vigorous.
[2] Series sent from Washington, D. C., to Minneapolis, Minn., April 8, 1907.
[3] Three inoculations were made from material from dried leaves, 7 from fresh material shipped in pots.
[4] Plus sign signifies "more than;" i. e., exact number of leaves pustuled not noted.
[5] Slugs destroyed 8 plants.
[6] Slugs destroyed 1 plant.
[7] Series sent from Minneapolis, Minn., to Washington, D. C., October 8, 1907.
[8] Several.
[9] Pustules not as vigorous as usual.
[10] Pustules not vigorous.
[11] Series transferred from Washington, D. C., to Minneapolis, Minn., March 19, 1908.
[12] Failure due to extreme heat in greenhouse.
[13] Inoculated from gg.
[14] Experiment discontinued.

TABLE III.—*Summary of experiments to determine the vitality of successive uredo generations of various grain rusts*—Continued.

PUCCINIA GRAMINIS SECALIS ON RYE.

Capital letter series.					Lower-case letter series.				
Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
	1907.		1907.			1907.		1907.	
A	Feb. 6	10	Feb. 19	[1] 7	a	Feb. 6	10	Feb. 19	4
B	Feb. 19	10	Mar. 5	4	b	Feb. 19	10	Mar. 5	4
C	Mar. 5	10	Mar. 25	8	c	Mar. 5	10	Mar. 25	5
D [2]	Apr. 4	4	Apr. 8	8	d [2]	Mar. 25	[3] 5	Apr. 8	3
E	Apr. 17	10	May 7	4	e	Apr. 17	[3] 9	May 7	2
F	May 8	6	May 26	([4])	f	May 8	10	May 26	([4])
G	May 27	5	June 13	([4])	g	May 27	6	June 13	([4])
H	June 14	4	June 26	[5] 0	h	June 14	9	June 26	[5] 0
	1909.		1909.			1909.		1909.	
AA [6]	July 7	10	July 21	10	aa [6]	July 7	10	July 21	10
BB	July 21	10	Aug. 2	8	bb	July 21	10	Aug. 2	8
CC	Aug. 2	10	.....	([7])	cc	Aug. 2	10	.....	([7])

PUCCINIA RUBIGO-VERA TRITICI ON WHEAT.

	1907.		1907.			1907.		1907.	
A	Feb. 6	10	Feb. 19	[1] 7	a	Feb. 6	10	Feb. 19	[1] 10
B	Feb. 19	10	Mar. 5	8	b	Feb. 19	10	Mar. 5	[1] 8
C	Mar. 5	10	Mar. 21	([4])	c	Mar. 5	10	Mar. 21	([4])
D [2]	Mar. 26	10	Apr. 8	0	d [2]	Mar. 26	10	Apr. 8	7
E [8]	Apr. 17	.....	.....	.....	e	Apr. 17	[3] 7	May 7	7
F	.....	.....	.....	.....	f	May 8	10	May 28	[9] +5
G	.....	.....	.....	.....	g	May 29	9	June 13	[9] +5
H	.....	.....	.....	.....	h	June 14	10	June 26	[9] +5
I	.....	.....	.....	.....	i	June 27	10	July 9	3
J	.....	.....	.....	.....	j	July 10	10	July 23	10
K	July 24	10	Aug. 8	[10] 7	k	July 24	10	Aug. 8	10
L	Aug. 9	8	Aug. 21	0	l	Aug. 9	10	Aug. 21	0
L	Aug. 22	[11] 6	Sept. 10	5	l	Aug. 22	[11] 6	Sept. 10	5
M	Sept. 11	5	Sept. 27	5	m	Sept. 11	9	Sept. 27	9
N [12]	Sept. 28	9	Oct. 8	([4])	n [12]	Sept. 28	10	Oct. 8	([4])
O	Oct. 24	8	Nov. 8	3	o	Oct. 16	8	Oct. 29	8
P	Nov. 9	7	Nov. 20	7	p	Oct. 31	10	Nov. 18	10
Q	Nov. 20	9	Dec. 10	9	q	Nov. 20	10	Dec. 10	10
			1908.					1908.	
R	Dec. 12	10	Jan. 7	10	r	Dec. 12	10	Jan. 7	10
	1908.					1908.			
S	Jan. 7	10	Jan. 25	10	s	Jan. 7	10	Jan. 25	10
T	Jan. 25	10	Feb. 15	10	t	Jan. 25	10	Feb. 15	10
U	Feb. 15	10	Mar. 3	10	u	Feb. 15	10	Mar. 3	10
V [13]	Mar. 3	10	Mar. 19	10	v [13]	Mar. 3	10	Mar. 19	10
W	Mar. 30	10	Apr. 13	10	w	Mar. 30	10	Apr. 13	10
X	Apr. 13	10	Apr. 28	7	x	Apr. 13	10	Apr. 28	10
Y	Apr. 28	10	May 13	10	y	Apr. 28	10	May 13	10
Z	May 13	10	May 26	10	z	May 13	10	May 26	10
AA	May 26	10	June 12	10	aa	May 26	10	June 12	10
BB	June 12	10	June 25	10	bb	June 12	10	June 25	10
CC	June 25	10	July 10	10	cc	June 25	7	July 10	7
DD	July 10	10	July 28	8	dd	July 10	10	July 28	10
EE	July 28	6	Aug. 12	2	ee	July 28	10	Aug. 12	5
FF	Aug. 12	5	Sept. 1	1	ff	Aug. 12	10	Sept. 1	7
GG [14]	Sept. 1	3	Sept. 17	([4])	gg [14]	Sept. 1	9	Sept. 17	([4])

[1] Pustules vigorous.
[2] Series sent from Washington, D. C., to Minneapolis, Minn., April 8, 1907.
[3] Inoculations made from material from dried leaves.
[4] Several.
[5] Very hot when inoculations were made, hence no infection.
[6] Not successive to preceding inoculations; material obtained directly from the field.
[7] Failure due to extreme heat in greenhouse.
[8] Material from D destroyed in transit.
[9] Plus sign signifies "more than;" i. e., exact number of leaves pustuled not noted.
[10] Inoculations made from j.
[11] Inoculations made from K and k.
[12] Series sent from Minneapolis, Minn., to Washington, D. C., October 8, 1907.
[13] Series sent from Washington, D. C., to Minneapolis, Minn., March 19, 1908.
[14] Letters III and hh were omitted in the series.

TABLE III.—*Summary of experiments to determine the vitality of successive uredo generations of various grain rusts*—Continued.

PUCCINIA RUBIGO-VERA TRITICI ON WHEAT—Continued.

Capital letter series.					Lower-case letter series.				
Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
	1908.		1908.			1908.		1908.	
II	Sept. 17	8	Oct. 2	8	ii	Sept. 17	10	Oct. 2	10
JJ	Oct. 2	10	Oct. 22	10	jj	Oct. 2	10	Oct. 22	10
KK	Oct. 22	10	Nov. 6	9	kk	Oct. 22	10	Nov. 6	8
LL	Nov. 6	10	Nov. 20	10	ll	Nov. 6	10	Nov. 20	10
MM	Nov. 20	9	Dec. 12	9	mm	Nov. 20	10	Dec. 12	10
			1909.					1909.	
NN	Dec. 12	10	Jan. 10	10	nn	Dec. 12	10	Jan. 10	7
	1909.					1909.			
OO	Jan. 10	10	Feb. 7	5	oo	Jan. 10	10	Feb. 7	2
PP	Feb. 7	7	Feb. 23	7	pp	Feb. 7	5	Feb. 23	5
QQ	Feb. 23	10	Mar. 14	10	qq	Feb. 23	10	Mar. 14	10
RR	Mar. 14	10	Mar. 30	10	rr	Mar. 14	10	Mar. 30	10
SS	Mar. 30	10	Apr. 12	7	ss	Mar. 30	10	Apr. 12	1
TT	Apr. 12	10	Apr. 27	2	tt	Apr. 12	10	Apr. 27	2
UU	Apr. 27	3	May 20	3	uu	Apr. 27	5	May 20	5
VV	May 20	10	June 14	10	vv	May 20	10	June 14	9
WW	June 14	8	June 26	7	ww	June 14	10	June 26	9
XX	June 26	10	July 7	7	xx	June 26	10	July 7	10
YY	July 7	10	July 21	8	yy	July 7	10	July 21	10
ZZ	July 31	10	Aug. 2	10	zz	July 21	10	Aug. 2	10
AAA	Aug. 2	10	[1]	[1]	aaa	Aug. 2	10	[1]	[1]

PUCCINIA SIMPLEX ON BARLEY.

	1907.		1907.			1907.		1907.	
A	Feb. 6	10	Feb. 19	10	a	Feb. 6	10	Feb. 19	10
B	Feb. 19	10	Mar. 5	10	b	Feb. 19	10	Mar. 5	10
C	Mar. 5	10	Mar. 26	[2]	c	Mar. 5	10	Mar. 26	[2]
D[3]	Mar. 26	10	Apr. 4	10	d[3]	Mar. 26	10	Apr. 4	8
E	Apr. 17	[4] [7]	May 7	7	e	Apr. 17	[4] 9	May 7	7
F	May 8	10	May 28	10	f	May 8	10	May 28	10
G	May 29	10	June 13	10	g	May 29	10	June 13	10
H	June 14	10	June 25	10	h	June 14	10	June 25	[2]
I	June 26	10	July 8	10	i	June 26	10	July 8	10
J	July 10	10	July 23	10	j	July 10	10	July 23	10
K	July 24	10	Aug. 7	10	k	July 24	10	Aug. 7	10
L	Aug. 9	10	Aug. 23	10	l	Aug. 9	10	Aug. 23	10
M	Aug. 24	10	Sept. 8	10	m	Aug. 24	10	Sept. 8	10
N	Sept. 9	10	Sept. 27	10	n	Sept. 9	10	Sept. 27	10
O[5]	Sept. 28	10	Oct. 8	[2]	o[5]	Sept. 28	10	Oct. 8	[2]
P	Oct. 24	10	Nov. 8	7	p	Oct. 16	10	Oct. 29	10
Q	Nov. 9	9	Nov. 18	9	q	Oct. 31	10	Nov. 18	10
R	Nov. 20	10	Dec. 10	10	r	Nov. 20	10	Dec. 10	10
			1908.					1908.	
S	Dec. 12	10	Jan. 7	10	s	Dec. 12	10	Jan. 7	10
	1908.					1908.			
T	Jan. 7	10	Jan. 25	10	t	Jan. 7	10	Jan. 25	10
U	Jan. 25	10	Feb. 15	10	u	Jan. 25	10	Feb. 15	10
V	Feb. 15	10	Mar. 3	10	v	Feb. 15	10	Mar. 3	10
W[3]	Mar. 3	10	Mar. 19	10	w[3]	Mar. 3	10	Mar. 19	10
X	Mar. 30	10	Apr. 13	10	x	Apr. 2	10	Apr. 13	10
Y	Apr. 13	10	Apr. 28	10	y	Apr. 13	8	Apr. 28	8
Z	Apr. 28	10	May 13	10	z	Apr. 28	10	May 13	10
AA	May 13	10	May 26	10	aa	May 13	10	May 26	10
BB	May 26	10	June 12	10	bb	May 26	10	June 12	10
CC	June 12	10	June 25	10	cc	June 12	10	June 25	10
DD	June 25	10	July 10	10	dd	June 25	10	July 10	10
EE	July 10	10	July 28	10	ee	July 10	10	July 28	10
FF	July 28	9	Aug. 12	[6] 2	ff	July 28	10	Aug. 12	[6] 3
GG	Aug. 12	4	Sept. 1	4	gg	Aug. 12	1	Sept. 1	1
HH	Sept. 1	10	Sept. 17	[7]	hh	Sept. 1	8	Sept. 17	.....

[1] Experiments discontinued.
[2] Several.
[3] Series sent from Washington, D. C., to Minneapolis, Minn.
[4] Inoculations made from material from dried leaves.
[5] Series sent from Minneapolis, Minn., to Washington, D. C., October 8, 1907.
[6] Extreme heat in greenhouse.
[7] Not recorded.

TABLE III.—*Summary of experiments to determine the vitality of successive uredo generations of various grain rusts*—Continued.

PUCCINIA SIMPLEX ON BARLEY—Continued.

Capital letter series.					Lower-case letter series.				
Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
	1908.		1908.			1908.		1908.	
II	Sept. 17	6	Oct. 2	4	ii	Sept. 17	7	Oct. 2	4
JJ	Oct. 2	10	Oct. 22	10	jj	Oct. 2	10	Oct. 22	10
KK	Oct. 22	10	Nov. 6	10	kk	Oct. 22	10	Nov. 6	10
LL	Nov. 6	10	Nov. 20	9	ll	Nov. 6	10	Nov. 20	10
MM	Nov. 20	9	Dec. 12	8	mm	Nov. 20	10	Dec. 12	9
			1909.					1909.	
NN	Dec. 12	7	Jan. 10	7	nn	Dec. 12	10	Jan. 10	9
	1909.					1909.			
OO	Jan. 10	8	Feb. 7	4	oo	Jan. 10	10	Feb. 7	7
PP	Feb. 7	10	Feb. 23	10	pp	Feb. 7	10	Feb. 23	10
QQ	Feb. 23	10	Mar. 14	10	qq	Feb. 23	10	Mar. 14	10
RR	Mar. 14	10	Mar. 30	10	rr	Mar. 14	10	Mar. 30	10
SS	Mar. 30	10	Apr. 12	8	ss	Mar. 30	10	Apr. 12	6
TT	Apr. 12	10	Apr. 27	10	tt	Apr. 12	10	Apr. 27	10
UU	Apr. 27	4	May 20	4	uu	Apr. 27	7	May 20	7
VV	May 20	10	June 14	10	vv	May 20	10	June 14	10
WW	June 14	10	June 26	10	ww	June 14	10	June 26	10
XX	June 26	10	July 7	0	xx	June 26	10	July 7	4
XX	July 7	10	July 21	10	yy	July 7	10	July 21	9
YY	July 21	10	Aug. 2	10	zz	July 21	10	Aug. 2	10
ZZ	Aug. 2	10	[1]	[1]	aaa	Aug. 2	8	[1]	[1]

PUCCINIA CORONATA ON OATS.

	1907.		1907.			1907.		1907.	
A	Feb. 6	10	Feb. 19	[2] 9	a	Feb. 6	10	Feb. 19	10
B	Feb. 19	9	Mar. 5	9	b	Feb. 19	10	Mar. 5	10
C	Mar. 5	9	Mar. 21	9	c	Mar. 5	10	Mar. 21	10
D [3]	Mar. 21	10	Mar. 30	10	d [3]	Mar. 21	10	Mar. 30	10
E	Apr. 17	[4] 10	May 7	10	e	Apr. 17	[4] 10	May 7	10
F	May 8	10	May 26	10	f	May 8	10	May 26	10
G	May 27	8	June 10	8	g	May 27	9	June 10	9
H	June 11	10	June 23	10	h	June 11	10	June 23	10
I	June 24	10	July 8	10	i	June 24	10	July 8	10
J	July 8	10	July 23	10	j	July 8	10	July 23	10
K	July 24	10	Aug. 8	10	k	July 24	10	Aug. 8	10
L	Aug. 9	10	Aug. 23	10	l	Aug. 9	10	Aug. 23	10
M	Aug. 24	10	Sept. 10	10	m	Aug. 24	10	Sept. 10	10
N	Sept. 11	10	Sept. 27	10	n	Sept. 11	10	Sept. 27	10
O [5]	Sept. 28	10	Oct. 8	[6]	o [5]	Sept. 28	10	Oct. 8	[6]
P	Oct. 24	9	Nov. 8	8	p	Oct. 17	10	Oct. 29	10
Q	Nov. 9	10	Nov. 18	10	q	Oct. 31	10	Nov. 18	10
R	Nov. 20	9	Dec. 10	9	r	Nov. 20	8	Dec. 10	8
			1908.					1908.	
S	Dec. 12	10	Jan. 7	10	s	Dec. 12	10	Jan. 7	10
	1908.					1908.			
T	Jan. 7	10	Jan. 25	10	t	Jan. 7	10	Jan. 25	10
U	Jan. 25	10	Feb. 15	10	u	Jan. 25	10	Feb. 15	10
V	Feb. 15	10	Mar. 3	10	v	Feb. 15	10	Mar. 3	10
W [3]	Mar. 3	10	Mar. 19	10	w [3]	Mar. 3	10	Mar. 19	10
X	Mar. 30	10	Apr. 13	10	x	Mar. 30	10	Apr. 13	10
Y	Apr. 13	10	Apr. 28	10	y	Apr. 13	10	Apr. 28	10
Z	Apr. 28	10	May 13	10	z	Apr. 28	10	May 13	10
AA	May 13	10	May 26	10	aa	May 13	10	May 26	10
BB	May 26	10	June 12	10	bb	May 26	10	June 12	10
CC	June 12	10	June 25	10	cc	June 12	10	June 25	10
DD	June 25	10	July 10	10	dd	June 25	10	July 10	10
EE	July 10	10	July 28	10	ee	July 10	10	July 28	10
FF	July 28	9	Aug. 12	8	ff	July 28	9	Aug. 12	8
GG	Aug. 13	10	Sept. 1	10	gg	Aug. 13	10	Sept. 1	10

[1] Experiments discontinued.
[2] Pustules vigorous.
[3] Series sent from Washington, D. C., to Minneapolis, Minn.
[4] Inoculations made from material from dried leaves.
[5] Series sent from Minneapolis, Minn., to Washington, D. C.
[6] Several.

TABLE III.—*Summary of experiments to determine the vitality of successive uredo generations of various grain rusts*—Continued.

PUCCINIA CORONATA ON OATS—Continued.

Capital letter series.					Lower-case letter series.				
Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
	1908.		1908.			1908.		1908.	
HH	Sept. 1	10	Sept. 17	[1]	hh	Sept. 1	10	Sept. 17	[1]
II	Sept. 17	10	Oct. 2	8	ii	Sept. 17	10	Oct. 2	8
JJ	Oct. 2	10	Oct. 22	10	jj	Oct. 2	10	Oct. 22	10
KK	Oct. 22	10	Nov. 6	10	kk	Oct. 22	10	Nov. 6	10
LL	Nov. 6	10	Nov. 20	9	ll	Nov. 6	10	Nov. 20	10
MM	Nov. 20	10	Dec. 12	10	mm	Nov. 20	10	Dec. 12	10
			1909.					1909.	
NN	Dec. 12	10	Jan. 10	8	nn	Dec. 12	9	Jan. 10	9
	1909.					1909.			
OO	Jan. 10	10	Feb. 7	10	oo	Jan. 10	10	Feb. 7	5
PP	Feb. 7	10	Feb. 23	10	pp	Feb. 7	10	Feb. 23	10
QQ	Feb. 23	10	Mar. 14	10	qq	Feb. 23	10	Mar. 14	10
RR	Mar. 14	10	Mar. 30	10	rr	Mar. 14	10	Mar. 30	10
SS	Mar. 30	10	Apr. 12	8	ss	Mar. 30	10	Apr. 12	6
TT	Apr. 12	10	Apr. 27	10	tt	Apr. 12	10	Apr. 27	10
UU	Apr. 27	7	May 20	7	uu	Apr. 27	9	May 20	9
VV	May 20	10	June 14	10	vv	May 20	10	June 14	10
WW	June 14	10	June 26	10	ww	June 14	10	June 26	10
XX	June 26	10	July 7	9	xx	June 26	10	July 7	10
YY	July 7	10	July 21	10	yy	July 7	10	July 21	10
ZZ	July 21	10	Aug. 2	10	zz	July 21	10	Aug. 2	10
AAA	Aug. 2	10	[2]	[2]	aaa	Aug. 2	10	[2]	[2]

PUCCINIA RUBIGO-VERA SECALIS ON RYE.

	1907.					1907.			
A	Feb. 6	10	Feb. 19	[3] 9	a	Feb. 6	10	Feb. 19	[3] 10
B	Feb. 19	10	Mar. 5	9	b	Feb. 19	10	Mar. 5	8
C	Mar. 5	10	Mar. 27	3	c	Mar. 5	10	Mar. 25	[4]
D[5]	Mar. 27	3	Apr. 8	1	d[5]	Mar. 27	3	Apr. 8	1
E[6]	Apr. 17				e	Apr. 17	[7] 2	May 7	1
F					f	May 8	10	May 26	[4]
G					g	May 27	10	June 10	[4]
H					h	June 11	10	June 25	[4]
I					i	June 26	10	July 8	7
J					j	July 10	10	July 23	10
K					k	July 24	10	Aug. 8	10
L					l	Aug. 9	10	Aug. 23	2
M					m	Aug. 24	10	Sept. 10	10
N	Sept. 11	10	Sept. 27	[8] 10	n	Sept. 11	10	Sept. 27	10
O[9]	Sept. 28	10	Oct. 8	[4]	o[9]	Sept. 28	10	Oct. 8	[4]
P	Oct. 16	6	Oct. 29	6	p	Oct. 16	5	Oct. 31	5
Q	Oct. 31	10	Nov. 18	9	q	Oct. 31	9	Nov. 18	9
R	Nov. 20	10	Dec. 10	10	r	Nov. 20	10	Dec. 10	10
			1908.					1908.	
S	Dec. 12	8	Jan. 7	8	s	Dec. 12	10	Jan. 7	10
	1908.					1908.			
T	Jan. 7	9	Jan. 25	10	t	Jan. 7	9	Jan. 25	10
U	Jan. 25	10	Feb. 15	10	u	Jan. 25	10	Feb. 15	10
V	Feb. 15	10	Mar. 3	10	v	Feb. 15	10	Mar. 3	10
W[5]	Mar. 3	10	Mar. 19	10	w[5]	Mar. 3	10	Mar. 19	10
X	Mar. 30	10	Apr. 13	10	x	Mar. 30	10	Apr. 13	10
Y	Apr. 13	10	Apr. 28	10	y	Apr. 13	10	Apr. 28	10
Z	Apr. 28	10	May 13	10	z	Apr. 28	10	May 13	10
AA	May 13	10	May 26	10	aa	May 13	10	May 26	10
BB	May 26	10	June 12	10	bb	May 26	10	June 12	10
CC	June 12	9	June 25	9	cc	June 12	10	June 25	10
DD	June 25	7	July 10	5	dd	June 25	10	July 10	10

[1] Not recorded.
[2] Experiments discontinued.
[3] Pustules vigorous.
[4] Several.
[5] Series sent from Washington, D. C., to Minneapolis, Minn.
[6] Lost in transit.
[7] Inoculations made from material from dried leaves.
[8] Inoculations made from m.
[9] Series sent from Minneapolis, Minn., to Washington, D. C.

TABLE III.—*Summary of experiments to determine the vitality of successive uredo generations of various grain rusts*—Continued.

PUCCINIA RUBIGO-VERA SECALIS ON RYE—Continued.

Capital letter series.					Lower-case letter series.				
Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Series letter.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
	1908.		1908.			1908.		1908.	
EE	July 10	6	July 28	6	ee	July 10	4	July 28	4
FF	July 28	9	Aug. 12	[1] 1	ff	July 28	6	Aug. 12	[1] 0
GG	Aug. 13	8	Sept. 1	5	gg				
HH	Sept. 1	10	Sept. 17	[2]	hh	Sept. 1	[3] 9	Sept. 17	[2]
II	Sept. 17	10	Oct. 2	10	ii	Sept. 17	10	Oct. 2	10
JJ	Oct. 2	10	Oct. 22	10	jj	Oct. 2	10	Oct. 22	10
KK	Oct. 22	10	Nov. 6	10	kk	Oct. 22	10	Nov. 6	10
LL	Nov. 6	8	Nov. 20	8	ll	Nov. 6	10	Nov. 20	10
MM	Nov. 20	7	Dec. 12	4	mm	Nov. 20	9	Dec. 12	9
			1909.					1909.	
NN	Sec. 12	10	Jan. 10	8	nn	Dec. 12	10	Jan. 10	10
	1909.					1909.			
OO	Jan. 10	7	Feb. 7	4	oo	Jan. 10	10	Feb. 7	5
PP	Feb. 7	10	Feb. 23	10	pp	Feb. 7	8	Feb. 23	8
QQ	Feb. 23	10	Mar. 14	10	qq	Feb. 23	10	Mar. 14	10
RR	Mar. 14	10	Mar. 30	10	rr	Mar. 14	10	Mar. 30	10
SS	Mar. 30	10	Apr. 12	10	ss	Mar. 30	10	Apr. 12	10
TT	Apr. 12	10	Apr. 27	10	tt	Apr. 12	10	Apr. 27	10
UU	Apr. 27	10	May 20	10	uu	Apr. 27	10	May 20	10
VV	May 20	10	June 14	10	vv	May 20	10	June 14	10
WW	June 14	8	June 26	8	ww	June 14	8	June 26	8
XX	June 26	10	July 7	10	xx	June 26	10	July 7	10
YY	July 7	10	July 21	9	yy	July 7	10	July 21	8
ZZ	July 21	10	[4]	[4]	zz	July 21	8	[4]	[4]

PUCCINIA GRAMINIS TRITICI ON BARLEY FROM WHEAT.[5]

Original inoculation made Nov. 13, 1906.				Original inoculation made Nov. 22, 1906.			
Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
1907.		1907.		1907.		1907.	
Apr. 17	10	May 7	[6] 8	Apr. 17	10	May 7	[6] 10
May 7	10	May 29	10	May 8	10	May 26	10
May 29	10	June 11	10	May 27	10	June 11	10
June 11	10	June 26	10	June 11	10	June 26	10
June 26	10	July 8	6	June 26	10	July 8	10
July 8	10	July 21	6	July 8	10	July 21	10
July 22	9	Aug. 6	8	July 22	10	Aug. 6	10
Aug. 7	8	Aug. 21	8	Aug. 7	10	Aug. 21	10
Aug. 22	8	Sept. 8	8	Aug. 22	10	Sept. 8	10
Sept. 9	10	Sept. 25	10	Sept. 9	10	Sept. 25	10
Sept. 26	10	Oct. 87	10				
Oct. 17	9	Oct. 29	9	Oct. 17	[8] 4	Oct. 29	3
Oct. 31	10	Nov. 18	10	Oct. 31	10	Nov. 18	10
Nov. 20	10	Dec. 10	10	Nov. 20	10	Dec. 10	10
		1908.				1908.	
Dec. 12	6	Jan. 8	6	Dec. 12	6	Jan 8	6
1908.				1908.			
Feb. 15	[9] 7	Mar. 3	7	Feb. 15	[9] 6	Mar. 3	6

[1] Extreme heat in greenhouse.
[2] Several.
[3] Inoculations made from GG.
[4] Experiments discontinued.
[5] The original material was obtained from wheat November 13 and 22, 1906. It was transferred to barley and was kept on barley continuously from that time. Notes on the inoculations from November 13 and 22, respectively, to March 30, 1907, have been mislaid or lost; but six series of successful inoculations were made in each case at Washington, D. C., and on March 30 the successfully inoculated plants were sent to Minnesota. The table gives the results from inoculations from this material, beginning with April 17, 1907.
[6] Pustules vigorous.
[7] Series sent from Minneapolis, Minn., to Washington, D. C., October, 8, 1907.
[8] Inoculations made from material pustuled September 25, 1907.
[9] Inoculations made from material pustuled January 8, 1908.

TABLE III.—*Summary of experiments to determine the vitality of successive uredo generations of various grain rusts*—Continued.

PUCCINIA GRAMINIS TRITICI ON BARLEY FROM WHEAT—Continued.

Original inoculation made Nov. 13, 1906.				Original inoculation made Nov. 22, 1906.			
Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
1908.		1908.		1908.		1908.	
Mar. 3	10	Mar. 19[1]	10	Mar. 3	10	Mar. 19	10
Mar. 30	10	Apr. 28	2	Mar. 30	10	Apr. 12	8
Apr. 28	10	May 13	10	Apr. 12	6	Apr. 28	6
May 13	10	May 26	10	Apr. 28	10	May 13	9
May 26	10	June 12	5				
June 12	10	July 10	2				
July 10	7	July 28	1	May 13	10	May 26	10
July 28	7	Aug. 12	[2] 1	May 26	10	June 12	2
Aug. 12	1	Sept. 1	1	June 12[3]			
Sept. 1	5	Sept. 17	5				
Sept. 17	9	Oct. 2	6				
Oct. 2	10	Oct. 24	10				
Oct. 24	10	Nov. 6	10				
Nov. 6	10	Nov. 20	10				
Nov. 20	10	Dec. 12	8				
		1909.					
Dec. 12	8	Jan. 10	8				
1909.							
Jan. 10	7	Feb. 7	2				
Feb. 7	7	Feb. 23	[4]				
Feb 23	16	Mar. 14	8				
Mar. 14	10	Mar. 30	10				
Mar. 30	10	Apr. 12	7				
Apr. 12	8	Apr. 27	7				
Apr. 27	6	May 20	6				
May 20	10	June 14	9				
June 14	10	June 26	10				
June 26	10	July 7	10				
July 7	10	July 21	10				
July 21	10	Aug. 2	10				
Aug. 2	10						

PUCCINIA GRAMINIS HORDEI ON WHEAT FROM BARLEY.[5]

Original inoculation made Nov. 14, 1906.				Original inoculation made Nov. 22, 1906.			
Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
1907.		1907.		1907.		1907.	
Apr. 17	8	May 7	7	Apr. 17	10	May 7	10
May 9	10	May 28	10	May 8	10	May 28	10
May 28	10	June 11	7	May 28	10	June 11	10
June 11	10	June 24	7	June 11	10	June 24	10
June 24	10	July 8	4	June 24	10	July 8	6
July 8	10	July 21	1	July 8	10	July 21	7
July 22	5	Aug. 6	2	July 22	10	Aug. 6	8
Aug. 6	8	Aug 21	8	Aug. 7	10	Aug. 21	10
Aug. 22	8	Sept. 8	8	Aug. 22	10	Sept. 8	10
Sept. 9	10	Sept. 25[6]	10	Sept. 9	10	Sept. 25	10
Oct. 17	9	Oct. 29	6	Oct. 16	9	Oct. 29	9

[1] Series sent from Washington, D. C., to Minneapolis, Minn.
[2] Extreme heat in greenhouse.
[3] Accidentally mixed with *Puccinia simplex;* discarded.
[4] Notes not taken.
[5] The original material was obtained from barley November 14 and November 22, 1906. It was transferred to wheat and was kept on wheat continuously from that time. Notes on the inoculations from November 14 and 22, respectively, to March 30, 1907, have been mislaid or lost; but six series of successful inoculations were made in each case at Washington, D. C., and on March 30 the successfully inoculated plants were sent to Minnesota. The table gives the results from inoculations from this material, beginning with April 17, 1907.
[6] Series sent from Minneapolis, Minn., to Washington, D. C., October 8, 1907.

TABLE III.—*Summary of experiments to determine the vitality of successive uredo generations of various grain rusts*—Continued.

PUCCINIA GRAMINIS HORDEI ON WHEAT FROM BARLEY—Continued.

Original inoculation made Nov. 14, 1906.				Original inoculation made Nov. 22, 1906.			
Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.	Date of inoculation.	Number of leaves inoculated.	Date matured.	Number of leaves pustuled.
1907.		1907.		1907.		1907.	
Oct. 31	8	Nov. 18	8	Oct. 31	9	Nov. 18	9
Nov. 20	10	Dec. 10	10	Nov. 20	10	Dec. 10	10
		1908.				1908.	
Dec. 12	10	Jan. 8	10	Dec. 12	10	Jan. 8	10
1908.				1908.			
Jan. 25	10	Feb. 15	4	Jan. 25	10	Feb. 15	10
Feb. 15	6	Mar. 3	6	Feb. 15	10	Mar. 3	6
Mar. 3	10	Mar. 19[1]	10	Mar. 3	10	Mar. 19[1]	10
Mar. 31	10	Apr. 13	9	Mar. 30	10	Apr. 13	9
Apr. 13	7	Apr. 27	7	Apr. 13	8	Apr. 27	8
Apr. 27	10	May 13	10	Apr. 27	10	May 13	10
May 13	10	May 26	10	May 13	10	May 26	10
May 26	10	June 12	10	May 26	10	June 12	10
June 12	10	June 25	10	June 12	10	June 25	10
June 25	10	July 10	10	June 25	10	July 10	10
July 10	10	July 28	7	July 10	10	July 28	7
July 28	10	Aug. 12	6	July 28	6	Aug. 12	1
Aug. 12	10	Sept. 1	9	Aug. 12	10	Sept. 1	5
Sept. 1	10	Sept. 17	[2]	Sept. 1	[2]	Sept. 17	[2]
Sept. 17	10	Oct. 2	10	Sept. 17	10	Oct. 2	10
Oct. 2	10	Oct. 24	10	Oct. 2	10	Oct. 24	6
Oct. 24	9	Nov. 6	9	Oct. 24	9	Nov. 6	5
Nov. 6	9	Nov. 20	6	Nov. 6	9	Nov. 20	9
Nov. 20	10	Dec. 12	9	Nov. 20	10	Dec. 12	9
		1909.				1909.	
Dec. 12	10	Jan. 10	9	Dec. 12	10	Jan. 10	8
1909.				1909.			
Jan. 10	10	Feb. 7	1	Jan. 10	10	Feb. 7	4
Feb. 7	3	Feb. 23	3	Feb. 7	7	Feb. 23	7
Feb. 23	10	Mar. 14	10	Feb. 23	8	Mar. 14	8
Mar. 14	10	Mar. 30	10	Mar. 14	10	Mar. 30	10
Mar. 30	10	Apr. 12	8	Mar. 30	10	Apr. 12	8
Apr. 12	10	Apr. 27	10	Apr. 12	10	Apr. 27	10
Apr. 27	8	May 20	8	Apr. 27	4	May 20	4
May 20	10	June 14	10	May 20	10	June 14	10
June 14	10	June 26	10	June 14	10	June 26	10
June 26	10	July 7	10	June 26	10	July 7	10
July 7	10	July 21	10	July 7	10	July 21	10
July 21	10	Aug. 2	10	July 21	10	Aug. 2	10
Aug. 2	10	[3]	[3]	Aug. 2	10	[3]	[3]

[1] Series sent from Washington, D. C., to Minneapolis, Minn.
[2] Not recorded.
[3] Experiment discontinued.

The lowered percentage of successful infections in July and August of 1907 and 1908 is noticeable and was due to the extreme heat in the greenhouses at the time of inoculation. The uredospore germinates either not at all or not nearly so well at the excessive temperatures of 90° to 100° F. and over, which then existed during parts of each day, as it does in more moderate temperatures, 55° to 75° F.; the germ tubes are injured and the host plants themselves become drawn and weak, reducing the chances for infection very markedly. *Puccinia coronata*, however, is noticeably resistant to heat and *P. rubigo-vera* on wheat is a close second, while *P. graminis* on oats is injured quickly and *P. graminis* on rye is killed by excessive temperatures.

The most important point brought out by these experiments is that for 52 generations there is no apparent diminution of the vitality of the uredo generation due to continuous culture and the absence of the æcidio or teleuto generations. For this length of time, at least, there is no need for a sexual generation. How long successive uredo generations can continue without lowered vitality has not been determined, but these experiments indicate that they may continue for a very long period and that the uredo generation may be all sufficient. It must be noted that the number of successive inoculations in these experiments far exceeds the probable number under ordinary field conditions because they were continued throughout the winter months. Since 8 to 12 days (even up to 20 days and over in unfavorable weather) are necessary for infection and since probably not more than 5 or 6 successive infections follow each other annually in field conditions, the inoculations in the above experiments are equivalent to 7 or 8 years of successive infection in the field.

WINTERING OF THE UREDO GENERATION.

HISTORY OF THE INVESTIGATIONS.

The question whether or not the uredo stage of rusts lives over winter either as mycelium or in the spore form has been a much mooted one ever since De Bary demonstrated the heteræcism of *Puccinia graminis* in 1865. This problem has been investigated by many scientists in different countries and localities.

Germany.—De Bary (12, p. 23) was one of the first of these investigators. He looked for the wintering of the mycelium of *Puccinia graminis* on *Agropyron repens* and *Poa pratensis*, but although heavily covered with rust in the field the same plants in the following spring and summer remained rust free. He concluded that the rust mycelium is annual only, even in perennial grasses.

Kühn (66, p. 401) found the uredo of *Puccinia coronata* in all stages of development on *Holcus lanatus* in the middle of winter and maintained that it developed without hindrance in the spring; on this account he considered a similar wintering in *Puccinia graminis* and *P. rubigo-vera* very possible.

According to Eriksson and Henning (39, p. 38), Blomeyer (20, p. 405) believed that *Puccinia graminis* was able to winter over in the uredo stage at Leipzig on account of the early appearance of *P. graminis* in the spring (latter part of May) at that place.

Klebahn (63, p. 64) says that neither does *Puccinia graminis* appear to winter in the uredo stage nor *P. coronifera avenae* nor *P. simplex*, because oats and barley rarely, if ever, are grown as winter grains in

Germany. He considers the wintering of the uredo of *P. dispersa* (*P. rubigo-vera*) and of *P. glumarum* to be possible.[1]

Denmark.—According to Eriksson and Henning (39, p. 38), Rostrup (87, p. 55) considered the wintering of the uredo of *Puccinia graminis* very possible in mild winters in Denmark, especially as it sometimes appears before the æcidium on the barberry.

Sweden.—Eriksson and Henning (39, pp. 40, 41, 131) were unable to find that Puccinia on *Agropyron repens*, *Dactylis glomerata*, and *Agrostis vulgare* winters over in the uredo stage in Stockholm. They were inclined to believe, however, that *Puccinia phlei-pratensis* winters in the uredo.

In a letter from Eriksson to the authors dated December 28, 1907, he states that his conclusions as to the wintering of the uredo of *Puccinia phlei-pratensis* published in Die Getreideroste, 1906, lack sufficient support; that conditions are very probably the same for this rust as for the cereal rusts—i. e., it does not winter in the uredo stage.

The wintering of the uredo of *Puccinia dispersa*, either as spore or mycelium, according to Eriksson and Henning, does not take place in Sweden (39, p. 218), and according to these authors the probability of the uredospore of *P. glumarum*, the yellow rust common in Scandinavia, England, and India, living over winter is very slight, at least in the vicinity of Stockholm.

England.—Plowright (85, p. 234) found uredospores in England on *Agropyron repens* in December, 1881, and again in March; whether *Puccinia graminis* or *P. rubigo-vera* is not absolutely clear. He adds:

> This spring our Norfolk and Suffolk wheats were much affected with rust; some of this may be and probably was due to the *Uredo linearis* kept alive from the previous autumn, but the bulk of it was due to the uredo of *Puccinia straminis* (*P. rubigo-vera*), which is always an earlier uredo than that of *P. graminis*.

The same author (84, p. 35) affirms that the uredo of *Puccinia rubigo-vera* can be found throughout the whole winter in England. Ward (99, p. 132) found viable uredospores of *P. dispersa* on Bromus during every month in the year.

Biffen (17, pp. 241–253) believes that the yellow rust *Puccinia glumarum* also winters in the uredo stage in England. He says:

> The uredospore stage seems to be sufficient to enable the fungus to tide itself over the winter, for it is possible to find pustules of rust on the foliage of self-sown wheat

[1] In an article which has appeared while this paper was in preparation Hecke (Naturwissenschaftliche Zeitschrift für Forst- und Landwirtschaft, vol. 9, pt. 1, Jan., 1911, pp. 44–53) brings forth experimental evidence to show that the uredo mycelium of yellow rust, *Puccinia glumarum*, winters over in the leaves of the winter grains at Vienna, Austria. He inoculated winter wheats in pots October 28 and November 21, 1909, left them in the greenhouse for three days, and brought them into the open, where they remained all winter. Pustules of yellow rust appeared March 28, 1911, on the inoculated leaves, while control leaves remained rust free. In this instance the incubation period of this rust must have been four and five months, during which time the mycelium remained practically dormant.

or sometimes on the ordinary autumn-sown crops even in the depths of winter. The twisted leaves lying on the soil form a series of sheltered, moist chambers, on the inner surface of which the rust pustules are occasionally present in great numbers. These may develop with rapidity in the early spring, and at times as early as March the whole of the plant's foliage may be yellow with rust. The winter's cold does not appear to injure these spores, for they germinate readily when brought into the laboratory, and there can be little doubt that they serve to start the epidemic in the spring, when conditions become favorable for infection. Under these circumstances it is not necessary to assume that the first appearance of any fungus in any season is dependent upon its being actually present in the embryo of the grain, spreading therefrom as the plant develops and ultimately producing its spores when the external conditions are favorable.

Australia.—McAlpine (74, p. 27) believed it probable that the red-rust spores survive the winter in Australia and reproduce the fungus again in the spring or summer.

Cobb (36, p. 186) says:

> During the past two years it has been proved that the wheat rusts, that is, *Puccinia graminis* and *P. rubigo-vera*, exist in the uredo stage all the year around in Australia.

McAlpine (76, p. 20), from further observations on the wintering of rusts in Australia, says:

> When the winter is mild and green vegetation flourishes, the mycelium of the rust fungus may continue to grow and may even produce spores; whereas, if the winter is severe and the mycelium does not remain in the perennial part of the plant, then the continuance of the fungus is likely to be by teleutospores, which can last through the winter on dead stems or other decaying vegetable matter. The so-called wintering of the uredo depends so much on the climate that in a mild climate the fungus may perpetuate itself exclusively by uredospores; whereas under severe conditions it has to resort to teleutospores.

He further observes that in Australia it is the heat and drought of summer which the rust must withstand, not the cold of winter, and hence *Puccinia graminis* produces only comparatively few teleutospores and lives over in the uredo stage in that country. During the winter it is found in abundance on volunteer grains.

The same author cites numerous instances of the germination of the uredospore during winter. He says (76, p. 22):

> The uredo may become inured to unfavorable conditions, such as drought or cold, and carry on the life of the species independent of the teleutospore.

Such adaptation is seen in this country in *Puccinia vexans* Farl., which, in addition to the ordinary uredo, has a specialized form, a thick-walled, strongly papillate amphispore which germinates only after a period of rest (31, pp. 22–25).

United States.—Bolley in 1889 (21, pp. 13, 14) proved by a series of observations that *Puccinia rubigo-vera* on wheat near Lafayette, Ind., can pass the winter as "healthy fungal mycelium within the tissues of the leaves," producing rust spores in abundance at the first appearance of warm weather in March. "The very early appearance

and prevalence of red rust, *Puccinia rubigo-vera*, is attributed in part to the ability of that species to winter its mycelium" (21, p. 14). Apparently the same instance is cited by him in a later publication (22, p. 107).

The same author in 1891 (23, p. 260) says:

> The red rust (uredo of *P. rubigo-vera* and *P. coronata*) is developed to a greater or less extent during all months of the year in States south of Tennessee. * * * In the States north of this line there seem to be isolated cases in which the mycelium may persist through winter, dependent, apparently, chiefly upon the point whether the attacked portion of the host persists or not.

Hitchcock and Carleton (57, p. 11; 29, p. 453) found in Kansas throughout the winter months (January 23–25, February 25, and March 1) viable uredospores of *Puccinia rubigo-vera* on wheat. They state:

> It would seem that the uredospores were not formed during the winter, but had retained their vitality since the preceding fall.[1]

Again, Bolley (24, p. 894) says that fresh uredospores of *Puccinia rubigo-vera* can be found in the United States throughout the winter in States south of Ohio, and although new spores are not formed in States as far north as Indiana and Kansas during the coldest periods, those already formed retain their viability.

Carleton (30, p. 21), in speaking of the uredo of *Puccinia rubigo-vera* on wheat, says that the conclusions of Bolley, Hitchcock, and Carleton as to the wintering of the uredo have been confirmed and reconfirmed by him both in Kansas and in Maryland.

> In the Southern States the leaf rusts of both wheat and rye not only live but grow all winter. * * * In latitudes below 40° in this country, leaf rust of wheat is able to pass a perpetual existence in the uredo stage on wheat alone, without intervention of any other stage.

Again he maintains (30, p. 44) that *Puccinia rubigo-vera secalis* lives over winter in a similar manner, and it is his opinion that this rust "readily passes the winter as a uredo in all parts of the United States." He found the uredo in great abundance in a patch of volunteer rye at Lincoln, Nebr., in November, 1897, and afterwards in midwinter in the same place. April 15, 1898—

> it was still present in considerable quantity, but was confined entirely to the leaves of the previous autumn's growth and had without question lived through the winter, though the leaves were still somewhat green.

Some of the uredospores germinated in water-drop cultures. Two days later the uredo was found in considerable quantity several miles from this locality.

> In neither case was there any production of new spores, and yet the spring was so far advanced that there could be no question about the continual growth of the rust.

[1] The minimum temperatures (F.) at this time were: For December, −9°; January, −1°; February, −6°.

He did not demonstrate the wintering of the uredo of *Puccinia coronata* on oats, *P. graminis tritici*, or *P. graminis avenae*, although it is his opinion that *P. coronata* passes the winter in the uredo stage in the warm latitudes of the United States (30, pp. 49, 57, 64).

In 1902–3, Christman (32, pp. 103, 104) showed that in the locality of Madison, Wis., the uredospores of *Puccinia poarum* would winter and germinate as late as March 13; of *P. rubigo-vera secalis* and *P. rubigo-vera tritici*, March 20. Numerous other collections and germinations were made throughout the winter from plants in exposed places, and the author concludes that—

> in the latitude of Madison and with a period of three months during which the temperature scarcely rises above the freezing point, viable uredospores may be obtained at practically any time during the winter.

In investigations during the winter of 1904–5, Bolley (28, p. 642) obtained a collection of viable uredospores of *Puccinia rubigo-vera* in December and January in Kansas, Oklahoma, Missouri, Illinois, Wisconsin, Minnesota, and North Dakota. Viable uredospores of *P. graminis* were collected late in October at St. Louis, and December 25 at Dallas, Tex. In January—

> quantities of them were being procured upon winter wheat at Riverside, Ill. Later some were procured in quack-grass at Lake City, Minn., and a quantity of viable spores were taken from the leaves of quack-grass and wild barley frozen in the ice at Fargo in March, 1905.

RECENT EXPERIMENTS ON THE WINTERING OF THE UREDOSPORE.

During the winter of 1906–7, the authors undertook to establish the extent of viability of the uredospore of various rusts in the vicinity of St. Paul, Minn. All material was collected on or near the Minnesota Agricultural Experiment Station farm.

In the early fall suitable plants of *Hordeum jubatum*, *Agropyron repens*, *A. tenerum*, winter wheat, and fall-sown barley were selected. These were left undisturbed in the open field at the University farm. They had become thoroughly infected by either *Puccinia graminis*, *P. rubigo-vera*, or both. During the fall and winter, collections of uredospores were made from all hosts, selected every month and at times at intervals of two weeks.

Portions of the various hosts were also collected November 20 and 23 (1906); these were kept outside, were buried in snow December 10, and left in this condition until March 20, 1907. Every month specimens from this supply were tested in the same manner as the material brought from the field.

All tests were made in distilled water in watch crystals placed under a bell jar and kept at ordinary living-room temperature or a little above. In many instances the percentage of spores that germinated was determined by actual count, but generally rough estimates only were made.

Dates of collection and germination and summary of results are given in Tables IV and V.

TABLE IV.—*Summary of experiments on the wintering of the uredospore at St. Paul, Minn.*

Date of collection and germination test.	Species.	Host plant.	Time of incubation.	Germination.
			Hours.	*Per cent.*
November 20, 1906	Puccinia graminis	Hordeum jubatum	22	95
December 14, 1906	do	do	40	26
December 27, 1906	do	do	24	50
January 25, 1907	do	do	22	30
February 15, 1907	do	do	24	75
March 16, 1907	do	do	18	50
April 15, 1907	do	do	26	35
November 20, 1906	do	Agropyron repens	22	50
December 14, 1906	do	do	40	3
December 27, 1906	do	do	24	32
January 25, 1907	do	do	22	5
February 15, 1907	do	do	24	25
March 16, 1907	do	do	18	50
April 15, 1907	do	do	26	5
November 20, 1906	do	A. tenerum	48	5
December 14, 1906	do	do	40	25
December 27, 1906	do	do	24	95
January 25, 1907				
February 15, 1907	P. graminis	A. tenerum	24	10
March 16, 1907	do	do	18	25
April 15, 1907	do	do	26	40
November 29, 1906	P. rubigo-vera	A. repens	22	50
December 14, 1906	do	do	40	10
December 27, 1906	do	do	24	50
January 25, 1907	do	do	22	50
February 15, 1907	do	do	24	0
March 16, 1907				
November 20, 1906	P. rubigo-vera	Winter wheat	40	15
December 14, 1906	do	do	40	20
December 27, 1906	do	do	24	12
January 25, 1907	do	do	22	25
February 15, 1907				
April 16, 1907				
November 20, 1906	P. simplex	Barley		
December 14, 1906	do	do	40	30
December 27, 1906	do	do	24	40
January 25, 1907				
February 15, 1907				
March 16, 1907				

TABLE V.—*Summary of germination results from uredo material kept buried in snow until germination tests were made.*

Date of germination test.	Species.	Host plant.	Time of incubation.	Germination.
			Hours.	*Per cent.*
December 10, 1906	Puccinia graminis	Hordeum jubatum	20	50
January 8, 1907	do	do	24	5
February 9, 1907	do	do	24	5
March 20, 1907	do	do	20	15
December 10, 1906	do	Agropyron repens	20	25
January 8, 1907	do	do	24	90
February 9, 1907	do	do	24	25
March 20, 1907	do	do	20	75
December 10, 1906	do	A. tenerum	20	40
December 10, 1906	P. rubigo-vera	A. repens	20	60
January 8, 1907	do	do	24	30
February 9, 1907	do	do	24	10
March 20, 1907	do	do	20	50
December 10, 1906	do	Winter wheat	20	25
January 8, 1907	do	do	24	3
February 9, 1907	do	do	24	5
March 20, 1907	do	do	20	10
December 10, 1906	P. simplex	Barley	20	15
January 8, 1907	do	do	20	10
February 9, 1907	do	do	24	50
March 20, 1907	do	do	20	10

The wide variation in percentage of germination in collections made at different times in these experiments is principally due to the fact that spores at the same stage of development and equally well protected can not be obtained twice in succession. It was noticeable that those spores which were just mature and remained well protected under the epidermis of the host were the most viable. In a large number of cases such spores seemed to be as healthy in the spring as they were in the fall. Those spores which broke through the epidermis, dropped off from the old mycelium, and rested loosely in the leaf sheath, seemed to lose their power of germination during the winter and would not germinate in the spring.

The winter of 1906–7 in Minnesota was not abnormal, and much of the rust material collected was dug from under the snow and ice. A thaw during a part of January incased much of the material in frozen snow. About February 15 there was another thaw, and the *Agropyron repens* material in particular became incased in ice, which disappeared during the latter part of March.

The tables show that a large per cent of the uredospores of *Puccinia graminis* on *Hordeum jubatum*, on *Agropyron repens*, and *A. tenerum*, collected from plants in the field, germinated throughout the winter, such germinations having been made November 20, December 14 and 27, January 25, February 15, March 16, and April 15. After April 15 such spores were extremely hard to find in the locality under consideration, as most of them had germinated in the warm, humid days of early spring. Uredospores of the same rusts on *Hordeum jubatum* and *Agropyron repens*, collected November 20 and 23, kept outside until December 10 and then buried in snow, germinated on December 10, January 8, February 9, and March 20. After that date the snow disappeared and the material could be kept no longer. The uredospores on *Agropyron tenerum* were tested only on December 10, on account of the scarcity of the material.

Similar experiments with *Puccinia rubigo-vera* gave successful germinations from material on *Agropyron repens* from the field November 29, December 14 and 27, and January 25, while the small amount collected on February 15 did not germinate; from material on *Triticum vulgare* (winter wheat) successful germinations were made November 20, December 14 and 27, and January 25, while after that date no material could be obtained; *Puccinia simplex* on barley collected in the field germinated November 20, December 14, and December 27. After that time no more could be found.

Material of all three of these leaf rusts collected on their respective hosts November 20 and 23, kept outside until December 10 and then buried in snow, germinated December 10, January 8, February 9, and March 20. After that date no trials were made.

The uredospores of *Puccinia graminis* on *Hordeum jubatum*, *Agropyron repens*, and *A. tenerum* obtained from the natural field habitat have thus been demonstrated to retain their viability until April 15, and material from the two former kept buried in the snow until March 20 has also been shown to remain viable. *Puccinia rubigo-vera* on *Agropyron repens* and *Triticum vulgare* from the field have been demonstrated to germinate as late as February 15, and *Puccinia simplex* on *Hordeum vulgare* as late as December 27. After these dates no material could be obtained. A large per cent of the uredospores collected in the fall and kept buried in snow since December 10 germinated as late as March 20, 1907. Bolley has shown that spores of *Puccinia rubigo-vera* collected in Minnesota April 9 and in North Dakota April 13, 1905, were viable (28, p. 649). Together with Bolley's and Christman's investigations cited above, these experiments demonstrate conclusively that it is possible for the uredospores of various stem and leaf rusts to retain their viability throughout the winter in Minnesota, North Dakota, and Wisconsin.

How commonly the wintering of the uredospore in these northern States takes place is yet to be determined. Where snow remains throughout the winter, preventing alternate freezing and thawing of material thus covered, the wintering of the uredospore is, perhaps, facilitated. Indeed, it is very probable that the uredospore survives the winter more easily in the north, where snow is continuous during the winter, than in localities where snow covers the ground only at intermittent periods. Then, there is probably as good a chance, if not better, for the uredospore to winter in northern Minnesota or southern Canada, as in southern Minnesota or Iowa. This view is also held by Bolley and Pritchard (28, p. 643).

From Kansas south, it has been proved by Hitchcock and Carleton (57, p. 11) that *Puccinia rubigo-vera* winters very easily in the uredo stage, and undoubtedly this also holds true for *P. graminis*. In the springs of 1908 and 1909, the authors personally observed wheat fields in Texas and Oklahoma. During the latter part of April, 1908, both *Puccinia graminis* and *P. rubigo-vera* were extremely abundant on wheats at San Antonio, Tex. Farther north, at Amarillo, Tex., *P. rubigo-vera* was well scattered April 30, though not plentiful. At Stillwater, Okla., May 7, this rust was abundant. Wheats at San Antonio, in 1909, were heavily rusted April 4, with both *P. graminis* and *P. rubigo-vera*, and the superintendent of the San Antonio Experiment Farm said that a rust was abundant in the grain plats in February.

There is, then, an abundance of rust spores in southern wheat fields in the early spring, and, according to investigations cited in this paper, there are also a large number of uredospores of *Puccinia*

graminis and *P. rubigo-vera* which have survived the winter in the north and are ready to infect the growing grain.

The great problem for rusts in many places of the South, however, is not how to live over the winter, but how to pass through the extremely hot months of July, August, and September. This is especially true of the cereal rusts in portions of eastern and southern Texas, as volunteer grain is scarce at that time; but in northwest Texas the authors noticed vigorous rust pustules of both *Puccinia graminis* and *P. rubigo-vera* on volunteer wheat during September, 1907, so that in the higher altitudes in the Southwest the rust does exist in the uredo form on volunteer grain in late summer and early fall. The early-sown fall wheat can thus become infected with spores from this source, as described later in this paper.

DISSEMINATION OF THE UREDOSPORE.

METHODS OF DISSEMINATION.

Rusts in the uredo stage have been shown to be present in parts of both the North and South at almost all times of the year, and in order to explain their constant menace to the crops of the country it remains only to determine their means of dissemination. Rust spores are extremely numerous, hundreds occurring in a single pustule. They are very light, much more so than dust particles, which have been known to be carried in the air for hundreds of miles and distributed over large territories in a few days. An example of the carrying power of the air is cited by Klebahn (63, pp. 66–68) who relates that dust clouds arising in northern Africa, March 9, 1901, were driven over a large part of the continent of Europe in the next two days. Corresponding dust showers were noticed March 9 and 10 in Tunis, West Tripoli, and Algiers; early March 10 in southern Sicily; night of March 10–11 in the East Alps; early March 11 in Maingebiet; at 4.30 in the afternoon in Hamburg; and a little after midnight in the Danish Islands (Stege auf Moen). The dust was composed of clay, fine quartz particles, and other minerals, supposedly derived from the African deserts.

Undoubtedly, rust spores, which are much lighter than these dust particles, can be carried more easily by the wind and air currents over as great, if not greater, distances. Rising into the air, these spores may reach the upper atmosphere and be carried hundreds of miles a day in whichever direction the air currents are moving. In this way innumerable rust spores may be carried from regions where they are plentiful, either by reason of the presence of the æcidial hosts, or overwintering uredos, to regions where grain is in a receptive condition. This interchange of spores between localities may take

place mainly from south to north in early spring and summer and from north to south in late summer and fall. Together with the wintering uredos in the North, such wind-carried spores from the South undoubtedly can cause early infection of the grains, and together with the spores on volunteer grains in the South the spores from the Northern States wafted south may serve to infect the winter grains as they come up in October and November.

That large quantities of rust spores are present in the air at various times has been proved by many investigators. Klebahn (63, pp. 69, 70) constructed cotton plates, leaving them in the open in trees in different places in Germany in the spring and summer at different periods. These cotton plates were then taken down and washed out carefully, and the water examined. Several thousand uredospores of *Puccinia graminis* and other rusts were found in each cotton mass, as well as innumerable spores of other fungi. Ecidiospores and teleutospores were found very sparingly. Klebahn concludes that numberless spores are contained in the air and large numbers fall on a proportionally small space. He believes that since grains are almost universally cultivated, and are scarcely ever rust free, tremendous numbers of rust spores are carried into the air in every grain-growing country, and, as a consequence, there is a universal distribution of them.

Experiments on this point have also been performed by the authors. On May 22, 1907, plates containing water were exposed for four hours at a time on top of one of the university buildings at Minneapolis, Minn., and also in an adjoining garden. On centrifuging this water and examining the sediment several uredospores were found, of both *graminis* and *rubigo-vera* types. Several teleutospores of *Puccinia graminis* were also found. E. C. Stakman performed similar experiments at St. Anthony Park, Minn., in April and May, 1910. Plates with water were exposed in the field, outside the laboratory window, and at the top of a water-tank tower at a height of 100 feet or more. The direction of the wind was southeast. April 11, in a plate exposed outside the laboratory window for four hours, several uredos of a *graminis* form were found. April 11 and 12, from a plate exposed for 48 hours in the field, several uredos were found; and on the same dates in a plate exposed for 48 hours on top of the water tower over 100 feet high, several uredospores of the *graminis* form were secured. On May 11, Stakman made a similar test and succeeded in germinating a uredospore of *Puccinia graminis* collected from the air at this time. These experiments of 1907 and 1910 were performed before uredospores began to appear in the field in new growth in that locality, and the spores must have come either from uredos wintering over in the North or from uredos borne from the wheat fields in the

South where fresh uredos of both *Puccinia graminis* and *P. rubigo-vera* forms are plentiful at this time of the year. This furnishes substantial evidence that Klebahn's suppositions are correct, and rust spores may be considered fairly universal in distribution.

VIABILITY OF THE UREDOSPORE.

That spores can resist desiccation in air and maintain their viability when transported long distances has been proved by Bolley (24, p. 892). In July, 1898, he demonstrated that uredospores of *Puccinia rubigo-vera*, exposed for 12 days on a dry watch glass placed in the sunlight, would germinate 80 to 100 per cent, and on August 4, spores placed in a similar place for 21 days would germinate from 5 to 10 per cent. July 25 and August 4, 1898, respectively, the same investigator proved that the uredo of *P. graminis* would give "good" germination after being exposed for 12 days on a watch glass in direct sunlight, and gave 8 to 15 per cent germination after 21 days on a watch glass in a similar position.

Ward (102, p. 13) found that uredospores of *Puccinia dispersa* germinated after being kept dry for 61 days; and Miss Gibson, working in his laboratory, kept æcidiospores of Phragmidium for 54 days and uredospores of chrysanthemum rust for 94 days, when they still germinated. Carleton (31, pp. 21, 22), February 3, 1898, germinated uredospores of *P. cryptandri* collected in Oklahoma, October 8, 1897, and kept as herbarium specimens, and got successful infection on *Sporobolus airoides* from inoculations made February 6 from the same material. This is an extreme case of the viability of the uredospore when kept in a dry condition.

The authors have numerous times shipped uredo material of the cereal rusts through the mails from Minnesota to Washington, D. C., and vice versa, and from Texas to Washington, D. C., and have experienced no difficulty in producing successful infection on growing plants, even after these spores had been lying in the laboratory for several days after their arrival. The uredospore is thus seen to be sufficiently resistant to be transported long distances in a dry condition by either the wind or other agencies.

FIRST APPEARANCE OF RUSTS IN THE SPRING.

From the facts cited concerning the viability of the uredospore and its almost universal distribution, the first spring infection of grains in northern latitudes and the infection of grains far removed from the æcidial hosts of the rusts may be explained. Careful observations on the first appearance of rusts in the spring were made at Minnesota in 1907, 1908, and 1909. In 1907, *Puccinia rubigo-vera* on winter wheat was common up to the middle of April, when

the old leaves died and the rust disappeared, not being noticed again until June 21. In 1908 this rust was first found in the field June 18, and in 1909, June 9. *P. graminis* was first found on winter wheat July 26, 1907, July 3, 1908, and July 5, 1909, while æcidia on barberries were producing spores in 1907 about June 15, in 1908 about June 1, and in 1909 between June 14 and 26. Generally speaking, *P. rubigo-vera tritici* and æcidia on barberries appear at St. Paul, Minn., about the middle of June, and *P. graminis tritici* the first half of July—that is, from two to three weeks after the other two.

Puccinia rubigo-vera is believed not to have any æcidial stage in this country. If this is so and the impossibility of direct infection from the teleutospore is granted, the appearance of this rust in spring must be accounted for by infection from wintering uredo, either as mycelium or spore, or by infection from wind-borne spores from fields farther south. Both methods are possible, and both undoubtedly may be employed. That viable uredospores of this rust have not been found between April 15 and the first part of June in the locality under consideration might furnish some argument that infection from wintering uredos is not possible. Considerable light is thrown upon this question by a study of the difference in length of incubation period of rusts under varying conditions. Under the cool temperatures of early spring the incubation period—that is, the time from inoculation until pustules appear—is lengthened from 7 to 10 days in warm weather to between 3 and 4 weeks and possibly more in cool weather. This lengthened incubation period under cool temperatures has been noticed many times by various investigators.

In 1910, experiments on this point were performed in warm and cool greenhouses at Washington, D. C. A large number of oat plants were inoculated with the uredo of *Puccinia graminis* February 3, 1910. Half of them were placed in a house where the temperatures ranged between 42° and 67° F., reaching 70° F. for an hour or two February 8 and 14, and the other half were placed in a greenhouse where the temperatures ranged between 62° and 90° F. On the plants kept in the cool house pustules began to appear after a period of 18 days, while on the plants kept in the warm house pustules were abundant after 8 days. *Puccinia graminis* on wheat under similar conditions began to show pustules after 16 days on plants kept in the cool house, while pustules were abundant after 6 days on plants kept in the warm house. Could the temperatures in the cool house have been kept consistently lower than those indicated, undoubtedly the incubation period would have been considerably lengthened. Christman (32, p. 106) made similar observations in 1903 at Madison, Wis. He noticed an early outbreak of uredospores of *Puccinia rubigo-vera* on winter wheat and rye between March 20 and April 3, 1903. This

disappeared, and from April 8, a period of about four weeks, it was impossible to find a single spore. On May 6 new leaves began to show a diseased appearance. On May 13 open pustules were found in abundance. He states further that he has found by experiments that in the cooler weather of spring the incubation period following inoculation with uredospores is lengthened to between three and four weeks, and this explains the existence of a period with no rust after the first attack.

The winter leaves die in the early spring and with them the winter mycelium, but not until it has produced uredospores which inoculate the new leaves. Then follows a period of incubation which may be lengthened more or less according to the temperature and other conditions in the spring.

This, then, is one way to account for the spring appearance of *Puccinia rubigo-vera* in the middle Northwest. The other way is the infection of the grains from spores carried in the air from the South. It has been shown in this paper that *P. rubigo-vera* winters in the vegetative uredo stage in Kansas and Nebraska, producing spores on the winter grains in March and April. This is true, also, of a large part of the Atlantic Coast States, particularly Maryland and Virginia. These spores may be carried by the winds farther north during the months of April and May, becoming generally distributed. Inoculation may then be cumulative—i. e., spores may fall on fields from time to time during several weeks in April and May without any apparent effect. Then, when moisture and temperature conditions become just right, a general, though sparing, outbreak may take place over large territories within a few days. After this first outbreak spores will be present in abundance and the attack may spread rapidly.

Puccinia graminis in the Middle Northwest makes its first appearance from two to three weeks after the appearance of æcidia on barberries. From this it may be argued that the first infection always comes from the æcidiospore. Barberries are grown as hedges and ornamental shrubs here and there in the Middle Northwest, and certainly are the cause of more or less local æcidiospore infection, but the appearance of *P. graminis* over large territories within a few days is to be accounted for in other ways. The wintering of the uredo in the North and also wind-blown spores from southern fields in the progressive northward march of this rust are, perhaps, the most important agencies in its first appearance, just as in the case of *P. rubigo-vera*. Another possibility is the transfer of the uredo of *P. graminis* from the wild grasses, especially *Hordeum jubatum*, *Agropyron repens*, and *A. tenerum*. Viable uredos have been found in these grasses as late as April 15, and undoubtedly occur even later in the season. That the *graminis* form on these grasses may affect wheat has been demonstrated; but the new crop of uredospores on

these grasses in the Dakotas and Minnesota generally appears later than the uredo in the cereals, so that the first infection, if it comes from them, must come from wintering uredos.[1]

Careful studies of the wintering of *Puccinia coronata* and *P. graminis* on oats have not been made and a discussion of them is omitted. Undoubtedly the first appearance of these rusts in the spring will also be found to result from wintering uredos and wind-borne spores.

EPIDEMICS.

GENERAL DISCUSSION.

At irregular intervals of several years wheat-rust epidemics, more or less general, occur throughout the country. That these depend to a great extent on climatological conditions is quite generally believed. Periods of excessive rainfall, followed by warm, muggy days, are supposed to be favorable to their development. Why this should be so is not generally understood, and numerous instances where epidemics have not occurred, even after such climatological conditions, might be cited. On the other hand, in some parts of the country, south-central Texas for instance, rust is abundant almost every year in spite of frequent droughts during the maturing period of the grain.

CONDITIONS FAVORABLE FOR AN EPIDEMIC.

At least three conditions must be fulfilled before an epidemic can occur: (1) A sufficient number of rust spores must be present on the growing grain to give the fungus a start; (2) the humidity and temperature conditions must be favorable for the germination of these spores and consequent infection; (3) the grain must be in a receptive condition.

The first condition, very probably, is satisfied almost every year in the main grain-growing regions by the presence of overwintering uredos, wind-blown uredospores, or æcidiospores. However, if such spores are unusually abundant, as they may be after a favorable

[1] A full discussion and consideration of Eriksson's mycoplasm theory published in Compt. Rend., 1897, pp. 475–477, and further treated in Eriksson's later publications, is omitted for lack of space. In this theory Eriksson holds that the rust fungus "lives for a long time a latent symbiotic life as a mycoplasma in the cells of the embryo and of the resulting plant, and that only a short time before the eruption of the pustules, when outer conditions are favorable, it develops into a visible state, assuming the form of a mycelium." External infection is given only secondary importance. This theory has been severely criticized by H. Marshall Ward in "History of Uredo dispersa Erikss., and the 'Mycoplasm hypothesis.'" Philosophic Transactions of the Royal Society, series B, vol. 196, pp. 29–46, and in "Recent Researches on the Parasitism of Fungi," Annals of Botany, vol. 19, 1905, pp. 1–45, and Klebahn (63, pp. 72–76). Eriksson defends his position in Arkiv für Botanik, vol. 3, 1905, pp. 1–54, and in later articles. The subject is still a live one and readers are referred to the various authors cited for full discussions of it. The authors of this bulletin have found no evidence which can be said to substantiate the mycoplasm theory. On the other hand, the wintering over of the rusts, as shown above, can be reasonably explained without the assistance of Eriksson's theory.

winter and spring, the first infection may be heavy and widespread and the chances for an epidemic may be increased in proportion. Thus, the presence and unusual rustiness of barberries in any one district and consequent abundance of æcidiospores in that district are favorable for a local epidemic, and an abundance of uredospores produced on winter grains in mild climates, or wintering in colder climates and then distributed by the wind, may have the same effect over wider areas. That such wintering uredos and wind-blown spores are usually present in sufficient quantities to give the rust a good start is fairly well established. The multiplication and dissemination of these spores may extend over a period of several weeks and may even be facilitated by periods of dry, windy weather under temperature and moisture conditions in which germination will not take place.

Whether or not these spores cause infection after falling on the grain depends upon various conditions. Sudden showers at this time undoubtedly wash off many of the spores before germination occurs, while fairly humid conditions and moderate temperatures are not only favorable but almost absolutely necessary for infection. Cool nights with an abundance of dew and humid, misty days in which the grain remains moist from 12 to 24 hours at a time are exceedingly favorable and are far better than periods of excessive rainfall, due to sudden showers, with periods of hot sunshine between. Contrary to the general belief, moderately cool and even subnormal temperatures are more favorable for spore germination and infection of the grain than higher temperatures. Thus, in the excessive temperatures which often occur in the Middle Northwest in July and August, it is exceedingly difficult to produce rust infection by many of the rusts even though moisture and other conditions are favorable.

Marshall Ward (98, p. 233) has shown that in the case of the brown rust of bromes, *Puccinia dispersa* Erikss., germination of the uredospore will not take place at temperatures much above 26° to 27.5° C. (78.8° to 81.5° F.) or below 10° to 12° C. (50° to 53.6° F.), will not germinate at all at 30° C. (86° F.), and will produce maximum germination at about 20° C. (68° F.). The different species and varieties or biologic forms of rusts vary somewhat in this respect, but moderately cool temperatures are more favorable for germination (and consequent infection) of the uredospore of most of them than excessively high temperatures. Even after infection has taken place excessive temperatures may inhibit to some extent the development of the rust, while moderate temperatures will aid its development.

The presence and germination of rusts being accounted for, it remains to be seen when the grains are in the most receptive condition. In 1908 and 1909 the authors investigated this point for

Puccinia graminis both in Texas and Minnesota. Hundreds of wheat plants in field conditions were inoculated with spores of *P. graminis* by pouring over the head and culm water filled with fresh spores. Plants at all stages of development, from the time when the head was still in the boot to the time when the grains were half filled, were used for the inoculations. It was found that plants inoculated from the time when the heads emerge from the boot until they are in full bloom rusted far more than plants inoculated either before or after this stage of development. Just why the wheat should be very susceptible to a rust attack at this time requires further study. There may be a particular physiological weakness due to the rapid growth and abundant elaboration of starch at this period and the susceptibility of the grain may be increased on that account. Whatever may be the cause, the critical period for wheat with regard to attacks of *P. graminis* is during the heading time, a period of about 10 days for any one locality. If for any reason this period is delayed or lengthened, the number of uredospores falling on each plant is very considerably increased, infections have a longer time in which to develop, and the danger of an epidemic is imminent.

CLIMATOLOGICAL CONDITIONS IN RELATION TO RUSTS IN 1903, 1904, AND 1905.

To determine how closely the conditions favorable for rust epidemics have been approximated in years of severe rust, a study has been made of the climatological conditions over the important wheat States in the Mississippi Valley from the Gulf to Canada for the years 1903, 1904, and 1905. Rusts were fairly abundant in 1903, though not strikingly so. In 1904 an epidemic occurred which was particularly severe over North and South Dakota, Minnesota, and parts of Iowa, while in 1905 the rust, though not epidemical, was present in great abundance, causing considerable damage in certain localities, particularly in North Dakota and South Dakota.

Wheat heads out in April in southern Texas; in May in northern Texas and Oklahoma, Kansas, and Missouri; in June in Nebraska and Iowa; and in July in South Dakota, Minnesota, and parts of Wisconsin. These three months, then, include the critical period for the several States, that is, the period when rust infection develops and an epidemic, if it occurs, gets its initial impulse. The critical period at any one place would normally not extend over 10 days or two weeks.

PRECIPITATION.

Table VI summarizes the precipitation records for several periods in 1903, 1904, and 1905 in the important wheat States mentioned above.

TABLE VI.—*Precipitation records, showing average monthly departure in inches from normal in several States in 1903, 1904, and 1905.*

1903.

Month.	Texas.	Oklahoma.	Kansas.	Missouri.	Nebraska.	Iowa.	South Dakota.	North Dakota.	Minnesota.	Wisconsin.	Average.
October	+0.47										
November	+4.30	+3.59	+0.64	+1.36							
December	− .20	+ .19	+ .22	+ .71	+0.70	+0.85					
January	+1.4	−5.7	−4.2	− .69	− .38	− .74	−0.18	+0.23	−0.22	−0.88	
February	+4.05	+2.57	+ .98	+ .41	+ .72	+ .09	+ .18	− .10	− .15	− .65	
March	+ .53	+ .50	+ .01	.00	− .43	− .53	− .20	− .39	+4.4	+ .56	
April	−1.90	−1.54	+ .23	− .10	− .69	− .03	− .70	− .56	+ .36	+ .35	
May		+1.57	+4.46	+2.10	+3.62	+4.52	+1.10	+ .70	+2.13	+1.43	
June					−1.65	−1.52	−1.04	−2.51	−2.38	−2.46	
July							+1.55	− .50	+1.50	+2.20	
Departure from normal:											
Accumulative	−8.65	+1.18	+2.34	+3.79	+ .89	+2.64	− .61	−3.13	+5.64	+ .55	+0.46
Average monthly	−1.23	+ .16	+ .33	+ .54	+ .12	+ .37	− .08	− .44	+ .80	+ .07	+ .06
Of 3 crop months—											
Accumulative	+2.68	+ .53	+4.70	+2	+1.28	+2.97	+1.61	−2.31	+6.01	+1.17	+2.06
Average monthly	+ .89	+ .17	+1.23	+ .66	+ .42	+ .99	+ .53	− .77	+2	+ .35	+ .68
Of month containing critical period	−1.90	+1.57	+4.46	+2.10	−1.65	−1.52	+1.55	− .50	+1.50	+2.20	+ .78

1904.

October	+0.11										
November	−2.48	−1.99	−0.04	−1.14							
December	− .92	−1.18	− .61	− .77	−0.52	−0.88					
January	−1.41	+ .12	− .30	+ .86	− .12	+ .21	−0.17	−0.14	−0.31	−0.71	
February	− .08	−1.07	−1.03	−1.34	− .53	− .63	+ .04	+ .21	+ .10	− .02	
March	−1.17	−1.08	− .13	+ .89	− .57	+ .35	− .99	+ .38	+ .05	+ .41	
April	+ .20	− .47	+ .91	+1.97	− .51	+ .74	− .75	− .17	− .73	− .80	
May		+ .23	+1.86	+ .22	+ .02	− .35	− .23	− .57	− .75	+ .17	
June					− .74	−1.05	+ .43	+1.74	+ .05	− .70	
July							− .16	− .38	+ .44	− .72	
Departure from normal:											
Accumulative	−5.55	−5.44	+ .66	+ .69	−2.99	−1.61	−1.83	+1.09	−1.25	−2.37	−1.86
Average monthly	− .79	− .77	+ .09	+ .09	− .42	− .23	− .26	+ .15	− .17	− .39	− .26
Of 3 crop months—											
Accumulative	−1.05	−1.32	+2.63	+3.08	−1.25	− .66	+ .04	+ .79	− .26	−1.25	+ .07
Average monthly	− .35	− .44	+ .87	+1.02	− .41	− .22	+ .01	+ .26	− .08	− .41	+ .02
Of month containing critical period	+ .20	+ .23	+1.86	+ .22	− .74	−1.05	− .16	− .38	+ .44	− .72	− .01

1905.

October	+0.44										
November	−1.46	−1.88	−1.01	−2.09							
December	− .39	−1.06	− .33	− .67	−0.39	+0.15					
January	− .67	+ .64	+ .07	− .29	+ .59	+ .06	+0.16	−0.25	−0.09	−0.04	
February	+ .81	+ .01	− .12	− .54	+ .06	+ .53	− .12	− .21	− .18	− .04	
March	+2.28	+1.93	+1.14	− .33	+ .19	+ .20	− .15	− .15	− .29	− .18	
April	+3.44	+1.58	− .33	− .58	+ .93	+ .14	−1.33	−1.39	− .93	−1.11	
May		+1.38	+ .45	+ .03	+2.04	+1.82	+3.37	+1.18	+2.13	+1.64	
June					+1.24	+1	+2.40	+ .68	+2.39	+3.20	
July							+1.28	+1.44	+ .59	− .31	
Departure from normal:											
Accumulative	+4.45	+2.60	− .13	−4.47	+4.66	+3.90	+5.61	+1.30	+3.52	+3.16	+2.46
Average monthly	+ .63	+ .37	− .01	− .63	+ .66	+ .55	+ .80	+ .18	+ .50	+ .45	+ .35
Of 3 crop months—											
Accumulative	+6.53	+4.89	+1.26	− .88	+4.21	+2.96	+7.05	+3.30	+5.11	+4.53	+3.89
Average monthly	+2.17	+1.63	+ .42	− .29	+1.40	+ .98	+2.35	+1.10	+1.70	+1.51	+1.29
Of month containing critical period	+3.44	+1.38	+ .45	+ .03	+1.24	+1	+1.28	+1.44	+ .59	− .31	+1.05

The precipitation records (Table VI) for this region for the seven months preceding harvest and the plotted monthly mean departure from normal (fig. 1) show that the precipitation in all the States except Kansas and Missouri averaged slightly above normal in 1903 (*A*), was below normal in 1904 (*B*), and was again above normal in 1905 (*C*). In considering the three months before and during the

heading of the grain, that is, the main growing period, it is seen that in 1903 the monthly precipitation was above normal in all the States with the exception of North Dakota, averaging 0.68 inch above (*D*); in 1904 the monthly precipitation was above normal in only three States, Kansas, Missouri, and North Dakota, being below normal in all the others, averaging slightly above normal for the district (*E*); and in 1905 the monthly precipitation was above normal in all the States with the exception of Missouri, averaging 1.29 inches above normal (*F*). In considering the month during which the grain in the different States heads out, it is seen that in 1903 the precipitation was below normal in Texas, Nebraska, Iowa, and North Dakota, and above normal in the other States, averaging 0.78 inch above for the district (*G*). In 1904 precipitation was above normal in some States and below normal in others, averaging about normal for the district (*H*). In 1905 precipitation was generally excessive over the whole region, with the exception of Wisconsin, averaging 1.05 inches above for the States collectively (*I*).

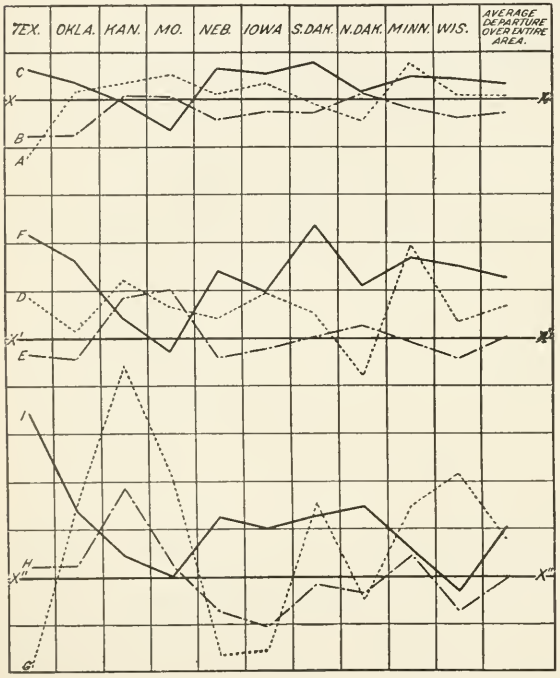


FIG. 1.—Precipitation chart, showing average monthly departure from normal in several States in 1903, 1904, and 1905. *X*, Normal line for a 7-month period preceding the maturity of the grain; *X'*, normal line for a 3-month period preceding the maturity of the grain; *X''*, normal line for 1 month including the heading period; *A*, *D*, and *G*, lines for 1903; *B*, *E*, and *H*, for 1904; and *C*, *F*, and *I*, for 1905. The lines show departure of precipitation from normal during the periods indicated, respectively, the distance between two adjacent horizontal lines representing 1 inch of rainfall.

In considering the precipitation record for the three years, whether for the seven months preceding harvest, for the 3-month growing period in each State, or for the month in which the heading period occurs in each State, it is found that the year 1905 was wetter than either of the other two. If rust depends wholly on the amount of precipitation, whether in a 7-month period, 3-month growing period, or 1-month heading period, the year 1905 should have had more

rust than either 1903 or 1904, while, as a matter of fact, the year 1904 had the most rust.

RELATIVE HUMIDITY.

If the development of rust were in direct proportion to the relative humidity over the whole region, 1905 should have had more than either 1903 or 1904, as the relative humidity during the month containing the critical period averaged about normal in 1903, about $1\frac{2}{3}$ per cent above normal in 1904, and approximately 6 per cent above normal in 1905 (78).[1] Of course a certain degree of humidity is necessary for rust development, but an excess over this degree apparently does not increase its virulence.

TEMPERATURE.

Table VII summarizes the temperature records for the several periods in 1903, 1904, and 1905 in the important States of the wheat-growing region.

TABLE VII.—*Temperature record, showing the average monthly departure (in degrees Fahrenheit) from normal in several States in 1903, 1904, and 1905.*

1903.

Month.	Texas.	Oklahoma.	Kansas.	Missouri.	Nebraska.	Iowa.	South Dakota.	North Dakota.	Minnesota.	Wisconsin.	Average.
October	0										
November	+ 4.9	+ 5.3	+4.8	+ 7.3							
December	− 1.1	− 2.3	−5.00	− 2.4	−4.6	− 3.5					
January	− .2	+ 1.4	+3.1	+ 1.1	+6.1	+ 3.5	+ 0.8	+ 2.7	+ 1.00	+ 1.8	
February	− 2.7	− 2.1	−2.6	0	−4.7	− .2	− 3.8	− 3.5	− 1.00	+ .8	
March	− 1.5	+ .1	+2.1	+ 4.8	+1.6	+ 6.6	+ 2.9	+ 4.5	+ 4.7	+ 8.6	
April	− 1.8	− 1.3	− .3	+ .4	+ .1	+ .3	0	+ 1.3	− .7	+ .3	
May		− 3.2	− .2	0	−1.2	+ 1.4	− .5	+ 3	+ .9	+ 1.5	
June					−5	− 5.6	− 2.2	− 1.6	− 2.6	− 4.1	
July							− 2	− 1.3	− 2.6	− 1.1	
Departure from normal:											
Accumulative	− 2.4	− 2.1	+1.9	+11.2	−7.7	+ 2.5	− 4.8	+ 2.4	− .30	+ 7.8	+0.85
Average monthly	− .34	− .3	+ .27	+ 1.6	−1.10	+ .35	− .68	+ .34	− .04	+ 1.1	+ .12
Of 3 crop months—											
Accumulative	− 6.0	− 4.4	+1.6	+ 5.2	−6.10	− 3.9	− .47	+ .10	− 4.5	− 3.7	−2.21
Average monthly	− 2.0	− 1.46	+ .5	+ 1.7	−2.03	− 1.3	− 1.56	+ .03	− 1.5	− 1.23	− .73
Of month containing critical period	− 1.8	− 3.2	− .2	0	−5	− 5.6	− 2	− 1.13	− 2.6	− 1.10	−2.26

1904.

Month.											
October	− 2.3										
November	− .8	− 1.8	−0.1	− 2.0							
December	+ .1	+ .8	+ .1	− 3.0	+1.3	− 3.9					
January	− 1.1	− .9	− .4	− 2.6	+ .1	− 4.2	− 2.5	− 3	− 4.8	− 6.9	
February	+ 5.4	+ 5.6	+2.6	− .2	+1.7	− 4.8	− 5	− 8.8	− 8.3	− 8.8	
March	+ 5.9	+ 5.9	+4.9	+ 2.5	+4.3	+ 2.4	+ 3	+ 6.9	+ .1	− .8	
April	− 1.1	− 2.4	−4.9	− 6.4	−3.7	− 5.2	− 3.6	− 4	− 4.8	− 6	
May		− 1.6	−2.5	− 1.9	− .6	− .8	0	+ .8	− .6	− .4	
June					−2.8	− 2.5	− 2.4	− 1.7	− 1.9	− 2.4	
July							− 3.7	− 3.6	− 3.6	− 3.4	
Departure from normal:											
Accumulative	+ 6.1	+ 5.6	− .30	−13.6	+ .3	−19	−14.2	−13.4	−23.9	−28.7	−8.67
Average monthly	+ .87	+ .8	− .04	− 1.9	+ .04	− 2.7	− 2.02	− 1.91	− 3.42	− 4.1	−1.23
Of 3 crop months—											
Accumulative	+10.2	+ 1.9	−2.5	− 5.8	−3.77	− 8.5	− 6.10	− 4.50	− 6.10	− 6.20	−3.11
Average monthly	+ 3.4	+ .6	− .8	− 1.9	−1.25	− 2.8	− 2.03	− 1.5	− 2.03	− 2.06	−1.03
Of month containing critical period	− 1.1	− 1.6	−2.5	− 1.9	−2.8	− 2.5	− 3.70	− 3.60	− 3.60	− 3.40	−2.67

[1] Accurate detailed records of relative humidity for this region during the years under consideration are not available, and tabulations are therefore omitted.

TABLE VII.—*Temperature record showing the average monthly departure (in degrees Fahrenheit) from normal in several States in 1903, 1904, and 1905*—Continued.

1905.

Month.	Texas.	Oklahoma.	Kansas.	Missouri.	Nebraska.	Iowa.	South Dakota.	North Dakota.	Minnesota.	Wisconsin.	Average.
October	+ 0.9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
November	+ .3	+ 2.5	+3.8	+ 3.6	.....	.....	.....	.....	.....	.....	.....
December	− .7	− .5	−1.3	+ .3	+0.9	+ 0.5	.....	.....	.....	.....	.....
January	− 3.5	− 7.8	−8.2	− 7.5	−5.7	− 7	− 5.8	− 5.3	− 5.5	− 6.8	.....
February	−10	−10.4	−8.8	− 8.6	−6.8	− 6.8	− 2.9	− 1.4	− 1.9	− 5	.....
March	+ 3.5	+ 5.5	+8.5	+ 7.8	+8.2	+ 9.1	+10.7	+14.5	+ 7.4	+ 4.2	.....
April	− 2	− 2.2	−1.5	− .1	−2.9	− 1.8	− 1.7	− 1	− 1.7	− 1.8	.....
May	.....	+ .2	−1.1	+ .1	−3.4	− 2.1	− 4.4	− 2.8	− 3.5	− 2.9	.....
June	.....	.....	.....	.....	− .5	− .3	− 2.3	− 3.5	− 1.8	− 1.2	.....
July	.....	.....	.....	.....	.....	.....	− 3.5	− 2.7	− 2.3	− 2.1	.....
Departure from normal:											
Accumulative	−11.5	−12.7	−8.6	− 4.4	−9.20	− 8.40	− 9.9	− 2.2	− 9.3	−15.6	−9.18
Average monthly	− 1.64	− 1.81	−1.22	− .62	−1.31	− 1.20	− 1.42	− .31	− 1.32	− 2.22	−1.31
Of 3 crop months—											
Accumulative	− 8.5	+ 3.50	+5.90	+ 7.8	−6.80	− 4.20	−10.2	− 9	− 7.6	− 6.2	−3.53
Average monthly	− 2.83	+ 1.16	+1.96	+ 2.60	−2.26	− 1.40	− 3.4	− 3	− 2.53	− 2.06	−1.17
Of month containing critical period	− 2	+ .20	−1.1	+ .10	− .50	− .30	− 3.5	− 2.7	− 2.3	− 2.1	−1.42

The temperature records (Table VII) for this region for the 7-month period preceding harvest and the plotted monthly mean departure from normal (fig. 2) show that in 1903 the average monthly temperature for the 7-month period varied less than one-half a degree F. from normal in all States except Missouri and Wisconsin, where temperatures were high, and in Nebraska, where temperatures were low, the average for the whole region being 0.12 degree F. above normal (*K*). In 1904 temperatures were subnormal in all States except Texas, Oklahoma, and Nebraska, and strikingly so in Iowa, North Dakota, South Dakota, Minnesota, and Wisconsin, the average for the region being 1.23 degrees below normal (*L*). In 1905 temperatures were again generally subnormal, but not to such an extent over the five last-named States as in 1904, although the general average below normal was slightly greater in 1905 than in 1904 (*M*). In considering the 3-month period before and during the heading of the grain it is seen that in 1903 the average monthly temperatures were subnormal, with the exception of Kansas, Missouri, and North Dakota, averaging 0.73 degree F. below normal (*N*); that these temperatures were more subnormal in 1904 than in 1903, with the exception of Texas and Oklahoma, averaging 1.03 degrees below normal (*O*); that they were again subnormal in 1905, but more irregularly so than in 1904, with a general average slightly greater than that of 1904 (*P*). In considering the month embracing the critical period it is seen that temperatures were subnormal with striking regularity over the entire region in 1904, averaging over 2½ degrees below normal in Nebraska and Iowa, almost 3½ degrees in Wisconsin, and over 3½ degrees in South Dakota, North Dakota, and Minnesota, with a general average of 2.67 degrees below normal (*R*).

The temperatures in 1903 (*Q*) and 1905 (*S*) were also subnormal during the critical period, but with much greater irregularity and not to such an extent as in 1904.

To recapitulate, it is seen that although the general average of temperatures for the whole area for the 7-month and 3-month periods in 1903, 1904, and 1905 were not much different, still in those States where the rust attack was most severe in 1904, namely, North Dakota, South Dakota, Minnesota, Iowa, and Wisconsin, temperatures for the 7-month period averaged generally much lower in 1904 than in 1905, and for the 3-month period averaged about the same as in 1905. During the 1-month period temperatures were consistently subnormal in 1904, averaging 2.67 degrees below normal for the whole region; temperatures were 3½ degrees below normal over South Dakota, North Dakota, Minnesota, and Wisconsin, this average being considerably lower than that of either 1903 or 1905. It is seen, then, that the unusually low temperature over this region was a very important factor, if not the determining factor, for the prevalence of rust in 1904. Low temperatures made the crop as a whole late.

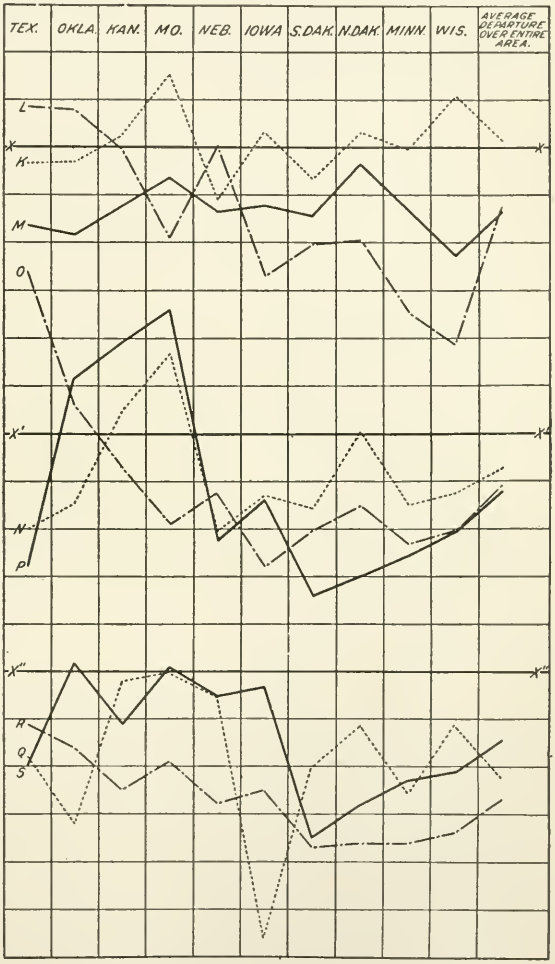


FIG. 2.—Temperature chart, showing average monthly departure from normal in several States in 1903, 1904, and 1905. *X*, Normal line for a 7-month period preceding the maturity of the grain; *X'*, normal line for a 3-month period preceding the maturity of the grain; *X''*, normal line for 1 month including the heading period; *K*, *N*, and *Q*, lines for 1903; *L*, *O*, and *R*, for 1904; and *M*, *P*, and *S*, for 1905. The lines show departure of temperature from normal during the periods indicated, respectively, the distance between two adjacent horizontal lines representing 1 degree F.

Growth was slow and the heading period was delayed and lengthened. The low night temperatures with abundant dews remaining late in the morning were most favorable for spore germination and infection of the growing grain, and a severe rust attack was the result. That rusts were also severe in parts of South Dakota and North Dakota in 1905 was to be expected from the low temperatures that also prevailed during that year in those States throughout the growing and heading period.

PREVENTION OF RUSTS.

In view of the almost universal distribution of rusts, the great variety of rust forms, their complicated life histories and relationships, the ease with which they are distributed, the apparent absence of any weak point in their life history, and the great influence of climatological conditions upon their development, it would seem that there is but little chance to control these fungi or to prevent losses caused by them from year to year. The worker on rusts from an economic standpoint has kept persistently at it, however, and investigations along many lines have been made, a few of which seem to give some promise of success in the future. Three main lines of experimentation have been pursued. These are (1) experiments in spraying, (2) experiments with soil treatments, and (3) experiments in the selection and breeding of varieties resistant to the disease. A comprehensive survey and treatment of these subjects must be reserved for the future, but a few of the more important points will be mentioned.

SPRAYING EXPERIMENTS.

Some of the first spraying experiments for rust prevention in this country were made by Kellerman and Swingle, in Kansas, in 1891 (61, p. 90). Two varieties of spring wheat, Fife and Bluestem, six varieties of barley, and one variety of oats were used in the experiment. The fungicides employed were flowers of sulphur, potassium sulphid, chlorid of iron, and Bordeaux mixture. Spraying was begun when the plants were 2 to 3 inches high and was repeated every eight days, on an average, for 11 successive times. Rains were unusually abundant during the season. Rust appeared plentifully on the sprayed plats and apparently no beneficial results followed the application of the fungicides. Pammel (82, p. 329) made similar experiments with ammoniacal carbonate of copper and Bordeaux mixture. Three applications were made, but were "entirely useless." Galloway (53, p. 198), in 1891–92, performed spraying experiments at Garrett Park, Md. He used a variety of spraying solutions, among which were Bordeaux mixture, ammoniacal copper carbonate, ferrous ferrocyanid, copper borate, ferric chlorid, ferrous

sulphate, cupric ferrocyanid, cupric hydroxid, potassium sulphid, flowers of sulphur, and sulphosteatite powder. Treatments were given when the plants were 2 to 4 inches high in the fall and continued until May 16—seventeen treatments in all.

In June, in spite of all these treatments, "not a leaf could be found that did not show the fungus." The treatments, furthermore, had no appreciable effect on the yield. Under Galloway's direction, Swingle performed similar experiments in Kansas the same year with Bordeaux mixture, ammoniacal copper carbonate, and potassium sulphid. In his experiments "Bordeaux mixture did to a considerable extent prevent rust, but the other preparations had little or no effect on the disease. In no case did the prevention of rust affect the yield to any appreciable extent." At Rockport, Kans., the same year, Bartholomew practically duplicated the Maryland experiments under Galloway's direction. Bordeaux mixture seemed to have a fairly good effect, and Bartholomew concluded that "while no plat was entirely free from rust it is nevertheless a fact that the ravages were reduced to a minimum on the 10-day plats sprayed with Bordeaux mixture and ammoniacal solution of copper carbonate." In summing up all of these experiments, Galloway concluded "that the spraying treatments did, in some cases at least, diminish the amount of rust and similarly increased the yield of straw and grain." Even with the most improved spraying methods known at that time Galloway believed spraying would be impracticable on a large scale. That there was a possibility of making it practicable in the future was conceded. Hitchcock and Carleton further carried on spraying experiments in Kansas in 1893 and 1894 (58, pp. 4–9). They used a large number of spraying solutions. Some of these, particularly potassium bichromate and ferric chlorid, were somewhat effective in preventing rust, but the investigators found it impossible to cover the foliage sufficiently to make them thoroughly efficient. They concluded that "although the rust can be largely decreased, we can not attain prevention as is done in such diseases as the grape mildew. Furthermore, it is extremely doubtful if spraying of wheat or oats would pay, even if effective."

Since these extensive spraying experiments very little work along this line has been done in the United States, although more or less desultory trials have been made. The trouble at all times in spraying for rust has been the impossibility of getting a spraying solution that will cover all parts of the leaves evenly. The more or less waxy bloom which occurs on the leaves of cereals causes the moisture to drop off very easily, and it is almost impossible with any kind of spraying apparatus to wet both surfaces of the leaves equally well. The areas to be covered are so extensive that the expense of spraying

would be very high. It would seem, however, that with modern machinery and the many and varied formulæ for spraying solutions in existence, interesting results might be obtained with further spraying experiments; particularly would this be true in the case of prevention of stem rust of wheat (*Puccinia graminis*), as we now know the critical period for its attack, namely, the heading time of the grains. It would seem possible to limit spraying operations to this period, particularly in years when it falls in a prolonged cold season, thus concentrating the spraying operations. Even under these conditions there is considerable doubt that spraying would ever be of practical value in preventing rust, but the possibilities justify further experiments.

The literature on spraying experiments for the prevention of rusts in foreign countries is extensive and can not be reviewed in this bulletin for want of space. Within the knowledge of the authors, no such experiments have been successful from an economic standpoint, though a few have shown some promise.

SOIL AND SOIL TREATMENTS.

That an excess of some elements in the plant food may predispose a plant more or less to an attacking disease, or that an excess of some other elements may have the opposite effect, rendering the plant more resistant, has not been firmly established. On the contrary, Ward (100, p. 138) has performed experiments to show that nutrition alone does not make for or against predisposition or immunity on the part of the host or virulence or impotence on the part of the parasite. That cereals will absorb sufficient quantities of any element originally in the soil, or which has been applied as fertilizer, to render them resistant to rust attack is thus problematical. If this were possible it would be a difficult matter to explain just how this resistance is obtained, whether from changed physiology, modified morphology of the host, or from some toxic effect against the fungous parasite. We know, for instance, that excess of certain salts in the soil will change not only the morphology but the physiology of cereals. Harter (54, p. 134) has shown that wheat plants grown in soils made saline by the addition of 0.7 to 1.4 per cent of sodium chlorid "modified their structure by depositing bloom on the leaf surface, by thickening the cuticle, and by reducing the size of the epidermal cells." In other words, the plants assumed xerophytic characters. Physiologically, transpiration was decreased in plants on soil sufficiently saline to cause increase in thickness of the cuticle, and was increased in plants in soil containing soluble salts in proportions too small to affect the measurements of the cuticle. Although, as will be discussed later, Ward has shown that the

morphology of grains has little or no effect upon the resistance, physiological effects, such as described, undoubtedly will influence the general resistance or predisposition of plants to disease in some degree, the extent of which has not yet been determined. Experiments in soil treatments for disease prevention have, however, been made from time to time, a few of which will be cited.

In 1891–92 Galloway (53, p. 208) at Garrett Park, Md., treated the soil with various chemicals, among which were flowers of sulphur, air-slaked lime, ferrous sulphate, Bordeaux mixture, potassium sulphid, ammoniacal copper carbonate, and potassium bichromate in various quantities and proportions. No practical results were apparent, and he concluded that "in no case did these chemicals have any appreciable effect on the prevalence of rust." On the other hand, Petermann (83, p. 15) claims that wheat on land fertilized with superphosphate rusted badly, while wheat under similar conditions, but manured with Martin slag (a commercial fertilizer), remained almost rust free. He was inclined to believe that the silicic acid present in the fertilizer was an effective agent in preventing rust.

Further experiments on the effect of fertilizers on crops, both in the United States and in Europe, have been exceedingly numerous in the last few years, but very little careful attention seems to have been given to their effect on cereal diseases. General observations have been made, however, and it is now well established that where there is an excess of nitrogen in the soil, other things being equal, grains are more severely attacked by rust than crops on soil containing less nitrogen (28, p. 659; 60, p. 245; 76, pp. 72, 73; 95, pp. 263–270). Where barnyard manure has been applied heavily the result is similar, and where grains are grown after a crop of clover, beans, or vetch, rusts may be expected. In fact, it may be generally stated that where soils are rich in nitrogen, producing rank and succulent plant growth, rust attacks will, as a rule, be most severe on account of increased succulence of the plants, increased rankness of growth, delay in drying out after showers and dews, and slight delay in the ripening period. On the other hand, phosphate of lime tends to shorten the ripening period and thus acts as a rust preventive to some extent. Careful observations and experiments along this line in the future should give both interesting and valuable results. Care should be taken, however, to differentiate the results in experiments on fertilizers with relation to rust resistance of cereals. In general, a rust attack is most virulent on a healthy plant. This is particularly true of succulent plants in thick stands. As delay in ripening and other effects may also be produced by fertilizers, their relationship to the rust must be carefully kept in mind. The effect of such

results on the rust attack might easily be erroneously attributed to the action of certain chemical constituents of various fertilizers on the rust itself. It seems probable that this is the case in the above-cited results attributed to nitrogen-bearing fertilizers, viz, that the fertilizer produced a very luxuriant growth on which the rust attack would naturally be virulent.

RESISTANT VARIETIES.

CAUSES OF RESISTANCE.

That some plants are far more resistant to the attacks of parasitic fungi than others of the same genus or species has long been noticed, and that this holds true with respect to grains is well established. Some remarkably rust-resistant wheats, such as the durums and the primitive einkorn wherever grown, Extra Squarehead in Sweden, American Club in England, and Rerrarf and Ward's Prolific in Australia, are well known. Some of these varieties, however, can not be said to be universally rust resistant, as one variety may be resistant to one or more species or biologic forms of rust in one country but will not necessarily hold the same balance toward other forms of rust, or in another country (51, p. 36; 39, pp. 340, 341; 44, p. 249; 43, pp. 141–144; 75, p. 27; 30, pp. 59, 60; 28, pp. 661, 662). Thus, for instance, Squarehead is more resistant toward *Puccinia glumarum* in Sweden than toward *Puccinia triticina*, and Rerrarf, while very resistant in Australia, breaks down completely in North Dakota. Numerous instances of this kind might be cited.

It has not yet been established to what character of the plant this elusive and seemingly erratic resistance is due. From a large number of inoculation experiments with the brown rust of bromes and from detailed histological investigations of the hosts, Ward (98, p. 303) found that there is absolutely no relation between differences in the morphology of the brome varieties expressed in length of hairs, number and size of stomata, thickness of epidermis, etc., and rust resistance. He concluded:

> Resistance to infection of the immune or partially immune species and varieties is not to be referred to observable anatomical or structural peculiarities, but to internal, i. e., intraprotoplasmic properties beyond the reach of the microscope and similar in their nature to those which bring about the essential differences between species and varieties themselves.

In the study of resistant and nonresistant wheats the same author (102, pp. 38, 39) showed that rust spores germinate on both susceptible and resistant varieties and gain entrance to them through stomata, but in the resistant varieties further progress is checked by the rapid deterioration and collapse of host cells around the entering fungus, while in the nonresistant varieties the host cells remain turgid

and healthy for a long time, giving abundant nourishment to the parasite. Marryat (70, pp. 129–137) had similar results in working with two wheats, American Club, a resistant wheat, and Michigan Bronze, a highly susceptible variety. She concluded:

> We are forced to fall back upon the theory that immunity to disease is due in these cases to the production of certain toxins and antitoxins by host or parasite, or both, which are mutually destructive.

Salmon (91, p. 88), working on the barley mildew, was similarly led to believe that disease resistance is due to physiological and not structural peculiarities. Bolley (26, pp. 180–182) is not certain whether disease resistance is due to structural or physiological characters, but believes it to be due to the latter, from having been able to develop resistance in every strain of potatoes, flax, or wheat with which he has worked. He further maintains:

> Under uniform conditions of rust infection, all wheats rise rapidly to a stage of marked resistance to general uredospore infection, whether caused by *Puccinia graminis* or *P. rubigo-vera*, which resistance seems to be characteristic for each variety concerned * * * . The facts point quite clearly to the probable influence of chemical agencies, perhaps toxins, arising from the direct existence of fungous attacks upon the hosts. In my mind there is not the slightest doubt but such attacks originate heritable resistance.

Biffen (16, p. 128), after making numerous hybrids between varieties resistant and susceptible with respect to rusts and studying the first and second generations, concluded that "immunity is independent of any morphological character." Orton (81, p. 457), in analyzing the nature of resistance of varieties, similarly concluded that "resistance is due to a specific protective reaction of the host cell against the parasite." To whatever the resistance may be due in the last analysis, it seems to be a peculiar, delicately balanced condition of the host against specific parasites, a balance which is not maintained in the same way toward any two species or varieties and which may be easily upset by change in environment of the host.

SELECTION AND BREEDING OF RESISTANT VARIETIES.

It has long been known that disease resistance is inheritable to a greater or less degree, and on this basis selection of resistant varieties and strains has been going on for some time. Biffen (15, p. 40; 16, pp. 109–128) has recently brought forth experimental results to prove that resistance and susceptibility of cereals to rust are Mendelian characters and are inherited in Mendelian proportions. He collected a large number of wheat and barley varieties of various degrees of resistance to the three rusts, *Puccinia glumarum*, *P. graminis*, and *P. triticina*, common in England, and then made crosses between resistant and susceptible varieties. The hybridizing was done in 1904, and results of growing these in 1905 and 1906 were reported. With

regard to yellow rust, he found that on crossing susceptible and resistant varieties the offspring was susceptible. Upon self-fertilization of these susceptible individuals, resistant and susceptible descendants were produced in the proportion of one of the former to three of the latter, that is, resistance was recessive to susceptibility, the degree of susceptibility being variable. When the degree of susceptibility differed in the two parents the hybrid resembled the more susceptible parent in that respect. More important still, the relatively resistant forms bred true to these characters in the succeeding generations. Bolley (27, pp. 182, 183), from several years' work in the selection and breeding of flax and wheat resistant to wilt and rust, respectively, came to similar conclusions, and in addition believes that unit characters of resistance may be originated even from a very susceptible variety by gradually subjecting the crop to disease from year to year. He maintains that these characters may later be inheritable.

The authors have been engaged in similar work since 1907, but sufficient results have not yet been obtained to pronounce definitely on the question of the application of Mendelian laws to resistance to rust in these experiments. Detailed results of this work are reserved for future publication.

METHODS USED IN SELECTION AND BREEDING.

From the foregoing it will be seen that there are three methods in use for the development of rust-resistant grains through selection and breeding. (1) A careful testing and selection of pure varieties to determine which are already resistant; (2) selection of the best individuals or bulk selection from some strain or variety from year to year under fairly constant disease conditions in the belief that disease resistance is accumulative; (3) hybridizing of desirable varieties with some variety of known resistance and selecting the resistant plants.

The first method is absolutely necessary before the third can be applied, while the second is possible for any worker along this line at any time.

In breeding for resistance to almost any disease, in order to insure rapid progress, the disease must be present every year in sufficient virulence to affect the crop under trial with more or less severity. Certain diseases, particularly rust, occur in epidemical proportions only at irregular intervals. This not only delays results in nonepidemical years but disturbs them in other ways. To overcome these objections, diseases must be promoted yearly on the breeding grounds in every possible way. In order to do this, special breeding plats are employed If one is working for resistance to flax wilt, the breeding plat must be on flax-sick soil; if for drought resistance, on ground particularly

subject to drought; and for rust resistance, on ground where a rust epidemic can be insured. In the case of rust these conditions can be promoted in several ways: (1) By keeping the breeding plats on fairly low ground, where moisture is plentiful but not excessive and where dews remain as long as possible; (2) by planting barberries around or through the plats when breeding for resistance to *Puccinia graminis*, or by planting buckthorns when breeding for resistance to *P. coronata;* (3) by planting winter grains at intervals through the plat where spring grains are being bred (since the rusts, as a rule, occur earlier on the winter than on the spring grains); (4) and most important, by collecting æcidio or uredospores in water and spraying on the plants with hand or knapsack sprayers during the evenings at the period when the grains are most susceptible. All of these methods, or modifications of them, are now in use by Bolley (25, p. 48; 27, pp. 177–182), in North Dakota; by Biffen, in England (16, p. 112), at the Cawnpore Agricultural Experiment Station, in India (55, pp. 54–57); and have been employed since 1907 by the Office of Grain Investigations in cooperation with the Minnesota Agricultural Experiment Station. In Minnesota the authors have established a plat where a very virulent rust attack was obtained, even in the season of 1909, in which no stem rust appeared in any of the fields in its vicinity and in which only local infections were reported throughout the spring-wheat States.

Breeding of this kind is extremely important and should be carried on by agronomists and plant pathologists at every experiment station where conditions are such that rust epidemics may occur at any time. To be effective, it must be extensive and must be persistently employed.

SUMMARY.

(1) Rusts are among the most serious diseases of grains in the United States, causing an estimated annual loss of fifteen to twenty million dollars. In 1904, in the three States, Minnesota, North Dakota, and South Dakota, the loss due to rusts, conservatively estimated, was as high as $10,000,000.

This paper deals only with the rusts of the small-grain crops, wheat, rye, oats, and barley, including *Puccinia graminis, P. rubigo-vera tritici, P. rubigo-vera secalis, P. coronata,* and *P. simplex.*

(2) Practically all these rusts are coextensive with their hosts in the United States, but are not serious in all localities. In general, the areas most affected are the valley of the Mississippi and its tributaries and certain coastal areas. In some years even the drier areas may be affected.

The stem rust of wheat is of great importance in the hard winter and the hard spring wheat belts, is frequent in Washington and Oregon,

is almost always virulent on the coast of California, and is severe and frequent in the southern half of Texas. The epidemic of 1904 was prevalent throughout the entire Mississippi Valley, extended into the wheat fields of the Canadian Northwest, and even invaded the dry lands.

Leaf rust of wheat is also coextensive with the wheat crop and is more common in many districts than stem rust. It occurs yearly over the eastern half of the United States. The losses caused by it are not comparable to those caused by stem rust.

Stem and leaf rusts of oats are coextensive with the oat crop. They usually occur together and are abundant east of the dry belt of the Great Plains region, are paramount in importance in the Southern States, and extend north to the Canadian border and even beyond.

Stem rust of barley is practically coextensive with barley, but is not often present in sufficient quantity to do serious damage.

Leaf rust of barley seems to be of recent introduction. It is economically one of the least important of the grain rusts.

Stem rust of rye is probably widely distributed in small quantities and is fairly common, but causes little injury.

Leaf rust of rye is widely distributed and very abundant, but causes little damage, as the rust is closely confined to the leaves and the rye matures too early to be appreciably damaged.

(3) Botanical characteristics, life histories, and physiological specializations of parasitic fungi may vary with the geographical distribution. European and American forms may be apparently identical morphologically, but are not necessarily identical in their life histories or physiological specialization.

That stem rusts on wheat, rye, oats, and barley, both in Europe and America, may produce their æcidia on barberry has been proved, but that they always do so and can not live for more than one season without passing on to barberry is disproved by experiment.

The æcidial stage of leaf rust of wheat is not known either in Europe or in this country. The uredo stage exists through the winter months, and the rust may live independent of an æcidial stage.

The æcidial stage of the crown rust of oats occurs in Europe on *Rhamnus frangula* L. and *R. cathartica* L. and in the United States on *R. lanceolata* Pursh., *R. caroliniana* Walt., and *R. cathartica*.

The æcidial stage of the leaf rust on rye occurs in Europe on *Anchusa officinalis* L. and *Lycopsis arvensis* L. It is believed that the European and American forms are identical.

The æcidial stage of the leaf rust on barley is not known for Europe or America. This rust seems not to have been previously reported in this country.

Rusts exhibit great variety in regard to complexity of life histories; some are confined to a single host species, some range over two or

more species of one host genus, while others range over two or more genera and often on different tribes of the same family. What appear to be the same forms macroscopically and microscopically are often physiologically different, and may consist of a large number of strains or varieties conveniently called biologic forms. This fact accounts, to a large extent, for the differences in results obtained by American and European investigators working on what are apparently the same species.

In an attempt to break down the barriers between biologic forms the writers have been able to transfer rusts in the uredo stage as follows: Stem rust of wheat (*Puccinia graminis tritici*) from wheat to wheat, rye, and barley, but not to oats; from wheat to barley and then to wheat and rye; and from wheat to barley successively three times and then to oats. Stem rust of barley (*P. graminis hordei*) from barley to barley, oats, rye, and wheat; from barley to wheat and then to barley, wheat, oats, and rye; and from barley to rye, to barley, and then to wheat, oats, and rye. Stem rust of rye (*P. graminis secalis*) from rye to rye and barley; from rye to barley and then to barley, oats, and rye; and from rye successively to barley, to barley, and to rye. Stem rust of oats (*P. graminis avenae*) from oats to oats and barley, but not to wheat or rye. Leaf rust of wheat (*P. rubigo-vera tritici*) from wheat to wheat, rye, and barley. Leaf rust of barley (*P. simplex*) from barley to barley only. Leaf rust of rye (*P. rubigo-vera secalis*) from rye to rye only. Leaf rust of oats (*P. coronata*) from oats to oats and barley, but not to wheat or rye.

There is a measurable difference in size between the uredospores of the stem rust on wheat and the stem rust on barley. In continuous culture experiments of wheat stem rust on barley and barley stem rust on wheat, the uredospore of the wheat stem rust approached the uredospore of the barley stem rust in size and the barley stem rust approached the wheat stem rust in size.

The following points in regard to biologic forms of rusts of cereals may be emphasized: (1) The stem rusts on wheat, barley, rye, and oats are undoubtedly biologic forms of the same species, *Puccinia graminis* Pers.; (2) these forms are not entirely confined to their hosts, but vary in range in part according to the host plants they have been recently inhabiting; (3) the leaf rusts on wheat and rye are more highly specialized than the corresponding stem rusts; (4) the stem rust on barley has ordinarily the widest, while the leaf rusts on barley and rye have the most restricted range; (5) under favorable conditions all the stem rusts can be carried successfully to the four cereals; (6) when rusts are transferred to uncongenial hosts, if pustules are produced they are small and weak; (7) two biologic forms may inhabit the same cereals without being identical; (8) by gradual variation

and adaptation to varying conditions a rust species widely distributed may form a number of strains or types, differing in physiological reactions; (9) the host plants exercise a strong influence not only on the physiological and biological relationships but in some cases even on the morphology of the uredospore.

(4) Rust life histories were very incompletely understood up to 1864–65, when De Bary demonstrated the heterœcism of *Puccinia graminis* Pers., but numerous citations in literature show that barberries in proximity to grainfields had long been believed harmful. From 1865 life-history work on the Uredineæ has made rapid strides and the relationships of many European and American forms of rusts, particularly those of *P. graminis*, have been demonstrated.

Whether or not the æcidium is an essential stage in the life history of rusts has long been questioned. Many authors believe it serves to reinvigorate the fungus, and this view has been strengthened since the recent discoveries of cell fusions and the origin of the binucleated condition in the æcidium of various rust species.

To test this invigoration theory continuous culture experiments from the uredospore of six different grain rusts were undertaken by the writers and 52 successive uredo generations of each rust grown without the intervention of any other spore form. At the end of these experiments cultures were as easily made and the rusts grew as luxuriantly as at the first inoculation. For this length of time, at least, there is no need for a sexual generation.

(5) Whether or not rusts live over winter in the uredo stage has been a mooted question. Investigators in Germany, Denmark, Sweden, England, and the United States have investigated this problem for different rusts with various results. In the United States it has been demonstrated by several investigators that forms of *Puccinia graminis* and *P. rubigo-vera* live over winter in the uredo stage. These results have been reenforced by experiments cited in this bulletin, and the possibility of wintering of the uredo of several rusts in the northern latitudes of the United States has been shown.

(6) Rusts in the uredo or æcidial stages are present in different parts of the country at all times. Like dust particles, which have been proved to be carried hundreds of miles by air currents, these rust spores may be carried from regions where they are plentiful to regions where grain is in a receptive condition.

That large quantities of rust spores are present in the air at various times has been proved by various investigators and by the writers.

(7) A severe rust epidemic was prevalent in the important wheat States of the Mississippi Valley in 1904. In an analysis of the climatological conditions of this region for the years 1903, 1904, and 1905, during the critical or heading period of the grain and during

the 3-month and 7-month periods preceding harvest, it is seen that 1905 had more precipitation than 1903 or 1904; the relative humidity was greater in 1905, but the average temperature, though about the same for the 7-month and 3-month periods during the 3 years, averaged 2.67 degrees subnormal over the whole area in 1904 during the month containing the critical period. It was 3½ degrees below normal in South Dakota, North Dakota, Minnesota, and Wisconsin, the region most affected by rust in 1904. This average was considerably lower than that of the same period in 1903 and 1905 over the same area. It is believed that this unusually low temperature in 1904 was a very important factor, if not the determining factor, for the rust epidemic of that year.

(8) Spraying experiments for the prevention of rusts have been tried from time to time by various investigators, but for the most part without satisfactory results. There is doubt that spraying will ever be of practical value for rust prevention, but as the critical period for wheat, with regard to the attack of stem rust, is now known, further spraying experiments limited to this period may give valuable results.

That excess of some elements in the plant food may predispose a plant to disease or render it more resistant has not been firmly established. That indirectly it will have some influence, by affecting either the physiology or the general growth of the host plant, is very probable.

Where soils are rich in nitrogen, other conditions being equal, rust attacks are, as a rule, most prevalent.

Experiments in soil treatments for disease prevention have been made by various investigators, but no very practical results have been reported. This field of work is promising and should be further investigated.

Some plants are more resistant to attacks of parasitic fungi than others, and it has not yet been definitely established to what character in the plant this resistance is due; but most authorities agree that resistance is due, as a rule, not to morphological but to physiological characteristics.

Disease resistance is inheritable to a greater or less degree, and Biffen has brought forth experimental results to show that resistance and susceptibility of cereals to rust are Mendelian characters. Other investigators have reached similar conclusions.

There are three methods in use for developing rust-resistant grains through selection and breeding: (1) Testing and selection of pure varieties to determine which are resistant; (2) selection of the best individuals, or bulk selection from some strain or variety from year to year under fairly constant disease conditions; (3) hybridizing of

desirable varieties with some variety of known resistance, and selecting the resistant plants.

In breeding for resistance to disease, the disease must be present every year. Rust occurs in epidemical proportions only at irregular intervals, and, therefore, in order to breed for resistance to rust special breeding plats must be employed, where the disease can be produced yearly by conditions particularly favoring its propagation. Wherever efficient breeding of rust-resistant cereals is to be done, such a breeding plat is absolutely necessary.

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U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 217.
B. T. GALLOWAY, *Chief of Bureau.*

ROOT-KNOT AND ITS CONTROL.

BY

ERNST A. BESSEY,
Professor of Botany, Michigan Agricultural College, and Collaborator, Bureau of Plant Industry.

ISSUED NOVEMBER 21, 1911.

WASHINGTON:
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Chief of Bureau, BEVERLY T. GALLOWAY.
Assistant Chief of Bureau, WILLIAM A. TAYLOR.
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COTTON AND TRUCK DISEASE AND SUGAR-PLANT INVESTIGATIONS.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., April 10, 1911.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 217 of the series of this Bureau a manuscript entitled "Root-Knot and Its Control," by Dr. Ernst A. Bessey, professor of botany, Michigan Agricultural College, formerly a plant pathologist in this Bureau and now a collaborator of the Bureau of Plant Industry. This bulletin presents the results and conclusions of studies made by the author while in the service of the Bureau.

Root-knot, which is widespread through the warm temperate and tropical zones of the whole world, is especially prevalent in this country in the South, and, as the bulletin shows, it is present even in the cold parts of the Northern States. It is also a very serious disease of greenhouse plants all over the country. Fortunately, it is almost exclusively confined to the lighter types of soils, causing little or no damage in stiff clays. Dr. Bessey has worked out under field conditions a practical method of holding the pest in check. The means of its control in greenhouses had already been worked out, so that the methods presented here for controlling the pest in greenhouses offer little that is new. The list of plants susceptible to this disease is more complete than any previous list published, containing more than double the names of any other list.

Respectfully,

Wm. A. Taylor,
Acting Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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ROOT-KNOT AND ITS CONTROL.

INTRODUCTION.

The disease of plants known as root-knot, beaded root-knot, root-gall, eelworm disease, big-root, and probably under other names has been present in the United States for many years and has caused losses whose extent can not be calculated. Although more abundant in the South, it is present, at least sporadically, in all but the most Northern or Northwestern States as an out-of-doors pest and is everywhere distributed in greenhouses.

SYMPTOMS OF ROOT-KNOT.

The presence of root-knot becomes noticeable when the affected plants become dwarfed or begin to die, but it is often present and causing a great reduction in the crop yield without the grower's knowledge. Indeed, it is probable that greater actual loss occurs from the form of the disease where, to the untrained eye, no signs are visible than in the case where the plants are actually killed, for a farmer soon learns by experience not to plant in infected regions those crops liable to total destruction, while he fails to notice a reduction in yield, especially if the disease be well established and not a recent introduction, so long as the affected plants do not show too great dwarfing or discoloration.

Aside from the killing or dwarfing of the plants in severe cases or the reduction of yield in less serious infections there are no very noticeable symptoms apparent on those parts of the plant above ground. If rainfall has been rather scanty during the summer, the affected plants first show the lack of sufficient water, while sometimes the wilting is apparent when the sun is hot, even with abundant soil moisture. Occasionally no discoloration is noticeable, but usually plants that are badly affected show a lighter shade of green than unaffected plants. Since, however, the disease usually occupies large areas when it has been long established, there would be no opportunity ordinarily to compare affected with unaffected plants in mass, so that this difference would be readily overlooked.

On the roots, on the contrary, very marked structural changes are apparent. Instead of being smooth and of uniform or slowly

decreasing diameter toward the tip, they show irregular enlargements which involve the whole root if it be small or sometimes only one side of a large root. (Pls. II and III.) These are not superficial swellings only slightly attached to the root, as in the case of the bacterial tubercles of leguminous plants, but are integral parts of the root itself. On small roots these swellings may vary from only slightly greater than the thickness of the root to twice as thick, and spherical to spindle shaped; on larger roots they are usually lateral, or in bad cases may involve all sides, making a gall many times the normal diameter of the root and covered with furrows and seams until the root loses all semblance of its normal appearance. Such compound knots may reach a diameter of 3 or, rarely, even more centimeters and a length many times as great.

HISTORY OF ROOT-KNOT.[1]

Root-knot has been known for many years both in the United States and abroad. It was apparently first mentioned in print by the famous mycologist Rev. M. J. Berkeley,[2] who described and figured roots of plants affected by this disease and recognized the animal nature of the organism causing it. The galls were observed by Greef on grass roots in 1864, but it was not until 1872 that the parasite received a name,[3] *Anguillula radicicola* Greef, after it had been observed several times on a number of different plants. In 1879 Cornu described this species, observed by him on sainfoin in 1874, as *A. marioni*. In 1882 and 1885 the well-known plant pathologist, Prof. A. B. Frank, described it as a serious enemy of a number of cultivated plants in Germany. In 1883 and 1884 C. Müller made a careful study of the organism causing the disease and placed it in the genus Heterodera under the name of *Heterodera radicicola* (Greef) Müller. He showed it to be a close relative of the destructive sugar-beet nematode *Heterodera schachtii* Schmidt, which has caused so much injury to the beet-sugar industry in Europe and which the writer found in 1907 in scattered localities in the United States. Treub in 1885 described as a parasite of sugar cane in Java what he considered to be a new species, naming it *Heterodera javanica*. This is considered now by most authors to be a synonym of *H. radicicola*.

In the United States the root-knot early attracted the attention of greenhouse men as a serious pest of roses, violets, and other plants. J. N. May states [4] that he saw the disease, which he calls "club-root," on violets in 1876. We find the florists' papers full of references to

[1] The full titles of all papers mentioned in this bulletin will be found in the "Bibliography," pp. 76–81. The *a*, *b*, *c* following a date, if given, refer to the first, second, and third papers published if more than one paper in that year is referred to.

[2] Berkeley, 1855.

[3] Greef, 1864 and 1872.

[4] May, 1888.

this trouble in the late eighties and early nineties. The first extensive investigation in this country was undertaken by Dr. J. C. Neal,[1] of the Florida Agricultural Experiment Station, for the Division of Entomology of the United States Department of Agriculture. Owing to lack of access to literature he did not identify it with the pest previously described in Europe, but gave it the name *Anguillula arenaria*. Dr. N. A. Cobb,[2] then of New South Wales, in the absence of specimens from America, provisionally accepted Neal's species as distinct from the European species, renaming the former *Tylenchus arenarius* and the latter *T. radicicola*. He described the injury caused by it in New South Wales, and gave recommendations as to treatment. In 1889 Prof. G. F. Atkinson, then connected with the Alabama Polytechnic Institute, at Auburn, Ala., described the disease, paying special attention to the life history of the parasite, which he correctly identified with the European species. In 1898 Stone and Smith, of the Hatch Agricultural Experiment Station, published the most complete account yet written of the treatment of the trouble in greenhouses, at the same time giving some excellent illustrations of the parasite in various stages of development.

In 1892 Göldi described a nematode parasitic on the roots of coffee in Brazil under the name *Meloidogyne exigua*. This proved subsequently to be identical with *Heterodera radicicola*. Finally, in 1901, Lavergne, evidently misled by an erroneous statement as to the dimensions of *Heterodera radicicola*, described this species from Chile as *Anguillula vialae*.

The foregoing is by no means a complete list of the publications on the subject but embraces the papers that bear on the question of its synonymy and its occurrence in this country.

The synonymy of the causal parasite is, then, as follows:

Heterodera radicicola (Greef) Müller, 1883.
Syn. *Anguillula radicicola* Greef, 1872.
marioni Cornu, 1879.
arenaria Neal, 1889.
vialae Lavergne, 1901.
Heterodera javanica Treub, 1885. (?)
Tylenchus arenarius Cobb, 1890.
radicicola Cobb, 1890.
Meloidogyne exigua Göldi, 1892.

The writer's investigations of the subject were begun in 1900, but were soon interrupted for a period of years. Not until 1905 was the work resumed in earnest and pursued with various interruptions until its completion. The work was done partly at Washington, D. C., but mainly at Miami, Fla., at the Subtropical Laboratory and Garden of the Bureau of Plant Industry, and at Monetta, S. C.,

[1] Neal, 1889. [2] Cobb, 1890.

the majority of the field experiments being made at the last-named place. In addition to this, trips were made to the various parts of the country where the disease occurs or was suspected to occur.

The caring for the experimental plats at Monetta, as well as the making of many of the observations on these experiments, was performed by Mr. J. M. Johnson, without whose services much of the writer's work would have been impossible. At Miami the cooperation of Mr. P. J. Wester, at that time gardener of the Subtropical Laboratory and Garden, was also of considerable assistance, although the experiments there were not on so large a scale as at Monetta.

PLANTS AFFECTED BY ROOT-KNOT.

The nematode causing root-knot seems to be one of the most omnivorous known. Neal, in 1889, reported about 65 species of plants as more or less subject to attacks by this pest. Reports by other investigators in different parts of the world and extensive experiments and observations by the writer have increased this number to 480 species and subspecies. Of this total number the writer has personally observed it on 291. The most complete list hitherto is that of Dr. Kati Marcinowski,[1] who lists 235 species (after subtracting hosts reported under two names). Almost all of the more important families of flowering plants are present in the list, as well as one gymnosperm and a fern. The plants include monocotyledons and dicotyledons, herbs and woody plants, annuals and perennials. Most of the garden plants are affected, as are many field crops.

The list of plants shown in Table I is sure to be largely added to as investigations of this disease are carried on, and is not to be looked on as being in any way final. It is true that the writer has made many hundred examinations of plants in badly infested soil that did not take the disease, but such a list is of far less value than that of plants known to be susceptible. In the list are given (1) the scientific name of the plant;[2] (2) in parenthesis, the name under which it was reported, if different from the name now used; (3) the common English name, if any; (4) the name of the person first reporting it on that host; (5) the date of observation; and (6) the degree of injury. Where the disease is reported on the host for apparently the first time, the name of the first observer is omitted, the observation having been made by the writer. In all cases where the writer has seen the plant

[1] Marcinowski, Kati, 1909.

[2] The nomenclature followed is that used by the systematic botanists of the Bureau of Plant Industry. The list was submitted to the Office of Taxonomic Investigations of that Bureau, where it was revised by Mr. Homer C. Skeels. In a number of cases it would have been impossible, without seeing specimens, to determine to which of several segregates of a species the plant listed might belong, and in that case the original species name was retained, if still valid.

affected, whether previously reported or not, the name in the first column is preceded by an asterisk (*). In the last column the letters indicate the degree of injury only on those plants observed by the writer, the severest injury observed being reported, even though less severe cases have been observed—a=severe injury; b=nematodes abundant, but injury apparently not great; c=nematodes not abundant and no injury observed. It must be understood that under different circumstances many plants marked "a" would show little injury, while plants observed as uninjured and noted as "c" might easily be severely harmed under different conditions. Too much dependence can not, therefore, be laid on this column. In a number of cases the writer has grown in very badly infested fields plants reported by others as susceptible to root-knot, without the slightest signs of infection. Such cases are indicated in the list by a dagger (†). Some of these cases may be of species that are susceptible only under special conditions, while others may be due to erroneous observation on the part of the first observer or perhaps to the confusion of the bacterial root tubercle with the nematode gall. The former surmise may explain why the writer during a three years' residence in a part of Florida where the disease is very abundant failed to find it in any species of Citrus. Dr. H. J. Webber and Prof. P. H. Rolfs, who have studied plant diseases in Florida for many years, confirm this. Yet Dr. J. C. Neal [1] reports it as occurring on lemon, orange, and bitter-sweet orange, while a similar report is made by Lavergne from Chile.[2]

In the list those names added on the authority of Marcinowski [3] are indicated by a double dagger (‡) before the name of the plant.

TABLE I.—*List of plants susceptible to root-knot.*

[An asterisk (*) is used to show those plants which the writer has found affected with root-knot, and a dagger (†) those which he has grown in infested fields without infection, while a double dagger (‡) shows the names of susceptible plants added on the authority of Marcinowski. In the last column a=severe injury; b, nematodes abundant, but injury apparently not great; c, nematodes not abundant and no injury observed.]

Name of plant.	Name of observer.	Date of observation.	Character of injury.
**Abelmoschus esculentus* (L) Moench. Okra	Neal	1889	a
**Abroma augusta* L. f			b
**Abrus precatorius* L. Paternoster bean			c
**Abutilon avicennae* Gaertn. Chinese hemp			b
Abutilon sp	Atkinson	1889	
**Acacia dealbata* Link			b
Acacia, several species from Australia. Wattle.	C. P. Lounsbury [4]	1908	
Achyranthes sp	Neal	1889	
Ageratum conyzoides L	Breda de Haan	1899	
Ageratum sp	Zimmermann	1900–1	
Agropyron repens (L) Beauv. (*Triticum repens*). Quack-grass.	Greef	1872	

[1] Neal, 1889. [2] Lavergne, 1901. [3] Marcinowski, 1909. [4] In letter.

TABLE I.—*List of plants susceptible to root-knot*—Continued.

Name of plant.	Name of observer.	Date of observation.	Character of injury.
Ajuga reptans L.	Trotter	1905–1	.....
Alliaria officinalis Andrz. (*Erysimum alliaria*).	Trotter	1905–1	.....
**Allium ascalonicum* L. Shallot	.....	.....	b
**Allium cepa* L. Onion	.....	.....	b
**Allium fistulosum* L. Welsh onion	.....	.....	b
**Allium porrum* L. Leek	.....	.....	c
**Althaea rosea* (L) Cav. Hollyhock	.....	.....	a
**Amaranthus atropurpureus* Roxb	.....	.....	c
**Amaranthus caudatus* L. Love-lies-bleeding	.....	.....	c
**Amaranthus graeczans* L. (*A. albus*). Tumbleweed.	.....	.....	b
**Amaranthus hybridus* L. Slender pigweed	.....	.....	c
**Amaranthus hybridus forma hypochondriacus* (L.) Rob. Prince's feather.	.....	.....	c
**Amaranthus palmeri* S. Wats	.....	.....	c
Amaranthus retroflexus L	Atkinson	1889	.....
**Amaranthus spinosus* L. Spiny amaranth	Neal	1889	c
**Amaranthus tricolor* L	.....	.....	a
**Ammi copticum* L	.....	.....	c
Amygdalus communis L. (*Prunus communis*). Almond.	Neal	1889	.....
**Amygdalus persica* L. Peach	....do	1889	a
**Ananas sativus* Schult. f. Pineapple	.....	.....	b
Andropogon schoenanthus L	Breda de Haan	1899	.....
Anemone apennina L	Trotter	1905–1	.....
**Anethum graveolens* L. Dill	.....	.....	c
Angelica archangelica L	Licopoli	1877	.....
Angelica sylvestris L	....do	1877	.....
**Angelonia gardneri* Hook	.....	.....	a
**Anthemis cotula* L. Mayweed	.....	.....	b
**Antirrhinum majus* L. Snapdragon	.....	.....	a
**Apium graveolens* L. Celery	Janse	1892	a
†*Arachis hypogaea* L. Peanut	Neal	1889	.....
Arctium sp. Burdock	Selby	1896	.....
**Argyreia nervosa* (Burm.) Bojer	.....	.....	b
Aristolochia clematitis L	Frank	1896	.....
**Arrhenatherum elatius* (L.) Beauv. Tall meadow oat-grass.	.....	.....	c
‡*Artemisia absinthium* L	Cobb	1901	.....
Artemisia caudata Michx	Neal	1889	.....
Asclepias sp. Milkweed	Frank	1896	.....
**Asparagus officinalis* L. Asparagus	.....	.....	b
Aster sp	Sturgis	1893	.....
‡*Astrantia carniolica* Wulf	Dalla Torre	1892	.....
‡*Astrantia major* L	....do	1892	.....
**Atriplex semibaccata* R. Br. Australian saltbush.	.....	.....	c
**Avena sativa* L. Oats	Halsted	1891	c
**Basella rubra* L. Heart-leaved basel	.....	.....	a
Begonia coccinea Hooker. (*B. rubra*)	Selby	1896	.....
Begonia metallica L. Smith	....do	1896	.....
Begonia olbia Kuntze. (*B. olvia*)	....do	1896	.....
Begonia rex Putz	Molliard	1900	.....
**Bellis perennis* L. Daisy	.....	.....	b
**Benincasa cerifera* Savi. Wax gourd	.....	.....	a
Berberis vulgaris L. Barberry	Frank	1885	.....
**Beta vulgaris* L. Beet	....do	1885	a
Bihai pulverulenta (Lindl.) Kuntze. (*Heliconia pulverulenta*).	Ross	1883	.....

TABLE I.—*List of plants susceptible to root-knot*—Continued.

Name of plant.	Name of observer.	Date of observation.	Character of injury.
**Boerhaavia decumbens* Vahl			c
**Boerhaavia erecta* L.			c
Bosea amherstiana (Moq.) Hook. f. (Rodetia).	Trotter	1905–2	
**Boussingaultia basselloides* H. B. K. Madeira vine.	Neal	1889	b
‡*Bouvardia* sp.	Mosseri	1903	
**Brassica campestris* L. Rutabaga	Atkinson	1889	c
**Brassica juncea* (L.) Cass. Chinese mustard			c
**Brassica napus* L. Rape			a
**Brassica nigra* L. Mustard			c
**Brassica oleracea botrytis* L. Cauliflower, broccoli.			b
**Brassica oleracea capitata* L. Cabbage	Neal	1889	b
**Brassica oleracea viridis* L. Kale, collard	do	1889	b
**Brassica pekinensis* (Lour.) Skeels. Chinese cabbage.			b
**Brassica rapa* L. Turnip	Atkinson	1889	c
Buddleia sp.	Neal	1889	
Bursa bursa-pastoris (L.) Britt. (*Capsella bursa-pastoris*). Shepherd's purse.	do	1889	
**Cajan indicum* Spreng. Pigeon pea			b
Cananga odorata (Lam.) Hook. and Thom. Ylang-ylang.	Breda de Haan	1899	
**Canavali ensiforme* (L.) DC. Jack bean			b
**Capriola dactylon* (L.) Kuntze. Bermuda grass.	Mosseri	1903	c
**Capsicum annuum* L. (including *C. cordiforme*). Red pepper.	Neal	1889	a
**Cardiospermum halicacabum* L. Balloon vine			a
**Carica papaya* L. Papaya or melon pawpaw			a
**Carissa bispinosa* (L.) Desf.			c
Carpinus betulus L. Beech	Trotter	1905–1	
**Carthamus tinctorius* L. Safflower			c
**Carum carvi* L. Caraway	Frank	1885	c
Cassia mimosoides L.	G. A. Gammie[1]	1908	
†*Cassia tora* L. (*C. obtusifolia*). Wild senna, coffee bean.	Atkinson	1889	
Castanea sativa Miller (*C. vesca*). Chestnut	Trotter	1905–2	
**Catalpa speciosa* Warder. Catalpa			a
**Cecropia palmata* Willd.			c
**Centaurea cyanus* L. Cornflower			a
Centratherum reticulatum (DC.) Benth	G. A. Gammie[1]	1908	
**Ceratonia siliqua* L. Carob or St.-John's-bread.			c
**Chaetochloa italica* (L.) Scrib. German millet			c
**Chenopodium album* L. Lamb's quarters			c
**Chenopodium anthelminthicum* L. Wormwood.	Atkinson	1889	c
**Chenopodium boscianum* Moq.			c
Chenopodium botrys L. Jerusalem oak	Neal	1889	
**Chenopodium* sp. (Not any of the preceding)			c
Chrysanthemum cinerariaefolium (Trev.) Vis.	Gvozdenović	1902	
‡*Chrysanthemum leucanthemum* L. Oxeye daisy.	Darboux and Houard.	1901	
**Chrysanthemum* sp. Chrysanthemum			b
**Cicer arietinum* L. Chick-pea			b
**Cichorium endivia* L. Endive	Kamerling	1903	a
Cichorium intybus L. Chicory	Licopoli	1877	
Cinchona sp. Peruvian bark	Barber	1901	

[1] In letter.

TABLE I.—*List of plants susceptible to root-knot*—Continued.

Name of plant.	Name of observer.	Date of observation.	Character of injury.
Circaea intermedia Ehrh	Tischler	1902	.....
Circaea lutetiana L. Enchanter's nightshade	.....do	1902	.....
**Citrullus vulgaris* Schrad. Watermelon	Neal	1889	a
Citrus aurantium L. (*C. vulgaris*). Bitter orange.	.....do	1889	.....
Citrus aurantium sinensis L. (*C. aurantium*). Sweet orange.	.....do	1889	.....
Citrus limonum Risso. Lemon	.....do	1889	.....
‡*Clematis florida* Thunb	Chifflot	1900	.....
‡*Clematis hybrida* Hort	.....do	1900	.....
‡*Clematis lanuginosa* Lindl. and Paxt	.....do	1900	.....
**Clematis paniculata* Thunb	.....	.....	a
‡*Clematis patens* Morr. and Decais	Chifflot	1900	.....
Clematis vitalba L	Cornu	1879–2	.....
‡*Clematis viticella* L	Chifflot	1900	.....
Clematis sp	Müller	1884	.....
**Coffea arabica* L. Coffee	Jobert	1878	a
Coffea liberica Hiern. Liberian coffee	Bouquet de la Grye	1899	.....
Coffea robusta Hort. Robusta coffee	Cramer	1906	.....
Coleus blumei Benth. (*C. verschaffelti*). Coleus	Frank	1885	.....
Coleus scutellarioides (L.) Benth. Coleus	Breda de Haan	1905	.....
Coleus sp. (Coleus var. sp.). Coleus	Neal	1889	.....
**Corchorus olitorius* L. Jute	.....	.....	b
**Coriandrum sativum* L. Coriander	.....	.....	c
**Coronopus procumbens* Gilib	.....	.....	c
Corylus avellana L. Filbert	Casali	1898	.....
**Cosmos bipinnatus* Cav. Cosmos	.....	.....	c
Crepis leontodontoides Allioni. Hawk's-beard.	Trotter	1905–1	.....
‡*Crepis pulchra* L	Darboux and Houard.	1901	.....
**Crotalaria juncea* L. Sunn hemp	.....	.....	c
**Croton glandulosus simpsonii* Ferg	.....	.....	c
**Cucumis melo* L. Muskmelon	Neal	1889	a
**Cucumis sativus* L. Cucumber	Berkeley	1855	a
**Cucurbita maxima* Duch. Squash	.....	.....	a
**Cucurbita moschata* Duch. Squash	.....	.....	a
**Cucurbita pepo* L. Pumpkin, squash	.....	.....	a
Cuminum cyminum L. Cumin	Frank	1885	.....
**Cyamopsis tetragonoloba* (L.) Taub. Guar	.....	.....	b
‡*Cyclamen europaeum* L. Cyclamen	Peglion	? 1902	.....
Cyclamen persicum Mill. Cyclamen	Osterwalder	1901	.....
**Cydonia oblonga* Mill. Quince	.....	.....	b
**Cyperus esculentus* L. Chufa	.....	.....	c
**Dactylis glomerata* L. Orchard grass	.....	.....	c
Dahlia pinnata Cav. (*D. variabilis*). Dahlia	Neal	1889	.....
Datisca cannabina L	Trotter	1902	.....
**Daucus carota* L. Carrot	Licopoli	1877	a
Desmodium sp	Barber	1901	.....
**Deutzia crenata* S. and Z. Deutzia	.....	.....	a
**Dianthus barbatus* L. Sweet William	.....	.....	c
**Dianthus caryophyllus* L. Carnation	Trelease ? Lotsy	1894 1892	a .....
**Dianthus chinensis heddewigi* Regel. Pink	.....	.....	b
**Dianthus plumarius* L. Pink	.....	.....	b
‡*Dieffenbachia* sp	Schlechtendal	1886	.....
‡*Dioscorea illustrata* Hort. Yam	Queva	1895	.....
**Diospyros kaki* L. f. Japanese persimmon	.....	.....	a
**Diospyros virginiana* L. Persimmon	.....	.....	c

TABLE I.—*List of plants susceptible to root-knot*—Continued.

Name of plant.	Name of observer.	Date of observation.	Character of injury.
Dipsacus fullonum L. Teasel	Frank	1885	.....
‡*Dipsacus sylvestris* Huds	Hieronymus	1890	.....
Dodartia orientalis L	Greef	1872	.....
**Dolicholus intermedius* (T. and G.) Vail	.....	.....	c
**Dolichos biflorus* L	.....	.....	a
**Dolichos lablab* L. Hyacinth bean or Bonavist bean.	.....	.....	a
**Dolichos umbellatus* Thunb	.....	.....	c
Dracaena rosea Hort. Dragon tree	Frank	1885	.....
**Eclipta alba* (L.) Hask	.....	.....	b
‡*Elcocharis palustris* (L.) R. Br	Lagerheim	1905	.....
**Eleusine coracana* (L.) Gaertn. Ragi millet	.....	.....	c
**Eleusine indica* (L.) Gaertn. Wire-grass	.....	.....	c
**Elichrysum bracteatum* (Vent.) Andr. Immortelle.	.....	.....	b
Elymus arenarius L. Downy lyme-grass	Warming [1]	1877	.....
**Emilia sagittata* (Vahl.) DC. Scarlet tassel flower.	.....	.....	b
**Eruca sativa* Mill. Roquette	.....	.....	c
**Erythrina americana* Mill. Coral tree	.....	.....	b
Erythrina cristagalli L	Licopoli	1877	.....
**Eschscholtzia californica* Cham. California poppy.	.....	.....	a
Eupatorium capillifolium. (Lam.) Small. (*E. foeniculaceum*).	Neal	1889	.....
Euphorbia cyparissias L. Cypress spurge	Licopoli	1877	.....
**Euphorbia nutans* Lag	.....	.....	c
Euphorbia peplis L. Leafy spurge	Trotter	1905–1	.....
**Euphorbia pilulifera* L	.....	.....	c
**Fagopyrum vulgare* Hill. Buckwheat	.....	.....	c
**Festuca elatior* L. Meadow fescue	.....	.....	c
**Festuca ovina* L. Sheep fescue	.....	.....	c
**Ficus aurea* Nutt. Strangling fig. Wild rubber plant.	.....	.....	b
**Ficus carica* L. Fig	Neal	1889	a
**Ficus elastica* Roxb. Rubber plant	.....	.....	b
**Ficus* sp.[2] (from Natal)	.....	.....	a
**Ficus* sp.[2] (from Mexico)	.....	.....	a
Filicinae, genus and species not stated. Fern.	Stone and Smith	1898	.....
**Foeniculum vulgare* Hill. Sweet fennel	.....	.....	b
**Fragaria chiloensis* (L.) Duches. American strawberry.	.....	.....	b
Fragaria vesca L. European strawberry	Trotter	1905–1	.....
‡*Fuchsia* sp. Fuchsia	Mosseri	1903	.....
Galinsoga parviflora Cav	Tarnani	1898	.....
**Gardenia jasminoides* Ellis (*G. florida*). Cape jasmine.	Neal	1889	a
**Gladiolus* sp. Gladiolus	.....	.....	b
**Glycine hispida* (Moench) Maxim. (Soja bean.) Soy bean.	Frank	1882	a
**Gossypium barbadense* L. Sea Island cotton	Neal	1889	b
**Gossypium hirsutum* L. Upland cotton	do	1889	b
**Grabowskia glauca* Hort	.....	.....	b
**Hardenbergia monophylla* (Vent.) Benth. Australian sarsaparilla.	.....	.....	a
**Hedysarum coronarium* L. Sulla	.....	.....	c
Helianthus annuus L. Sunflower	Neal	1889	.....
**Helianthus debilis* Nutt. Sunflower	.....	.....	c
**Helianthus tuberosus* L. Jerusalem artichoke	.....	.....	b

[1] According to Ritzema Bos (1900–1) this injury is due to another nematode, *Tylenchus hordei*.
[2] Species distinct from the preceding.

TABLE I.—*List of plants susceptible to root-knot*—Continued.

Name of plant.	Name of observer.	Date of observation.	Character of injury.
Heliotropium sp. Heliotrope	Stone and Smith	1898	.....
**Heteropteris* sp.	.....	.....	b
Hibiscus coccineus Walt. Rose mallow	Neal	1889	.....
**Hibiscus rosa-sinensis* L. Hibiscus	.....	.....	b
**Hibiscus sabdariffa* L. Roselle	.....	.....	a
**Hibiscus syriacus* L. Rose of Sharon	Neal	1889	b
**Hicoria pecan* (Marsh) Britt. Pecan	....do	1889	a
Hordeum sativum. Barley	Trotter	1905–1	.....
Hypericum perforatum L. St.-John's-wort	....do	1905–1	.....
Hyssopus sp. Hyssop	Frank	1896	.....
Iberis umbellata L. Candytuft	Neal	1889	.....
**Ilysanthes dubia* (L.) Barnh.	.....	.....	c
Impatiens balsamina L. (*Balsamina hortensis*). Balsam.	Frank	1885	.....
Impatiens kleinii Wight and Arn.	G. A. Gammie [1]	1908	.....
**Ipomoea batatas* (L.) Poir. Sweet potato	.....	.....	b
Ipomoea bona-nox L. Moonflower	Stone and Smith	1898	.....
**Ipomoea cathartica* Poir. Wild morning-glory	.....	.....	c
**Ipomoea fuchsioides* Griseb. Fuchsia-flowered morning-glory.	.....	.....	a
Ipomoea lacunosa L.	Atkinson	1889	.....
**Ipomoea purpurea* L. Roth. Morning-glory	Neal	1889	a
**Ipomoea quamoclit* L. Cypress vine	....do	1889	a
**Ipomoea setosa* Ker.	.....	.....	b
**Ipomoea syringaefolia* Meissn. Tree morning-glory.	.....	.....	b
**Ipomoea* sp.[2] Indian potato	.....	.....	c
**Iresine paniculata* (L.) Kuntze	.....	.....	b
Iris sp. Iris	Brick	1905	.....
Ixora aurea Hort.	Cornu	1879–1	.....
Ixora chinensis Lam. (*Ixora flammea*)	....do	1879–1	.....
Ixora crocea Hort.	....do	1879–1	.....
‡*Ixora fraseri* Hort.	Darboux and Houard.	1901	.....
Ixora sp.[2]	Cornu	1879–1	.....
Jacquemontia tamnifolia (L.) Griseb. (*Ipomoea tamnifolia.*)	Atkinson	1889	.....
Juglans cinerea L. Butternut	Neal	1889	.....
**Juglans regia* L. Persian (English) walnut	....do	1889	b
**Juglans rupestris* Engelm. Arizona walnut	.....	.....	a
‡*Juncus gerardi* Loisel.	Lagerheim	1905	.....
Kadsura sp. (*Cadsura*)	Breda de Haan	1899	.....
**Konig maritima* (L.) R. Br. Sweet alyssum	.....	.....	c
**Kraunhia sinensis* (Sims) Greene. Wistaria	.....	.....	c
**Lactuca sativa* L. Lettuce	Frank	1882	a
**Lagenaria vulgaris* Ser. Gourd	Neal	1889	a
**Lamium amplexicaule* L. Dead nettle	.....	.....	b
Lantana horrida H. B. K. Lantana	J. J. Thornber [1]	1907	.....
**Lathyrus cicera* L. Lesser chick-pea	.....	.....	b
**Lathyrus odoratus* L. Sweet pea	.....	.....	a
**Lathyrus sativus* L. Bitter vetch	.....	.....	a
**Lathyrus tingitanus* L. Tangier pea	.....	.....	a
**Lens esculenta* Moench. Lentil	.....	.....	a
Leontodon hastilis L. Hawkbit	Frank	1885	.....
‡*Lepidium sativum* L. Garden peppergrass	Voigt	1890	.....
**Lespedeza bicolor* Turcz. Bush clover	.....	.....	c
†*Lespedeza striata* (Thunb.) Hook. Japan clover.	Atkinson	1889	.....

[1] In letter.

[2] Species distinct from the preceding.

TABLE I.—*List of plants susceptible to root-knot*—Continued.

Name of plant.	Name of observer.	Date of observation.	Character of injury.
**Ligustrum ovalifolium* Hassk. California privet.	.....	.....	c
**Linaria canadensis* (L.) Dumont. Toadflax	.....	.....	c
Linum angustifolium Huds	Trotter	1905–1	.....
**Linum usitatissimum* L. Flax	Sorauer	1906	a
**Lippia nodiflora* (L.) Michx. Frog-fruit	.....	.....	a
**Lobelia crinus* L	.....	.....	a
**Loniccra japonica* Thunb. Japanese honeysuckle.	.....	.....	c
**Lotus corniculatus* L. Bird's-foot trefoil	Atkinson	1889	b
Lotus sp	Trotter	1905–2	.....
**Leucaena glauca* (L.) Benth	.....	.....	c
**Lucuma rivicoa angustifolia* Miq. Ty-ess	.....	.....	c
**Luffa cylindrica* (L.) Roem. Sponge gourd	.....	.....	a
**Lupinus albus* L. White lupine	.....	.....	b
**Lupinus angustifolius* L	.....	.....	c
**Lupinus luteus* L. Yellow lupine	.....	.....	a
**Lupinus termis* Forsk	.....	.....	b
**Lycopersicon esculentum* Mill. Tomato	Neal	1889	a
Malus sylvestris Mill. (*Pyrus malus*). Apple	Selby	1896	.....
**Malva rotundifolia borealis* (Wallm.) Masters. Wild mallow.	.....	.....	c
**Manihot utilissima* Pohl. Cassava	Neal	1889	c
**Marrubium vulgare* L. Horehound	Atkinson	1889	a
**Medicago sativa* L. Alfalfa, or lucern	Frank	1882	b
†*Meibomia mollis* (Vahl) Kuntze. Florida beggarweed.	Rolfs	1898	.....
**Meibomia stricta* (Pursh) Kuntze	.....	.....	c
**Melia azedarach* L. Umbrella tree	.....	.....	c
**Melilotus alba* Desr. White sweet clover, or Bokhara clover.	Atkinson	1889	b
**Melilotus indica* (L.) All	.....	.....	c
**Melothria crassifolia* Small	.....	.....	a
Mesembryanthemum sp. Fig marigold	Neal	1889	.....
Modiola caroliniana (L.) Don. (*M. multifida*)	Atkinson	1889	.....
Mollugo pentaphylla L. (*M. stricta*)	G. A. Gammie[1]	1908	.....
**Mollugo verticillata* L. Carpet weed	.....	.....	c
**Momordica charantia* L. Balsam apple	.....	.....	a
**Morus alba multicaulis* (Perr.) Loud. Mulberry.	.....	.....	b
**Morus alba tatarica* (L.) Loud. Mulberry	.....	.....	b
**Morus nigra* L. Mulberry	.....	.....	b
**Morus rubra* L. Mulberry	.....	.....	b
Mulgedium macrophyllum (Willd.) DC	Müller	1884	.....
Musa cavendishii Lamb. (*Musa chinensis*). Dwarf banana.	Ross	1883	.....
**Musa cavete* Gmel. Bruce's banana	.....	.....	a
Musa paradisiaca dacca (Horan) Baker (*M. dacca*). Dacca banana.	Ross	1883	.....
Musa paradisiaca sapientum (L.) Kuntze. Banana.	Delacroix	1904	.....
Musa rosacea Jacq	Müller	1884	.....
**Musa textilis* Nec. Manila hemp	.....	.....	b
**Nicotiana sanderae* Hort	.....	.....	a
**Nicotiana tabacum* L. Tobacco	Janse	1892–2	a
Nolana sp	Neal	1889–1	.....
**Ocimum basilicum* L. Basil	Breda de Haan	1899	b
Oldenlandia sp	G. A. Gammie[1]	1908	.....
Onobrychis viciaefolia Scop. Sainfoin	Cornu	1879–2	.....

[1] In letter.

TABLE I.—*List of plants susceptible to root-knot*—Continued.

Name of plant.	Name of observer.	Date of observation.	Character of injury.
**Ornithopus sativus* Brot. Seradella			c
**Oxalis corniculata* L. Sheep sorrel			c
Oxalis stricta L.	Tarnani	1898	
**Paconia* sp. Peony			a
**Paliurus spina-Christi* Mill. Christ's-thorn			b
**Panax quinquefolium* L. Ginseng	Van Hook	1904	a
Papaver rhocas L. Poppy	Tarnani	1898	
Papyrius papyrifera (L.) Kuntze (*Broussonettia papyrifera*). Paper mulberry.	Neal	1889	
**Passiflora incarnata* L. Passion flower			a
**Passiflora pfordti* (=×*P. alato-cærulea* Lindl.)			a
Passiflora sp	Magnus	1888	
**Pastinaca sativa* L. Parsnip	Atkinson	1889	c
**Pelargonium zonale* (L.) Ait. Geranium			b
**Pentagonia physalodes* (L.) Hiern			b
**Perilla frutescens* (L.) Britt. Perilla			b
†*Persea gratissima* Gaertn. f. Avocado	Lavergne	1901	
**Petroselinum sativum* Hoffm. Parsley			c
**Petunia hybrida* Vilm. Petunia			a
**Phaseolus aconitifolius* Jacq. Aconite-leaved bean.			b
**Phascolus angularis* (Willd.) Wight. Adsuki bean.			a
**Phaseolus calcaratus* Roxb. Seeta bean			a
**Phascolus lunatus* L. Lima bean	Neal	1889	b
**Phaseolus max* L. Green gram, or mung bean			b
**Phaseolus radiatus* L. Green gram			b
**Phascolus retusus* Moench. Metcalfe bean			a
**Phaseolus vulgaris* L. (incl. *P. nanus*). Bean.	Neal	1889	b
Physalis peruviana L. Cape gooseberry	C. P. Lounsbury [1]	1908	
Physalis sp	Atkinson	1889	
**Phytolacca americana* L. (*P. decandra*). Pokeweed.	do	1889	b
**Pilea serpyllifolia* (Poir) Wedd. Artillery plant.			c
Piper betle L. Betel pepper	Zimmermann	1900–2	
Piper nigrum L. Pepper	Delacroix	1904	
**Piriqueta tomentosa* (Willd.) H. B. K.			b
**Pisum arvense* L. Field pea			c
**Pisum sativum* L. Garden pea	Neal	1889	b
**Pithecolobium saman* (Jacq.) Benth. Rain tree.			a
Plantago lanceolata L. Rib-grass	Licopoli	1877	
Plantago major L. Plantain	Frank	1885	
**Plantago* sp [2]			c
Platanus sp. Plane tree	Gándara	1906	
Plectranthus sp	Frank	1885	
**Pluchea purpurascens* (Swartz) DC.			a
**Plumbago capensis* Thunb. Cape leadwort			b
Poa annua L. Annual bluegrass	Greef	1872	
‡*Poa pratensis* L. Kentucky bluegrass	Henning	1898	
Podranea ricasoliana (Tanf.) Sprague (*Tecoma mackennii*).	C. P. Lounsbury [1]	1908	
**Polianthes tuberosa* L. Tuberose			a
Polygala oleifera Hort.	Breda de Haan	1899	
**Polygonum hydropiperoides* Mich.			c
Polygonum sp.	Tarnani	1898	
**Portulaca grandiflora* Hook. Portulaca			b

[1] In letter.

[2] Species distinct from the preceding.

TABLE I.—*List of plants susceptible to root-knot*—Continued.

Name of plant.	Name of observer.	Date of observation.	Character of injury.
**Portulaca oleracea* L. Purslane	Neal	1889	b
‡*Primula auricula* L. Primrose	Dalla Torre	1892	.....
‡*Primula carniolica* Jacq. Primrose	do	1892	.....
Prunus armeniaca L. Apricot	Neal	1889	.....
Prunus cerasifera Ehrh. (*P. myrobalanus*)	do	1889	.....
Prunus domestica L. Plum	do	1889	.....
Prunus japonica Thunb. (*P. nana* and *P. lanceolata*).	do	1889	.....
**Prunus virginiana* L. Choke cherry			c
**Prunus* sp.[1] (from Mexico). Cherry			a
**Psidium guajava* L. Guava			b
**Punica granatum* L. Pomegranate			a
Pyrus communis L. Pear	Frank	1882	.....
Quercus suber. Cork oak	Ducomet	1908	.....
**Radicula armoracia* (L.) Robinson. Horseradish.			b
**Radicula walteri* (Ell.) Greene			c
**Raphanus sativus* L. Radish	Neal	1889	b
**Reseda odorata* L. Mignonette			b
‡*Rhinanthus cristagalli* L. Rattlebox	Darboux and Houard.	1901	.....
‡*Ribes rubrum* L. Currant	Cobb	1901	.....
**Rosa chinensis manetti* Dippel. Manetti rose			b
**Rosa laevigata* Michx. Cherokee rose			b
**Rosa setigera* Michx. Rose			b
Rosa sp. Rose	Halsted	1891	.....
Rubus idaeus L. Raspberry	Selby	1896	.....
Rubus subuniflorus Rydb. (*R. villosus*). Blackberry.	Neal	1889	.....
Rubus trivialis Mich	do	1889	.....
**Rumex acetosa* L. Sorrel			b
**Rumex* sp.[1] Dock			b
**Saccharum officinarum* L. Sugar cane	Breda de Haan	1899	b
Salix babylonica L. Weeping willow	Neal	1899	.....
Salvia sp. Sage	Frank	1896	.....
‡*Sanicula europaea* L. Wood sanicle	Cornu	1879–2	.....
Scabiosa columbaria L	Sorauer	1906	.....
Schizonotus sorbifolius (L.) Lindl. (*Spiraea sorbifolia*).	Neal	1889	.....
**Scolymus hispanicus* L. Spanish oyster plant			b
**Scorzonera hispanica* L. Black salsify			a
Sedum (several species)	Greef	1872	.....
Sempervivum glaucum Ten	Licopoli	1877	.....
**Sempervivum tectorum* L	do	1875	c
Senecio vulgaris L	Trotter	1905–1	.....
**Sesban bispinosa* (Jacq.) Steud			a
**Sesban macrocarpa* Muhl			b
Sesuvium maritimum (Walt.) B. S. P. (*S. pentandrum*).	Neal	1889	.....
**Sesuvium portulacastrum* L			c
**Sida rhombifolia* L			b
**Sida spinosa* L	Atkinson	1889	b
**Smilax glauca* Walt			c
**Solanum carolinense* L. Horse nettle			c
Solanum dulcamara L. Bittersweet	Mosseri	1903	.....
**Solanum melongena* L. Eggplant	Atkinson	1889	a
**Solanum nigrum* L. Nightshade			c
**Solanum rostratum* Dun. Buffalo bur			b
**Solanum tuberosum* L. Potato	Neal	1889	a

[1] Species distinct from the preceding.

TABLE I.—*List of plants susceptible to root-knot*—Continued.

Name of plant.	Name of observer.	Date of observation.	Character of injury.
**Solanum* sp.[1]			b
Sonchus arvensis L. Sow thistle	Tarnani	1898	
Sonchus oleraceus L.	Frank	1885	
**Spergula arvensis* L. Spurry			c
Spermadictyon suaveolens Roxb. (*Hamiltonia spectabilis*).	Cornu	1879–1	
**Spinacia oleracea* L. Spinach			b
**Spiraea cantoniensis* Lour. Spiræa			b
**Spondias lutea* L. Hog plum			a
‡*Stephanotis* sp.	Voigt	1890	
**Stizolobium pachylobium*. Piper and Tracy			b
†*Stizolobium pruriens* (L.) Medic	Piper and Cobb[2]	1910	b
†*Stizolobium deeringianum* Bort (*Mucuna utilis*). Velvet bean.	Rolfs	1898	
Strelitzia nicolai Reg. and Koern. Bird-of-paradise flower.	Ross	1883	
**Syncarpia glomulifera* (Sm.) Niedenz			c
**Tamarindus indica* L. Tamarind			c
**Tanacetum vulgare* L. Tansy			b
Taraxacum officinale Weber. Dandelion	Licopoli	1877	
**Tetrapanax papyrifer* (Hook.) Koch. Japanese paper plant.			a
Thea sinensis L. Tea	Barber	1901	
‡*Theobroma cacao* L. Chocolate or cacao	Ritzema Bos	1900	
Theophrasta crassipes Lindl.	Cornu	1879–1	
**Thunbergia fragrans* Roxb.			a
**Tragopogon porrifolius* L. Salsify	Atkinson	1889	a
**Trichosanthes cucumeroides* (Ser.) Maxim.			a
**Trifolium alexandrinum* L. Egyptian clover, Berseem.			c
**Trifolium incarnatum* L. Crimson clover	Frank	1885	a
**Trifolium pratense* L. Red clover	do	1885	a
**Trifolium repens* L. White clover	Sheldon	1905	a
**Trigonella foenum-graecum* L. Fenugreek			b
Triticum aestivum L. (*T. sativum*). Wheat	Sorauer	1906	
Triumfetta rhomboidea Jacq.	G. A. Gammie[2]	1908	
**Tropaeolum majus* L. Nasturtium			c
**Tropaeolum minus* L. Dwarf nasturtium			c
**Ulmus campestris* L. European elm			a
**Verbascum thapsus* L. Mullein			c
Verbesina occidentalis (L.) Walt. Crownbeard	Neal	1889	
**Verbesina virginica* L. (*V. sinuata*). Crownbeard.	do	1889	c
**Veronica peregrina* L. Speedwell			c
**Veronica tournefortii* Gmelin			c
‡*Viburnum lantana* L. Wayfaring tree	Frank	1896	
‡*Viburnum tinus* L. Laurestine	Kieffer	1901	
**Vicia atropurpurea* Desf.			c
**Vicia faba* L. Horse bean			b
**Vicia fulgens* Battand. Scarlet vetch			c
**Vicia hirsuta* (L.) S. F. Gray			b
**Vicia monanthos* (L.) Desf.			a
**Vicia narbonensis* L. Narbonne vetch			b
**Vicia pseudocracca* Bertol.			c
**Vicia sativa* L. Vetch			b
**Vicia villosa* Roth. Hairy vetch			b
**Vigna repens* Baker			b

[1] Species distinct from the preceding.

[2] In letter.

TABLE I.—*List of plants susceptible to root-knot*—Continued.

Name of plant.	Name of observer.	Date of observation.	Character of injury.
**Vigna unguiculata* (L.) Walp. (*Vigna catjang, Dolichos catjang*). Cowpea.	Neal	1889	a
**Viola odorata* L. Violet	Halsted	1891	a
Vitis aestivalis Michx. Grape	Neal	1889	.....
Vitis labrusca L. Grape	Licopoli	1877	.....
Vitis serianaefolia (Bunge) Maxim. (*Cissus aconitifolia*).	Cornu	1879–2	.....
**Vitis vinifera* L. Old World grape	Neal	1899	a
**Washingtonia filifera microsperma* [1] Beccari. California fan palm.	.....	.....	b
**Washingtonia gracilis* [1] Parish	.....	.....	b
Willughbaea scandens (L.) Kuntze. (*Mikania scandens*).	Neal	1899	.....
**Zamia floridana* DC	.....	.....	b
†*Zea mays* L. Maize or Indian corn	Neal	1889	.....

[1] Seed received under this name from Dr. O. Beccari.

PLANTS NOT AFFECTED BY ROOT-KNOT.

Among the plants grown by the writer in infected land without their becoming infected with root-knot in the slightest degree were several species of Stizolobium, the genus to which the velvet bean belongs, viz, *Stizolobium lyoni*, *S. pruriens*, *S. hirsutum*, and the velvet bean and one or more other unidentified species of this genus.[1] Many of the grasses seem to be resistant. Thus the writer has failed to find the nematode on crab-grass (*Syntherisma sanguinalis*), redtop (*Agrostis alba*), Johnson grass (*Andropogon halepensis*), some varieties of oats (*Avena sativa*)—but some are susceptible—*Bromus schraderi*, *Eustachys petraea*, some varieties of barley (*Hordeum vulgare*), *Lolium perenne*, Japanese barnyard millet (*Echinochloa frumentacea*), broomcorn millet, or proso (*Panicum miliaceum*), pearl millet (*Pennisetum* sp.), timothy (*Phleum pratense*), rye (*Secale cereale*), the various forms of sorghums, milos, Kafir corn, etc. (*Andropogon sorghum*), wheat (*Triticum aestivum*), but see list of susceptible plants. The same is true of corn (maize, *Zea mays*) as of wheat. *Euchlaena luxurians* was also free. Several Compositæ seem to be free from the trouble even where the nematodes are very abundant in the soil. Thus, *Bidens leucantha* and *B. bipinnata* always were found free. *Gnaphalium purpureum*, *Helenium tenuifolium*, species of Solidago, Zinnia, etc., were also free. The absence of nematodes in the plants above enumerated is far less significant than their presence in other plants, for conditions may have been unfavorable, and yet under other con-

[1] Rolfs, however, 1898, reports root-knot on the velvet bean, and recently Prof. C. V. Piper has found it in abundance on plants of *Stizolobium pruriens*, S. P. I. 21566, grown in a greenhouse in Washington, D. C. Evidently under certain conditions some strains may be susceptible, but as a rule it is immune.

ditions they might have shown root-knot. However, it is probable that the above-named plants will show themselves nematode resistant in most cases.

CROSS-INOCULATION EXPERIMENTS.

It has been suggested by several investigators that *Heterodera radicicola*, like *Tylenchus dipsaci*, may show the development of strains preferring certain hosts and exhibiting a reluctance to attack others, although these different strains are morphologically indistinguishable.[1] This explanation has been suggested for the fact recorded by Stone and Smith [2] that lettuce often is not attacked in beds in greenhouses where other crops suffer great injury. The writer accordingly made a number of cross-inoculation experiments to determine, if possible, to what extent the nematodes of certain generally grown crops were interchangeable. The experiments were performed as follows: Pots of soil were sterilized in an autoclave for about an hour and a half, sometimes longer, at a temperature of 125° C. While this was perhaps not long enough to kill all bacterial spores in the center of the pots, the temperature attained showed itself to have been high enough to kill all nematode larvæ or eggs. In the sterilized soil were placed affected roots of the plant used as a source of the nematodes. These roots were first carefully washed (sometimes in water containing a small amount of formaldehyde) to remove all adhering dirt in which conceivably larvæ or eggs of other strains of nematodes might be present. These pots were planted with seeds of plants to be tested as possible hosts of the nematode, either at the same time or a few days after the roots were put into the pots. Except when it was certain that the water was nematode free, it was boiled and cooled before using it to water the pots. Experiments made in this manner showed that the root-knot nematodes were mutually interchangeable in the following plants: Red clover (*Trifolium pratense;* Pl. III, fig. 2), white clover (*T. repens*), crimson clover (*T. incarnatum*), cowpea (*Vigna unguiculata*), strawberry (*Fragaria chiloensis*), tree morning-glory (*Ipomoea syringaefolia*), sunflower (*Helianthus debilis*), horse bean (*Vicia faba*), ginseng (*Panax quinquefolium*), purslane (*Portulaca oleracea*), fig (*Ficus carica*), papaya (*Carica papaya*), catalpa (*Catalpa speciosa*), tomato (*Lycopersicon esculentum*), and Old World grape (*Vitis vinifera*). These all also affect the following, for which the reverse inoculation experiments were not made: Lettuce (*Lactuca sativa*), green gram (*Phaseolus radiatus*), tobacco (*Nicotiana tabacum*), squash (*Cucurbita moschata*), cucumber (*Cucumis sativus*), and muskmelon (*C. melo*).

[1] Prof. J. Ritzema Bos (1900) reports that *Tylenchus dipsaci* becomes so adapted to a host plant after growing on that species only for several generations that it will not attack with any severity the species upon which it grew before until several generations have passed.

[2] Stone and Smith, 1898, p. 30.

The various families of plants represented in the foregoing list and the fact that the infections were obtained easily and very pronouncedly would seem to indicate that the nematode causing root-knot of the plants experimented with, including some of those most generally affected in the field, is not as yet very markedly differentiated into strains peculiar to certain hosts. It is still possible, and indeed quite likely, that had seeds of the same host as that furnishing the roots from which the nematodes came been sown in the pot along with the other seeds the latter would have shown less infection than the other plants. Unfortunately, however, various circumstances prevented this line of experiments from being carried out.

Observations in the field seem to bear out the results of the pot experiments. The writer has been unable to detect any special adaptation to any one species of plant. Indeed, peaches were attacked very badly when planted where cowpeas had been grown for several years. Figs and the Old World grape are the plants through which the parasite has been introduced into many new districts, which could hardly have been done so thoroughly and rapidly if the nematode had become in a manner specialized upon them.

DISTRIBUTION OF ROOT-KNOT.

Root-knot was first observed by Berkeley [1] on greenhouse plants in England It was next reported by Greef [2] on out-of-doors plants in Germany. Since then it has been observed in many parts of Germany, France, Italy, Austria, Holland, Sweden, and Russia. In Africa it is abundant in parts of Algeria, occurring even in some of the Saharan oases, Egypt, German East Africa, Transvaal, Cape Colony, and Madagascar; in Asia it occurs widespread in India, Ceylon, and to some extent in China and Japan. In the East Indies, Java and Sumatra are badly infested. No authentic reports have been received of the presence of this pest in the Philippines, but it is probably to be found there. Several of the Australian States are infested, and the pest is not unknown in New Zealand. In South America it has been reported from Chile, Argentina, and Brazil. It seems also to be widespread throughout the West Indies. In Mexico it is prevalent at many points.

In the United States the root-knot is to be found in sandy soil now or previously in cultivation in most parts of North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, and Texas, as well as at many points in California. It is not abundant in New Mexico or Arizona, although proving destructive in some of the irrigated districts of the latter. It is very evidently of recent introduction there, as in many parts of Texas. In the interior of the

[1] Berkeley, 1855. [2] Greef, 1864.

West the writer has observed it, only sporadically it is true, in Utah and Colorado and at one place in Nebraska. It is reported, and the writer has seen specimens, from Arkansas. Oklahoma, Tennessee, and Kentucky have no reports of it in the open, but it is probably present to some extent, since it is found along the Ohio River in West Virginia and also in northern Pennsylvania. It occurs, but not in great abundance, in Delaware, Maryland, and Virginia The New England States appear to be almost free from the trouble, so far as outdoor plants are concerned, although it has been observed in Connecticut and Rhode Island. The most northerly points where it has been observed out of doors in this country are at various points in New York State, on ginseng and alfalfa; northern Indiana; Menominee, in the Upper Peninsula of Michigan; and the locality in Nebraska already mentioned. In the last three instances all the evidence indicates that the disease was directly imported from other localities and was not indigenous to that locality. The important point is, however, and this will be reverted to, that this nematode is able to maintain itself in regions where the winter's cold may be very intense

All of the localities named above are those in which the root-knot nematode has been found out of doors, not merely on plants partially protected during the winter, but in soil not at all protected from the severest winter cold. In addition to these localities it is almost universally present in this country in greenhouses and has in a number of instances become more or less established out of doors in their immediate vicinity, where it is protected by compost heaps, etc., from the extreme cold. In the most northern States it need not be feared that the pest will ever become widely distributed.

A careful study of the distribution of the disease convinces the writer that root-knot is of comparatively recent introduction in the regions west of the Mississippi. Indeed, it is possible to trace its arrival in parts of Texas, Arizona, and southern California, it having appeared in recent years after the land had been in cultivation for a long time with no signs of injury from such a pest. In Texas the introduction and spread of the nematode has been accomplished almost entirely by means of infected nursery stock, mainly figs, mulberries, and peaches; in Arizona and California figs and the Old World grape seem to be the responsible plants. The scattered localities in the North where the trouble occurs often reveal, on careful inquiry, the source of the infestation. Ginseng has been responsible for several outbreaks, the nematodes doubtless having been introduced in the moist earth in which the seeds were packed. In other cases nursery stock, such as peaches or even apples, has been responsible; sometimes the soil thrown out from greenhouses has

spread the trouble, and in some cases the manner of introduction can not be determined.

Close analysis of all the earlier reports and of the existing distribution of root-knot has convinced the writer that we have to deal with a pest originally tropical or subtropical in its distribution and not native to any part of the United States. In this the writer comes to a conclusion at variance with that of Neal,[1] who believed that it was native to the Southern States. If that were the case, however, it ought to be found on uncleared land where no crops have ever been grown, but that is not generally the case. Indeed, it is the general practice, when nematode-free land is needed, to go to uncleared land. To be sure, nematodes are occasionally found in such land, but almost always it can be shown to have been previously in cultivation, perhaps many years ago, or to be so situated that soil from infested fields could be washed upon it.

The general trade in exotic plants which began over a hundred years ago and grew rapidly, in the course of which ornamental and useful plants from the Tropics, especially of the Americas, were carried to European conservatories and gardens and also to our shores, may very probably have served to introduce the pest into the temperate regions of both the Old World and the New World. In all likelihood the Spaniards introduced this nematode into Florida directly from the West Indies or Central America, for it is found in parts of southern Florida that were in cultivation more than 75 years ago, but where now dense forests have grown up, as well as in clearings with no signs of recent cultivation. Yet even here it does not seem to occur in land absolutely unused in the past.

Whether the Old World or New World Tropics were the original home can not be decided now, as it is widely distributed in both. Perhaps its wide distribution in Africa, India, the East Indies, China, and Japan and the fact that another species of the same genus (*Heterodera schachtii* Schmidt) is apparently native in Europe would warrant the conclusion that it is probably of Old World origin.

THE CAUSAL PARASITE.

Upon breaking across a medium-sized or large knot and holding the broken surface so as to reflect the light a close observer will often see one to many clear to almost pearly white rounded bodies, considerably smaller than half the diameter of a pinhead, projecting from the surface. With a hand lens they are easily seen, but for the unaided eye they are sometimes very difficult to detect, on account both of their minuteness and of their transparency. In knots that have been cut across they are usually not visible, as they col-

[1] Neal, 1889.

lapse when touched by the knife. These objects are the mature females of the nematode *Heterodera radicicola* (Greef) Müller. Each is capable of laying several hundred eggs, more than 500 having been counted by the writer in one case where the nematode was still actively laying eggs.

EGG.

The eggs (Pl. I, figs. 1 and 2) are ellipsoidal bodies, sometimes symmetrical, more often slightly curved, and therefore somewhat kidney shaped. They are usually a little over twice as long as broad. Out of 71 different lots of egg masses measured by the writer, representing nematodes from 63 different hosts, the length varied from 67 to 128 μ and the width from 30 to 52.5 μ. The greatest ranges observed in any one lot of eggs were 67 to 108 by 33 to 42 μ, 88 to 128 by 33 to 44 μ, 81 to 112 by 33.5 to 40 μ, and 84 to 119 by 35 to 52.5 μ. These represented in each case eggs from the same nematode, showing how variable in size they may be. The average range of all measurements was 85 to 98 by 34 to 40 μ with an absolute average of more than 500 eggs measured of 92 by 38.4 μ. These dimensions agree closely with those given by Müller,[1] who studied this nematode in Germany, his figures being 94 by 38 μ. On the other hand, Frank,[2] also working in Germany, gives the figures as 80 by 40 μ. Stone and Smith[3] give the length as 100 μ.

When the writer first examined the eggs from different hosts he thought that there might be a possibility of distinguishing different races of the nematode by the variations in the size of the eggs, but the variability in size, even among the eggs from the same nematode, soon demonstrated that no results of value could be obtained in this direction. It seemed to be true, however, that the smaller, less strongly developed females often produce the smaller eggs. Thus, a nematode situated near the surface of a root, where the pressure was not so great, was often larger and had larger eggs, but this rule has so many exceptions that it can not be considered as being in any way general.

The egg consists of a densely granular body in which a lighter spot, the nucleus, can occasionally be seen, inclosed in a tough, elastic, transparent coat, or shell, probably chitinous in nature. When the mother nematode is so situated that she has plenty of room to deposit her eggs so that they are not laid with difficulty, they usually leave her body unsegmented. On the other hand, if the eggs as they are laid are crowded together so that considerable force has to be used to lay each egg, the oviposition is delayed and segmentation begins before the later eggs leave the body. Only exceptionally, however, do the eggs develop so far as to contain fully developed

[1] Müller, 1883. [2] Frank, 1885. [3] Stone and Smith, 1898.

larvæ by the time they are laid. Where this does occur it is mostly only the last eggs produced and which the mother nematode has not had the strength to force out against the large mass of eggs already laid. In this the root-knot nematode differs quite markedly from the sugar-beet nematode (*Heterodera schachtii* Schmidt), in which a comparatively large part of the eggs produced remain within the body of the mother and undergo segmentation and finally escape from the shell, eventually escaping to the outside through the openings in the body wall after the death of the old nematode.

Segmentation of the eggs begins very soon in any case and proceeds rapidly. It was not determined exactly how long the embryonic development required, but it is apparently not over two or three days in warm weather (much longer in cool).

The eggs were laid at the rate of 10 to 15 a day in the cases observed by the writer, although in some cases egg laying may proceed even more rapidly. They are surrounded by a slimy or gelatinous substance, which incloses them and evidently acts as a protection. This is secreted by the nematode with the eggs, as was observed on isolated mature females under the microscope. It is at first quite liquid and colorless, but soon becomes rather firm and light brown in color toward the outside. This is the structure that has been called by some investigators the egg sack (Eiersack); for example, Voigt [1] and Strubell.[2] The latter applied the term to the similar structure in the sugar-beet nematode (*Heterodera schachtii*), and, erroneously, denied its occurrence in *H. radicicola*. Occasionally the remains of the male may be found entangled in this slimy mass. It is probable in such cases that after fertilizing the female the male died and when the eggs were laid the egg mass surrounded his remains. The eggs at the outer portion of the mass are usually either hatched or contain larvæ, while those next to the body of the nematode are not segmented.

This egg mass is sometimes as large as the adult female and can be seen readily when the latter partly projects from the root.

LARVA.

The larva (Pl. I, figs. 3 and 4) emerges from the egg through a hole which it pierces in the shell, usually at one end. It is a slender, cylindrical animal, blunt at the anterior and tapering at the posterior end to a pointed tail. The larvæ when they emerge from the egg are 375 to 500 μ in length [3] and about 12 to 15 μ in greatest

[1] Voigt, 1890.

[2] Strubell, 1888.

[3] Stone and Smith (1898) give the length of the larva as 350 μ, but this is considerably less than the measurements made by the writer. They give the egg length as 100 μ, showing that they were not dealing with eggs below the normal size.

thickness. The average length is 420 to 475 μ. The structure of the larva is comparatively simple, consisting essentially of a tube (the alimentary canal) within a tube (the body wall), the space between (the body cavity) being filled with a liquid and minor structures (fig. 1). The body cavity has no opening to the exterior. The alimentary canal opens anteriorly at the end of the body, but posteriorly it opens in the median ventral line about one-eighth of the distance forward from the tip of the tail (i. e., 50 to 65 μ). The body wall consists of an external cuticle and a dermal layer of cells beneath which are the four "fields" of obliquely longitudinal muscle cells. Longitudinal tissue masses springing inward from the dermal layer at the median dorsal, ventral, and lateral lines separate the muscles into the four "muscle fields" mentioned. Only occasionally the opening of the excretory canal can be made out in the larva, but it is quite distinct in the mature male. It is in the ventral median line, opposite or slightly posterior to the esophageal bulb. These details of structure are clearly shown in the accompanying text figures (figs. 1, 2, and 3), contributed by Dr. N. A. Cobb.

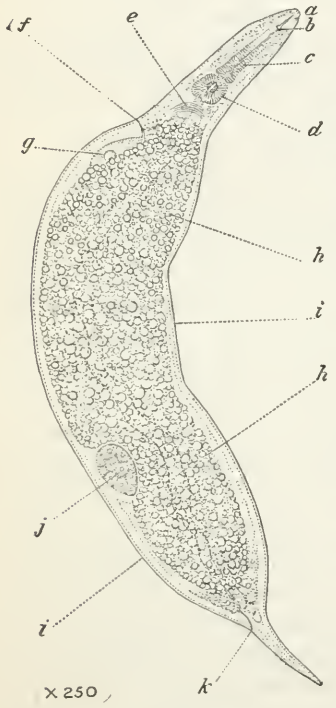

FIG. 1.—*Heterodera radicicola*. Half-grown female (?) individual shortly before the final molt: *a*, Anterior end; *b*, spear; *c*, esophagus; *d*, esophageal bulb; *e*, nerve ring; *f*, excretory pore; *g*, gland; *h*, thick wall of alimentary canal; *i*, body wall; *j*, beginning of reproductive organs; *k*, anus. Magnified 250 diameters. Drawn by W. E. Chambers.

The alimentary canal consists first of a buccal spear (Pl. I, fig. 4) 10 to 15 μ long (usually about 12 μ), a chitinous organ, pointed at the anterior end and with three small knobs at the posterior extremity and pierced its whole length by a fine canal. Connected with the basal knobs are retractile and exsertile muscles. This spear is used by the nematode in boring its way out of the egg and through plant tissues, and through it the nourishment is apparently drawn, for its canal is continuous with the lumen of the remainder of the alimentary canal. This spear lies in a cavity, the buccal cavity, from which it may be exserted. At the base of the spear begins the slender esophagus, 40 to 50 μ long, which expands then into the thick, muscular-walled esophageal bulb (figs. 2 and 3). This is a stout, muscular body, often nearly spherical, but more often a little longer

than broad, about 10 by 7 μ. The thick walls inclose a small lumen which can be expanded and contracted by the muscular action, thus acting in the manner of a pump in connection with the esophagus and spear (fig. 3). The expansion and contraction of the bulb are often synchronous with motions of the spear. Immediately behind the bulb the alimentary canal is rather narrow for a very short distance and then widens out rather abruptly into the comparatively thick-walled digestive portion which fills the body

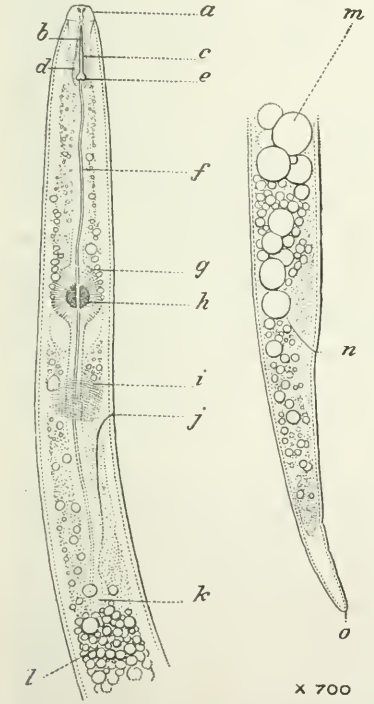

FIG. 3.—Larva of *Heterodera radicicola*: *a*, Anterior end; *b*, *c*, and *e*, spear; *d*, buccal cavity; *f*, esophagus; *g* and *h*, outer and inner portions, respectively, of esophageal bulb; *i*, nerve ring; *j*, excretory pore; *k* and *l*, lumen and thick wall, respectively, of alimentary canal; *m*, fat globule (?); *n*, anus; *o*, posterior extremity. Magnified 700 diameters. Drawn by W. E. Chambers.

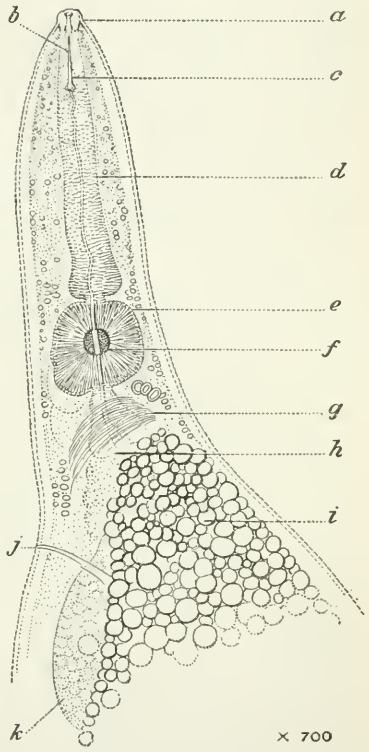

FIG. 2.—Anterior portion of the same nematode shown in figure 1: *a*, Anterior end; *b* and *c*, free and inclosed portions, respectively, of spear; *d*, esophagus; *e*, outer wall, and, *f*, central portion of esophageal bulb; *g*, nerve ring; *h*, second bulb; *i*, thickened wall of alimentary canal; *j*, excretory pore; *k*, gland. Magnified 700 diameters. Drawn by W. E. Chambers.

cavity and continues unchanged to a point shortly anterior to the anus. The anterior part of this digestive portion is not clearly marked off as a second bulb, as is the case in some species of Tylenchus. Immediately behind the esophageal bulb, surrounding the short, narrow portion of the canal, can be seen occasionally the nerve ring. About 25 to 40 μ anterior to the anus the walls begin to become thicker and the canal tapers, the anal opening itself being rather small.

Except anterior to the digestive portion of the alimentary canal the body cavity is small. There are no signs as yet of the reproductive organs, nor can the sexes be distinguished.

The larvæ are actively motile, but not so active as many of the free-living forms. Unlike the larvæ of some nematodes parasitic upon plants, for example, *Tylenchus tritici*,[1] *T. dipsaci*,[2] and a species of Aphelenchus discovered by Dorsett [3] on the violet and studied by the writer, the larvæ of *Heterodera radicicola* are not very resistant to unfavorable conditions. The other nematodes mentioned are uninjured by desiccation for long periods, by cold, many acids, etc. Thus, the wheat nematode has been revived after having been left dry for 27 years. The Aphelenchus referred to remained alive in kerosene emulsion for two days in contact with a drop of kerosene. Osmic-acid fixatives killed it but slowly, as was true of chromic acid, mercuric chlorid, and other strong poisons. On the other hand, the larvæ of *Heterodera radicicola*, although able to remain alive in water for a few days, soon die and decay, although damp or wet soil, provided the air supply is good, is favorable to their existence. Drying out is usually fatal to them in a comparatively short time.

The larvæ of the root-knot nematode are able to remain alive in the soil for months without entering upon a parasitic existence. The writer has been unable, however, to find any evidence that they take any nourishment from the soil; at least they undergo no development until they enter the roots of some plant, for if the soil be kept free from vegetation for two years they all die. Even one year without food is sufficient to kill large numbers of them.

In the normal course of development the larvæ, having encountered a root, seek its growing point and batter their way into it by the aid of the buccal spear (Pl. I, fig. 17). They then take up a position entirely within the root and parallel to its longitudinal axis, the anterior end pointing away from the root tip. This position may be in the plerome, or perhaps as frequently, if not more often, in the periblem. In the former case the nematode lies within the central cylinder as the root develops, in the latter case in the cortex. In either case the anterior end of the nematode is usually in close connection with the cells surrounding the conductive tissues. In the case of larvæ which hatch from eggs produced within the root, some bore their way out into the surrounding soil and enter new roots, as described above, while others burrow along in the tissues of the root and settle down, usually in the fleshy cortex. Thus an old nematode gall will contain nematodes in all stages of development and at a

[1] Davaine, 1857. Münter, 1866. Needham, 1745, 1775. Baker, 1753.
[2] Ritzema Bos, 1892.
[3] Dorsett, 1899.

depth below the surface of the root of even 5 or more centimeters. The latter has been observed by the writer in roots of sweet potato (*Ipomoea batatas*) at Miami, Fla.

Within the tissues the larva becomes fixed in position and remains quiet except for occasional movements of the spear and esophageal bulb. Whether all the nourishment is taken through the hollow spear or some is absorbed directly through the skin was not determined. It seems probable, however, that the former is the case, especially in view of the fact that the female occasionally bursts the surrounding tissues of the root, so that she lies outside the latter except for the anterior portion, which remains buried in the tissues.

Growth begins almost immediately. This is mainly, however, in thickness and only slightly in length (Pl. I, figs. 5, 6, 7, and 8). By the time a gain of 10 per cent in length has taken place the thickness has increased five to ten fold. This increase in thickness is confined to the region anterior to the anal opening and in the main posterior to the esophageal bulb. The alimentary canal posterior to the bulb becomes greatly enlarged. In a week or ten days the larvæ of both sexes are spindle shaped. By the end of the fifteenth to twentieth day the diameter is about a fourth of the length and the differentiation of the sexes becomes apparent (Pl. I, figs. 9 and 13). According to Stone and Smith [1] the female nematode sheds her skin four or five times during the course of development, the first time just before leaving the egg and the other two or three times before the final molt, when she becomes sexually mature. The writer has been unable to confirm this statement. In none of the specimens examined was any sign of shedding the skin apparent on leaving the egg, although on this point the evidence is slight, as special attention was not given to it. On the other hand, no trace of old skins could be found surrounding the developing larvæ within the galls up to the time of differentiation of the sexes. It seems possible that the investigators referred to may have been misled by the fact that an injured nematode sometimes secretes a new cuticle underneath the old or on account of the circumstance that the molting may commence at one point long before it is visible elsewhere. If these extra molts do actually occur it seems strange that no signs are to be found of the cast-off skins around the nematode.

The writer's observations lead him to the following conclusions: The sexes are alike (externally at least) up to about the fifteenth day, or sometimes longer. Then a new skin becomes visible underneath the old, from which it becomes separated at various points. In the female the most marked change is that of the shape of the posterior end of the body, which no longer possesses the tail it had

[1] Stone and Smith, 1898, p. 22.

before the new skin was formed. At first the remnants of the old skin are visible as an empty skin attached to the rounded posterior portion of the nematode (Pl. I, fig. 9), but soon the growth of the latter obliterates the cavity left and all signs of it disappear. The anus, which before this time occupied a median ventral position some distance anterior to the tip of the tail, now becomes terminal, and immediately ventral to it but also occupying a position almost terminal on the rounded posterior portion appears the prominent genital opening, a horizontal opening with two rather thick and prominent lips (Pl. I, fig. 10). The anterior portion has undergone but little change. Apparently fertilization must take place at about this time, for soon the external genitalia become so modified that this would become impossible. The lips become smaller, the opening less prominent, and eggs begin to develop.

Up to the last molt the larvæ of both sexes are alike, at least externally. The writer's very numerous observations do not allow him to confirm the statement of Atkinson [1] that the female can be distinguished before this period by the lack of a pointed tail, that of the male being pointed. In all the writer's observations, as previously described, the larvæ are indistinguishable until the last molt. Then the still small but sexually mature female may be seen, without a tail, in the old larval skin which has a tail.

ADULT FEMALE.

The mature female rapidly increases in thickness, becoming eventually flask shaped to pear shaped with a length of 400 to 1,300 μ and a thickness at the point of greatest diameter of 270 to 500 μ, or even 750 μ (Pl. I, fig. 12). The average of many measurements is about 800 μ for the total length, 500 μ at the point of greatest diameter, the length of the less enlarged anterior portion being 240 μ and its diameter just before the region of great thickening begins 150 μ. This not greatly enlarged anterior portion usually extends to a little posterior to the bulb. The body then enlarges abruptly, this posterior portion being approximately spherical.

Up to the last molt the spear of the female retains the dimensions and shape it had in the larva. As is characteristic of all spear-bearing nematodes, the old spear is shed with the cuticle at the time of molting, a new spear being formed in its place. This new spear is usually smaller both in length and thickness than the larval spear, and the knobs at its base are less prominent. It is usually 10 to 12 μ long as against 12 to 15 μ (rarely 10 μ), characteristic of the larva.

[1] Atkinson, 1889.

The fully mature egg-laying female is of a glistening pearly white color. The enlarged posterior portion is smooth and shows no markings, except that the internal organs are visible where they approach the surface. The comparatively little enlarged anterior portion shows faintly the transverse cuticular markings so characteristic of the mature male.

The bulk of the body of the sexually mature but not yet egg-laying female is occupied by the enormously dilated alimentary canal (Pl. I, fig. 11). The anus is a small round terminal opening, while the genital opening is a transverse slit slightly ventral to the anus and bordered by two more or less well-marked lips. This opens into a short, thick-walled vagina about 16 to 20 μ in diameter (including the walls). At its upper end it is abruptly contracted into a tube 8 to 10 μ in diameter, which soon divides into two tubes, the uteri. These are at first slender but slightly coiled tubes, leading forward (usually lateroventrally) and gradually increasing in diameter. Just before the ovary is reached each uterus expands into a spherical portion, about 16 μ in diameter, apparently the receptaculum seminis. Above this lie the cylindrical ovaries filled with the rudimentary eggs in the form of a sort of parenchyma. At this time the whole reproductive system if straightened out would not be more than 300 to 400 μ long. After fertilization the uteri undergo a most remarkable elongation and become very much coiled and tangled as they become filled with the fertilized ova. Although the body of the nematode increases rapidly in thickness, the increased space thus afforded is not sufficient, the alimentary canal becomes pushed to one side, and much of the space originally occupied by it is occupied by the uteri.

Egg laying had already begun, in the earliest cases observed by the writer, 29 days after the seed of the host plant (*Pisum sativum*, the garden pea) in these experiments was planted in soil known to be infested with the nematodes. Since germination of the seed is not immediate it is probably safe to assert that during warm weather the period from the time the larva enters the root until it begins egg laying is not over 25 days. This is somewhat longer during cooler weather, i. e., in the early spring and in autumn.

In most cases the greater part of the eggs are laid in an unsegmented condition. However, if the nematode is buried deeply in the tissues so that their pressure impedes egg laying, the eggs may develop and the larvæ escape still within the body of the mother, so that the latter may be viviparous. The last few eggs often develop in a similar manner, the nematode having evidently become so weak that she could not deposit them before they underwent development.

MALE.

The development of the male after the larval stage differs greatly from that of the female. Within the old larval cuticle a new cuticle is formed. The nematode pulls itself away from the old skin, remaining inclosed by it, however. The tail is rounded here, too, but the anus is ventral instead of terminal. The whole body now elongates very rapidly, becoming correspondingly slender (Pl. I, figs. 13 and 14). This necessitates a coiling in order still to remain within the old skin, until it is coiled two or three times. When this development is complete (Pl. I, fig. 15) it breaks its way out of the old cuticle, which has retained its larval shape, and passes through the tissues and probably even outside of the root in search of a female. Just prior to leaving the old larval skin after undergoing this metamorphosis the nematode does not molt again, as some assert.

The mature male differs greatly in many particulars from its appearance just previous to the last molt. The form is about like that of the larva on emerging from the egg, i. e., long and slender, differing, however, in the greater size and in the short, rounded tail. The length is usually 1,200 to 1,500 μ, the thickness 30 to 36 μ. The tail is short and rounded, not tapering, the distance from the anal opening to the posterior end of the body being not more than 13 to 18 μ. The cuticle over the whole body is very distinctly marked with transverse rings extending entirely around the body and 2 to 2.5 μ apart (shown in section in Pl. I, fig. 16). Except in profile it is only the furrows between the projecting segments of cuticle that are visible. These cuticular rings, which are also visible on the anterior portion of the mature female, are not visible, at least at ordinary magnification, in the larvæ.

The alimentary canal is essentially as in the young larva. The spear, however, deserves special notice. It is larger than in the larval stage or than in the mature female, being usually about 24 μ in length (rarely as short as 18 μ or as long as 28 μ). The knobs at its base are prominent. Above the knobs the sides are parallel for about half way and then taper to the finely pointed tip. The canal through the spear is rather distinct. The body wall is about 1.5 μ thick. However, at the truncate anterior end it is between 5 and 6 μ thick. The anterior 2.5 μ of this is a sort of hood, or cap, set off from the rest of the body by a sharp furrow. Lying in the terminal body wall, well below this hood and projecting but slightly into it, is a series of six radiating perforated lamellæ (apparently chitinous in nature), narrow at their anterior ends and broad basally. Viewed from the side they are approximately right triangles, the hypotenuse being somewhat wavy. The bases of the lamellæ radiate from a

common center, and the upright legs of the triangle surround a canal through which the spear passes. The bases are united into a small ring just around this canal and another ring unites the outer ends of the basal legs (Pl. I, fig. 16). Looked at from the anterior or posterior direction this apparatus resembles a wheel with six spokes. Distinct muscle strands run from the rim of this wheel to the knobs of the spear, as well as to the point where it begins to taper. It is probable that this peculiar organ is to help support and guide the spear as the male is battering his way through the tissues. A similar apparatus is present in *Heterodera schachtii*, the sugar-beet nematode. It was imperfectly described by Strubell,[1] but the writer's observation shows it to be essentially the same as in the root-knot nematode. It has also been reported, but not correctly described, for a Tylenchus species.

The reproductive organs of the male consist in all cases examined by the writer of a single testis, a tube blind at the anterior end and running parallel to the alimentary canal, into which it opens just before the anal opening is reached. Atkinson reports that there are two of these reproductive organs, as is the case with some other nematodes. In all the specimens examined by the writer, however, including specimens from Indiana, South Carolina, and Florida, using the oil immersion lens and viewing the nematodes from different sides, there was not the slightest evidence of a second testis. Cobb [2] also mentions its presence, and as both he and Atkinson are accurate observers it must be that sometimes this occurs. In fact, Atkinson himself later found specimens in which the testis was single.[3] According to the writer's own observation the right testis is the one that is missing, as the one present is placed somewhat asymmetrically, lying nearly in the left half of the body.

Lying on either side of the posterior portion of the alimentary canal and with their points entering the cloacal chamber are two peculiar, somewhat sickle-shaped bodies, the spicules. These are curved bodies, tapering toward the posterior end, about 35 μ long, measured on the chord connecting the two ends. No accessory piece is present, although a thickening near the apical portion may represent one fused with the spicules. These spicules are of use only during the sexual process.

The excretory canal is plainly visible in the left lateral line, opening ventrally in the median line 160 to 170 μ from the anterior end of the body.

It seems probable that the mature males take little or no food and that they perish after having performed their function. The reason for this supposition is the fact that one often finds still actively

[1] Strubell, 1888. [2] Cobb, 1902. [3] Atkinson, 1889; see also Atkinson, 1896.

moving males in which the alimentary canal posterior to the bulb, or even including it, has entirely disintegrated, leaving the body cavity filled with a granular disorganized mass except for the long testis, which extends nearly to the esophageal bulb. The large buccal spear with its complicated guiding apparatus is doubtless to enable the animal to batter its way through the root tissues in its search for the female, as a much smaller spear serves the female for obtaining the necessary food.

OVERWINTERING.

The stage in which this nematode overwinters was made the object of considerable study. In the galls on annual plants examined in November it was found that in almost all cases the mature or partly developed nematodes, as well as the eggs, were dead, in many cases being filled with fungous threads. Larvæ, however, alive and actively motile, were found in abundance in and around the galls. It is probable, therefore, that it is in the larval stage that the nematodes from annual plants pass the winter, probably descending into the lower levels of the soil to avoid the cold. This latter point, however, was not determined. In cases where the death of the top of the plant had caused the death of the roots, the nematodes in the roots soon died also.

In roots of perennial plants, for example, European grape, fig, etc., the writer has repeatedly found living female nematodes in nearly or quite complete development at various periods in the winter and early spring, showing that in such roots the nematodes may survive not only in the larval stage, as previously described, but also as mature females ready to begin egg laying as soon as the weather becomes favorable.

COMPARISON WITH HETERODERA SCHACHTII.

In view of the fact that some authors[1] have questioned the correctness of keeping separate the two species *Heterodera schachtii*, the sugar-beet nematode, and *H. radicicola*, the cause of root-knot, it will be well to give briefly an account of the points of difference, especially since the writer has found the former to be a serious pest at several points in California and Utah, while the latter has been found as a serious sugar-beet pest at some other points. In tabular form the main differences are easy to point out.

[1] Stone and Smith, 1898; Atkinson, 1896.

TABLE II.—*Differences between Heterodera schachtii and H. radicicola.*

Points.	Heterodera schachtii.	Heterodera radicicola.
Effect on host	No galls.	Produces galls on roots.
Location of mature female.	External, anterior end only within tissues of root.	Usually entirely within tissues of root, more rarely the posterior portion, very rarely nearly the whole body external.
Shape of female, external appearance, etc.	Mostly lemon shaped, dull and flaky in appearance, no trace of transverse rings.	Pear or flask shaped, glistening and pearly white, transverse rings of cuticle often visible on anterior portion.
Eggs	Part deposited outside body, but most developing within it.	All but the last few deposited outside the body.
Larva	Buccal spear about 25 μ (Pl. I, figs. 18 and 19).	Buccal spear 10 to 15 μ, mostly 12 to 15 μ (Pl. I, figs. 3 and 4).
Mature male	Buccal spear about 30 μ (according to Strubell, 1888).	Buccal spear mostly about 24 μ.

That these nematodes are not the same is readily seen when they occur on the sugar beet. The one causes no conspicuous galls while the other makes the galls so characteristic of root-knot (Pl. II, fig. 1). Both are very destructive pests of this host, and there is not much choice as to which is the more harmful. Another difference not mentioned above is that *H. schachtii*, perhaps by virtue of its more powerful spear, is able to thrive and spread in stiffer soils than does *H. radicicola*. In Plate I the figures for the larvæ of *Heterodera radicicola* (figs. 3 and 4) and *H. schachtii* (figs. 18 and 19) are drawn to the same scale, respectively. The difference between the two species was emphasized in tabular form by Voigt in 1890.

METHODS OF SPREAD.

The larva of *Heterodera radicicola* is capable of active movement in the soil, and in this manner doubtless the disease is slowly spread. From some experiments made by Frank[1] he estimated the rate of progress at about 3 cm. per week. This would amount, during the warm weather, in which infection occurs, say May 1 to September 15, to about 75 cm., or about 30 inches. These figures are probably far too low. However, it is not through their own efforts that these nematodes are mainly spread. There are many means of transportation at their disposal. A very frequent one is running water. Thus, a field previously free from the pest sometimes shows its presence in those spots where surface water at a time of heavy rains has deposited a lot of soil from an infested field lying higher up. In this way the pest has been carried from infested fields even to uncultivated woods, as observed by the writer at one place. It has been suggested that heavy winds carrying large quantities of soil from one field to another may also transfer the nematodes, but in view of their susceptibility to injury by drying, this seems little likely. Especially is

[1] Frank, 1885.

this unlikely since the larvæ shun dry soil, and so would not be present in that part of the soil which is dry enough to be transported by the wind. More effective as means of transportation are the hoofs of animals, wheels of vehicles, farm implements, and men's boots. It is difficult to see how it would be possible to avoid conveying living nematode larvæ from one field to another on farm implements if they are left, as is too often the case, uncleaned on being transferred from one field to the next. Thus, a wagon and horses going from one field to another would, if the soil were at all damp, carry some of the damp earth, probably containing nematode larvæ, with them.

The foregoing explains the spread of nematodes after they have once been introduced into a locality. The introduction of nematodes into a new locality, however, must have some other manner of accomplishment. This seems to be in most cases along with nursery stock. Thus, the writer found that in parts of Texas the nematode appeared first in the soil near fig and mulberry trees obtained from farther east, which were noticed at the time of planting, several years ago, to have knotted roots. In this way the soil near the trees became infested and thence the disease spread, as previously described, to different points in the locality. Perhaps east of the irrigated districts the fig, mulberry, and peach are responsible more than any other plants for the spread of the disease. Since the putting into effect of good nursery inspection much of this source of infection has been cut off. In the irrigated districts of Arizona and California the vine was observed in several cases to be the plant at fault. The strawberry has been observed at a few points in the East as the plant upon which the pest was introduced. It is often badly affected without showing much injury. A case has been called to the writer's attention in which the disease was introduced into a garden in Washington, D. C., by asparagus roots from an infested field. The wide distribution of the disease in ginseng plantations is doubtless due to the setting out of small rooted plants from infested regions, as well as to the practice of some growers of packing the seed in damp earth. Should this come, as is natural, from the vicinity of the ginseng bed and this be affected by nematodes, the danger of sending nematodes along with the seeds is very great. The dirt used for packing is naturally thrown out at the point where the seeds are planted, and thus the larvæ, if present, are able to enter the soil and infect the young ginseng seedlings. Seed potatoes are also another known source of introduction of the disease.[1] In a personal communication Dr. N. A.

[1] Lounsbury (1904) regards the potato as perhaps the chief source of introduction and spread of this disease in South Africa.

Cobb expresses the same opinion based on his observations in New South Wales.[1]

For the North, where root-knot is mostly confined to greenhouses and hotbeds and their vicinity, perhaps one of the chief sources of infection is the soil that is thrown out of these beds at the end of the season. This soil, if infested, will spread the disease in the immediate vicinity, especially if it be put near some manure pile or compost heap which keeps the ground damp and warm during the winter.

EFFECT ON THE HOST.

The effect upon the root of the presence within it of the young nematode is usually the hypertrophy of some of the tissues. The parenchyma cells become abnormally large and multinucleate,[2] sometimes only a few, at other times a great many cells being involved in this hypertrophy. This abnormal enlargement of the cells leads to a displacement of the various tissue elements, so that the tracheary cells and vessels become separated and also show lateral displacement and often much distortion. Often in bad cases individual cells of a tracheary nature will occur entirely separated from others of the same kind. The amount of hypertrophic enlargement of the root depends upon the host on the one hand and upon the number of nematodes entering the root in the same vicinity on the other. In some roots the swelling is barely noticeable and is so small that as the female nematode enlarges she eventually is inclosed in the root only by the narrow anterior third of the body, the remainder being entirely external, in this particular showing great similarity to the sugar-beet nematode, whose galls are always of this nature. More often, however, the hypertrophy is so pronounced that the mature female is entirely concealed or reaches the surface only at the extreme posterior portion of the body. If many nematodes are present in the same general region of a susceptible root, the gall may be many times the normal size of the root (Pl. II, fig. 2). These galls are at first of soft tissues, but in some woody plants, the European elm, for example, some of the hypertrophied cells become lignified, inclosing the female nematode in a woody prison from which in all probability the larvæ would be unable to escape should egg laying continue after the lignification has begun. The structure of such a gall is like that of the burls that often occur on various trees.

A very frequent phenomenon, but one that is by no means universal or characteristic of any one group of plants, is the formation of numerous lateral rootlets above the gall. This is doubtless due

[1] The writer's attention has been called to the fact that in certain of the irrigated districts of the West this nematode has become a very serious potato trouble. On one occasion several carloads of potatoes were rejected on account of being infested with it.

[2] Tischler, 1902.

to the disturbed and to a large extent interrupted water supply and to the accumulation above the gall of food substances which would normally pass on to the root tip. They accordingly are made use of in the formation of lateral roots at that point. It is probably not different in its nature from the adventitious root formation in cotton and other plants just above the point of entry of the wilt fungus (*Neocosmospora vasinfecta*)[1] or, in fact, from that occurring when the end of a root is cut off or mechanically injured. The shape or size of the gall does not seem to depend upon the place the plant occupies in the current schemes of classification. The statement of Frank[2] that the galls of the dicotyledons are mostly of the round, tuberlike type, with lateral rootlets, while those of the monocotyledons are mostly spindle shaped, without lateral rootlets, is not confirmed by the writer's observations. Galls of both types may be found on the same plant (Pl. III, figs. 1 and 2) and appear to owe their differences to the number of nematodes entering at a given point, to the age and rapidity of growth of the root, and perhaps to other causes. On both the beet and the radish, as well as on many other plants, both types of galls and all gradations between may be found. Entrance to the plant by the larvæ is not confined to root tips or to passage from galls to the adjacent healthy tissues, although these are the usual ways by which a nematode reaches the point where it undergoes its subsequent development. Nematodes are also able to bore from the outside directly into the tender tissues of other parts of the roots, and even into stems. Thus, not only are the roots of potatoes attacked but even the tubers, while sometimes the prostrate stems of tomato plants as well as those buried beneath the ground in setting out the young plants are badly knotted. Indeed, Señor Romulo Escobar, of the Mexican Ministry of Agriculture, informs the writer by letter that in the State of Nuevo Leon the roots, stems, leaves, and even fruits of the watermelon are attacked when they are in contact with the ground. This is exceptional, however, and is possible only where the nematodes are very abundant and when the surface of the soil is constantly moist, so that they are in its uppermost layers.

Through the kindness of Mr. W. K. Winterhalter, then consulting agriculturist of the American Beet-Sugar Co., at Rocky Ford, Colo., analyses were made of sugar beets badly affected with root-knot and of healthy beets from the same field. Strange to say, in six samples each of healthy and diseased beets the average sugar content differed less than one-fifth of 1 per cent of the total weight of the beet, while the percentage of purity was equally as close in the two lots. In these points there also seems to be a marked dis-

[1] Orton, 1902, p. 10, fig. 1. [2] Frank, 1885.

tinction between the root-knot nematode and the true sugar-beet nematode (*Heterodera schachtii*), for the latter's presence not only reduces the size of the affected beets, but also greatly reduces their sugar content and usually lowers also the purity.

The greatest depth at which Frank observed nematode galls was 33 centimeters (about 13 inches). On the other hand, the writer finds that they may occur more than a yard below the surface of the soil. To be sure, these are only scattering galls, for the great majority of the nematode galls occur in the first foot of the soil. Indeed, in practical culture it has been found that if trees can be forced to root extensively at a depth of 16 inches or more they suffer but little from root-knot.

CONDITIONS FAVORING ROOT-KNOT.

SOIL.

Root-knot is essentially a disease of light soils. Wherever the soil is sandy or contains a fairly large proportion of sand, other conditions being favorable, the root-knot nematode may be expected to thrive when once introduced. In heavy soils, on the other hand, the disease seems never to be serious. In some of the writer's experiments affected plants were planted in pots of stiff clay soils, and not only was it almost impossible to obtain infection of susceptible plants placed in close proximity in the same pots, but even on the diseased plants the new roots remained free from the trouble. Similar experiences have been reported to the writer from various parts of the country where diseased trees were set out in stiff soil and after a few years seemed to be entirely free from the trouble. Contradictory statements sometimes find their way into print, but they are explicable in most cases when one understands the great popular confusion in the use of the words "heavy," "stiff," and "light" as applied to soils. Thus, in parts of Florida and South Carolina a very sandy, yellow soil containing only enough clay to hold it together while moist, is called "clay" or "heavy soil." It is clayey, to be sure, compared with some of the soils thereabouts, for sometimes the latter are almost pure sand. "Light" and "heavy" in the sense used in this bulletin have reference to those soils containing, respectively, little and much clay. Soils that dry out rather quickly, that do not cake hard on drying, and that are easily crumbled to a fine granular mass are favorable to these nematodes, while the reverse is the case for the difficultly permeable, hard-caking, clayey soils. This applies only to the root-knot nematodes, as the writer's investigations have not gone into this point with reference to other sorts. It is known that the sugar-beet nematode will thrive in some of the heavier as well as in light soils.

MOISTURE.

A certain degree of moisture is necessary for the maintenance of the life of the nematode in the soil. Experiments by the writer, Frank,[1] and others have shown that the larvæ of the root-knot nematode, unlike those of many other nematodes, are destroyed by being dried in the laboratory. Observations by the writer in New Mexico, Arizona, and California confirm this abundantly, for in those communities the root-knot is practically confined to the irrigated land. This does not mean that the soil must be wet, for that is not necessary. The soil, however, must have sufficient moisture in it to be properly called a moist soil, though not enough to fill the air spaces and interfere with proper aeration. Thus, we have reports from South Africa,[2] Argentina,[3] and Chile [4] which state that the nematodes grow only in wet soils. This, in the light of conditions existing in America, evidently means not what we would call wet, but merely moist, in the eastern and southern part of the United States, but what many people in irrigated districts would not hesitate to call wet in contradistinction to the dry, unirrigated soils. Prof. P. H. Rolfs,[5] Dr. N. A. Cobb, and others report experiments which would seem to prove that drying of nematode-containing soil does not entirely kill out the *Heterodera radicicola*. This will be discussed more in detail later.

On the other hand, soils that are water-logged for a considerable part of each year are usually free from the trouble. Some observations on the effects of floods on nematodes led the writer to believe that flooding for a few days would destroy them, but field experiments in Arizona and California showed that keeping the soil submerged for five days was not sufficient to kill out the nematodes, at least not those inclosed within the root galls of the trees and vines growing in the fields. Yet it is certain that very wet soils are free where this is long continued, and long periods of flooding kill out the nematodes. Thus, in the Everglades of southern Florida there occur islands, parts of which are never flooded and parts of which are out of the water ordinarily, but submerged for two to six months of the year. Truck growers occupy some of these islands and find that the root-knot nematode is abundant above the high-water level—i. e., where the land is never flooded, but absent in the zone that is flooded every year.

TEMPERATURE.

As long as the soil is not too dry, the higher the temperature the more actively the nematodes seem to develop. On the other hand, they seem to become practically inactive when the soil temperature falls below 50° F. Yet they are capable of remaining alive when

[1] Frank, 1885. [2] Lounsbury, 1904. [3] Huergo, 1902, 1906. [4] Lavergne, 1901. [5] Rolfs, 1894.

exposed to great cold. The writer saw root-knot abundant on ginseng in a slat shed in Menominee, Mich., where the soil a year or so before froze to a depth of more than 3 feet and where outside the shed water pipes 6 feet beneath the surface were frozen, so the writer was informed. In spite of this cold the nematode injuries were bad the next year. In York, Nebr., where the temperature goes below zero every year and sometimes reaches nearly or quite to −30° F., this nematode survived the winter in peony roots which remained out of doors without protection. In New York State ginseng and alfalfa are both more or less affected with root-knot, while in West Virginia, along the Ohio River, clover is badly affected. It thus becomes apparent that cold alone does not destroy the pest in the soil. To be sure, Bailey[1] placed soil containing root-knot nematodes in boxes and set some of the boxes out of doors through the winter. In the spring the boxes kept indoors still had living nematodes, as shown by gall formations upon plants grown from seeds sown there, while the boxes left out of doors were free from nematodes. It seems probable that the soil in this case dried out in the freezing process sufficiently to kill the nematodes. Ordinarily, however, the frozen soil remains in connection with soil moisture below, and so the drying out and consequent destruction of nematodes does not occur.

The root-knot nematode does not become active in the soil and begin to penetrate the roots of susceptible plants until the soil begins to be warm. In the tropical and subtropical regions plants are subject to attack the year around, but the farther north one passes the longer is the winter period of comparative immunity from injury by this pest. Thus, in Miami, Fla., there is no dormant period for the nematode. In northern Florida, however, crops planted in the latter part of November or in December show comparatively little injury, nor does the injury begin to be severe until the middle of February or early in March. On the other hand, plants sown in October are infected before the soil becomes cool and are badly injured, the nematodes continuing to develop and spread within the tissues when it is too cool for them to spread outside through the soil. At Monetta, S. C., about half way between Columbia and Augusta, Ga., in the writer's experiments no infection by nematodes could be obtained before the middle of April, while it was the middle of May before they became really active. By the end of September or the middle of October their activity had begun to decline.

Frank[2] assumed that the chief period of infection was in the spring. He was in error in this statement, for the writer's experiments show that the nematodes are more active in midsummer and that infec-

[1] Bailey, 1892, pp. 157–158. [2] Frank, 1885.

tions occur more freely the warmer the weather, except where lack of rain permits the soil to dry out, in which case both plants and nematodes cease to thrive.

CONTROL OF ROOT-KNOT.

The problem of the control of root-knot is one that varies much according to the place infested, the kind of plants grown, the methods of culture followed, etc. We may distinguish between small, intensively cultivated lots of soil, such as we find in greenhouses, hotbeds, and seed beds, and field culture. Each group may be subdivided in accordance with the answer to the question whether the crops are annual or long lived. For the first great division, owing to the value of the crops raised and the amount of capital invested, methods of combating a disease may be used that would be barred from field crops or other crops on larger areas of land, because the expense would not be justified in view of the comparatively low earning power of the land. Furthermore, the actual monetary loss to the crop due to a given disease may be far greater in the restricted areas of intensive culture than in large fields where each plant is of relatively less value. So, for example, root-knot may affect a field of cowpeas and actually reduce the crop one-half, but unless the field were very large that might not equal the loss sustained by a grower of cucumbers, lettuce, or tomatoes whose whole greenhouse crop has been totally destroyed by the same pest.

GREENHOUSES, SEED BEDS, ETC.

LIVE STEAM.

Probably the most satisfactory method for destroying the root-knot in greenhouses and seed beds is the use of live steam under considerable pressure. This has been advocated by various persons, viz, May, Galloway, Selby, and Rudd,[1] but it was as a result of careful experiments by Stone and Smith [2] that it became generally used. The method recommended by them is a modification of that recommended by Galloway and others. The scheme is essentially as follows: At the bottom of the bench or bed are laid either iron pipes perforated with $\frac{1}{16}$-inch holes every few inches or drain tiles. Live steam is passed into these and escaping from the holes of the iron pipes or between the ends of adjacent tiles heats the soil to such a degree that all animals and most plants (except, of course, bacterial spores) are killed. The pipes must be placed at intervals short enough to permit the spaces between the rows of piping to be thoroughly permeated by the steam. This distance varies with the soil, but 12

[1] May, 1896; Galloway, 1897; Rudd, 1893; Selby, 1896.

[2] Stone and Smith, 1898.

inches is close enough for all general purposes, and even 2 feet is not too far in deep beds if the sterilization is kept up long enough. The bed should be covered with straw, boards, sacking, or something of the kind to permit the upper layer of soil to become heated through. The pipes or tiles in the soil should be arranged lengthwise in the beds, with the steam inlet in a crosspiece of piping running across the bed, from which the longitudinal rows take their origin. A similar crosspiece at the other end may be used, but is not absolutely necessary. There should be no open ends of pipes or tiles; otherwise all the steam will escape out of these and not through the cracks or small holes. Depending upon the pressure of steam used, the time necessary for sterilization will vary from half an hour to even two hours when the pressure is poor.

A method often recommended to determine whether the steam has passed long enough, and one that has considerable merit, is to bury raw potatoes at the surface of the soil underneath the covering of straw, boards, or sacking. When all these potatoes are found to be cooked the steam can safely be turned off. Stone and Smith recommend the use of a special boiler so that steam at fairly high pressure can be used, not under 40 pounds per square inch, preferably more. Even 80 to 100 pounds pressure is not too high if obtainable, as it shortens the time necessary and also prevents the soil from becoming as wet as with lower pressure.

Not only are all nematodes killed by this treatment, but also all insects and other noxious animals, as well as all fungi and their spores. Many bacteria are killed, too, but not all of their spores, the survival of the latter being desirable in view of what we know of the value of soil bacteria.

This method has some disadvantages. Thus, it can not be used for beds occupied by living plants. Furthermore, care must be taken on the one hand not to leave the soil soggy and on the other not to dry it out too much. The latter is, however, a much less serious matter than the former.

FRESH SOIL.

For greenhouses, cold frames, seed beds, etc., where a steam-heating plant is lacking and where it would not pay to incur the expense of installing a boiler for the purpose of using it for soil sterilization, the desired results can be obtained by the use of fresh soil each year. This should be taken from some place in the woods or from a field where the nematode is known not to occur. The old soil should be placed where it can do no harm in the way of spreading the disease. If it can be allowed to become perfectly dry for some weeks before taking it out, the danger from the old soil is greatly reduced. The

framework of the beds should be thoroughly whitewashed with strong, hot whitewash, freshly made from good quicklime, or it may be painted with formaldehyde or some other disinfectant of this nature. This is to kill all larvæ or eggs that might be in the dirt adhering to the cracks. In selecting new soil it will always be well to examine the roots of susceptible plants growing where the soil is to be obtained in order to determine whether or not root-knot is present. This method has given good satisfaction where carried out in the North. It is applicable, however, only to small greenhouses that do not require much new soil. Large greenhouses can be far better taken care of by sterilizing the soil in the benches.

It often happens that to obtain fresh soil is not desirable in view of the character of the soil in the vicinity. Perhaps it has taken some years to bring up the soil in the beds to the desired lightness, humus content, etc., and to have to take new soil every year would be a hardship. In such cases steaming should be made use of if possible. If it is not feasible, a formaldehyde solution has shown itself of considerable value.

FORMALDEHYDE.

The formaldehyde method consists essentially of treating the soil with a weak solution of commercial formaldehyde (or formalin). It has been found that a solution of 1 part commercial (36 to 40 per cent) formaldehyde in 100 parts water is effective against the root-knot nematode in shallow beds when applied at the rate of 1 to 1½ gallons (or more in the case of very absorbent soils) to every square yard of soil surface. For deep beds the quantity must be increased. Care must be taken that all parts of the soil are reached and thoroughly wetted by the solution. Upon the thoroughness with which it is done depends largely the success of the process. After the formaldehyde solution has soaked in the soil should be thoroughly stirred, so that all parts may be exposed to the disinfectant. Before setting into the soil any plants or sowing any seeds the excess of formaldehyde must be allowed to escape by evaporation or, if necessary, be washed out by flooding the bed. The former is preferable. The writer has not found the germination of seeds interfered with when 10 days are allowed to elapse between the treatment and the sowing of the seeds, especially if the soil be allowed to become rather dry and be stirred in the meanwhile.

This formaldehyde treatment has been used with success at the Ohio Agricultural Experiment Station [1] in the forcing house and seed beds. It was applied primarily to prevent certain damping-off fungi from destroying the seedlings, but it was found that the nematodes were sometimes destroyed also or greatly reduced in numbers. How-

[1] Selby, 1906.

ever, as a means of combating nematodes it is not recommended by Prof. Selby. The strength of the solution used there was about 1 to 1½ parts commercial formaldehyde to 400 of water, which is less than that found to be really effective against this nematode.

The treatment of living plants in the greenhouse to destroy root-knot is fraught with considerable difficulty. Means that will destroy the nematodes are mostly injurious to the plants containing them. Thus, steaming or drying and freezing the soil can not be thought of, as these processes are fatal to the plants. So, too, the use of carbon bisulphid has in a similar way proved not feasible. It is still possible, however, that certain plants less susceptible to this chemical, if perfectly dormant and rather dry, might escape without serious injury when enough of it was used to kill the nematodes present. This must be determined by experiment. Under certain conditions the use of the formaldehyde solution has been found efficacious with some kinds of roses. Many plants are killed outright by the treatment, but roses, at least some sorts, are less susceptible to injury. The first experiments in this line were performed in February, 1902, in the greenhouses of Mr. Loosé, a florist of Alexandria, Va., under the direction of Mr. A. F. Woods, of the Bureau of Plant Industry. The writer cooperated in so far that he examined the roots for nematodes after the experiment. The following extracts from Mr. Loosé's report of the experiment indicate the methods used:

In the early part of February a bed of Bridesmaids, 150 feet long and 3 feet wide, 4 inches soil, was thoroughly saturated, using 50 gallons of the 1 per cent mixture. The plants did not seem to suffer from the application, and one week later we were able to see young healthy roots making their appearance, while the old fibrous roots were entirely decayed. We then treated in the same manner Bride, Kaiserine, Chatanays, Nephetos, Beauty, Liberty, and Meteor with equal success as to freeing the soil of the pest.

Some strong-growing varieties, however, such as Beauties, Chatanays, and Kaiserine, suffered and lost much of their foliage. Even some of the soft growth wilted during the sunny part of the day. My experience in this treatment is that care should be taken to harden the plants by lower temperature and keeping the beds dry, being careful, however, to give the plant a good watering 12 hours before applying the mixture. * * * The cut of roses on February 10, at the time when we applied the remedy, had dwindled down to 250 a day. It remained practically stationary during the four following weeks. We were able, however, to notice that the foliage was regaining its normal color and the plants were starting strong growths. By April 1 our cut had increased to 500 daily, mostly prime stock, and by the middle of April it had resumed its normal cut of 1,000.

As a matter of experiment we left a few plants untreated at the ends of some of the benches, and to-day, May 10, they are practically worthless, showing effectually that the spring weather had nothing to do with the improvement. The roots of the untreated plants looked like a ball of fern roots used for orchid potting, full of galls and matted, plants making a weakly growth, foliage pale, and flowers insignificant. On the contrary, the plants treated last February have healthy strong roots, making fine growth and the foliage of the very best color.

The mixture was applied with the hose connected to a force pump at the rate of 4 pounds of formaldehyde to 50 gallons of water, the treating of 15,000 plants requiring 200 pounds of formalin, worth about 18 cents a pound, making the treatment quite inexpensive considering the result.

Since this experiment this method has been tried in a number of places and with success where the proper precautions were taken. Doubtless other plants might be treated similarly, but the method should be tried with caution, even for roses, until it is ascertained that the plants will not be killed.

MISCELLANEOUS.

Plants for which the formaldehyde treatment can not be used can often be benefited by the following treatment: Remove them from the soil, wash the roots clean, and cut away every diseased root, burning them. Top the plant to correspond with the amount removed from the roots and plant in nematode-free soil. Such severe treatment is too injurious to some plants, and about all that can be done then is to give them plenty of well-aerated soil with an abundance of fertilizer, so as to stimulate root growth to more than counterbalance the roots that are reduced in value by the entry of the nematodes into them.

It is possible that by transplanting diseased plants to stiff clay soil the number of nematodes will be so reduced that a subsequent transplantation to more suitable soil will find them free from the disease.

On purchasing rooted plants, unless they come from a place known to be free from root-knot, it will always be best to put them into a quarantine bench for several months. If at the expiration of this time they show no signs of the trouble, they can safely be removed to their permanent quarters. Of course the soil in the quarantine bed must be renewed whenever it becomes infested with the nematodes.

Moderate quantities of soil can be freed from the pest by putting it at the beginning of winter in a place where it will be exposed to the cold and subject to drying out at the same time. Thus, it can be thrown upon boards in a comparatively thin layer. The boards will keep the nematodes from passing downward into the ground as the soil dries out. At the same time the boards keep the moisture from the soil beneath from passing by capillarity up into the soil from the beds. The continued drying and freezing, especially if the soil be occasionally stirred, is fairly effective in killing off the nematodes.

CONTROL OF ROOT-KNOT IN THE FIELD ON PERENNIAL CROPS.

The treatment of perennial crops in the field is of a greatly different nature from that of plants in the greenhouse, cold frame, or seed bed, for a process that could be applied with profit to such valuable soil as that in greenhouses, etc., might, indeed mostly does, prove too

expensive for ordinary use in large fields where the crop value per given area is far lower. The methods to be applied differ according to whether the land is used for annual or short-lived crops or is possessed by a long-lived crop, such, for example, as fruit trees. In the former case the treatment can be begun after the crop is off, while in the latter it must be of such a nature that the trees present do not receive injury. The latter problem will be discussed first.

In the South the trees most generally affected seriously are the peach, fig, mulberry, and walnut, while in California and Arizona the Old World grapevine is seriously affected in addition. Many other plants are subject to great injury elsewhere, such as coffee in Brazil, Mexico, and the East Indies; papaya (*Carica papaya*) in Florida and the Tropics; shrubs like tea in Ceylon and India, etc. By consulting the list of plants subject to the disease it will be seen that many are woody plants and that of these a number besides those mentioned are seriously injured by the disease.

CHEMICALS.

Of the various treatments proposed, the use of chemicals has offered a wide field for investigation and one that is by no means thoroughly explored as yet. The more promising chemicals tested by the writer are mentioned in the following paragraphs:

Carbon bisulphid.—This has been used in Europe for the phylloxera on vine roots where the plants were dormant, without serious injury to the vine. The writer's experiments, however, lead him to look upon it with suspicion. Many plants were very quickly killed by it and others seriously injured. Its use should not be attempted without first testing its effect upon one or two trees. These should preferably be dormant, at least not in an actively growing condition. The root hairs are killed outright, so the plant must not be where it will actively transpire until new root hairs are formed. The usual method of procedure is to make holes in the ground to a depth of several inches or a foot or more, the carbon bisulphid being poured or injected into these holes and the latter covered up with dirt before the liquid volatilizes. The fumes penetrate the soil and destroy nearly all living things. Extreme care must be used in handling this chemical, as its fumes are poisonous and exceedingly inflammable, being explosive when enough air is mixed with them.

Carbon bisulphid will doubtless be of value in an orchard or grove where it is desired to replace certain trees or fill vacant places with new plants. By its use the spots where the old trees stood or where vacant places are to be filled can be thoroughly disinfected. After a week or two the trees can be set out and, the soil being free from nematodes, can make quite a start before the nematodes from the

soil outside of the disinfected patch can get to their roots. In deep sandy soil the writer found not all the nematodes destroyed by the use of 2 ounces of carbon bisulphid per square yard, but when 4 ounces were used they were exterminated. The size of the area to be treated depends upon the size and rapidity of growth of the trees to be planted, the faster they grow the smaller being the area to be treated. For the best results the chemical must be placed at a depth of several inches below the surface, the opening being firmly closed so that the vapors will have to diffuse throughout the soil. In France special forms of apparatus have been devised for this purpose in combating phylloxera. They consist of a reservoir for the liquid and a hollow rod which can be inserted to any desired depth, a measured quantity of the liquid then being forced out into the soil. In the writer's experiments, however, use was not made of these rather expensive contrivances, but of a simple dibble consisting of a pointed piece of broomstick. Holes were made to the depth of a foot to the number of eight or nine to the square yard. The desired amount of carbon bisulphid was poured into them, each being closed at once by the foot and the earth firmly pressed down to prevent the escape of the vapors into the air. About a teaspoonful to each hole is sufficient, or about 4 ounces to the square yard.

Potassium sulphocarbonate.—Potassium sulphocarbonate in the form of a solution of 1 part, by weight, to 5 parts of water to be applied in little trenches dug around the diseased trees is recommended by Gándara.[1] According to him, 4,000 liters of the solution suffice for a hectare—i. e., about 425 gallons per acre. His experiments were with nematode-affected coffee. This treatment he reports as being successful, but too expensive for general use. The writer's results, however, were not so successful. Papaya plants (*Carica papaya*), about 18 to 20 months old and with roots badly affected with root-knot, were used. The chemical, diluted as directed by Gándara, was applied to some trees in little ditches and to some in numerous holes about a foot deep. After it had all soaked in, the soil was watered thoroughly, as it was very dry, so that the chemical might the better soak evenly through the soil. In a day or two some of the old leaves dropped, showing that the roots had suffered some injury; but at the expiration of a few weeks the roots were found to be as badly knotted as ever, proving that for the papaya, at least, this process is ineffective. The high cost of the chemical, moreover, would make its use utterly impracticable.

Formaldehyde.—In view of the comparative success obtained with formaldehyde solution on roses it was tested on papaya trees out of doors. A ridge of earth was made around each tree at a distance of

[1] Gándara, 1906.

about 5 feet, so as to retain the solution. One part of commercial formaldehyde (about 40 per cent strength) was mixed with 100 parts of water. About 25 gallons were applied to each tree—i. e., about 3 gallons to the square yard. In some cases water was applied afterwards to cause the solution to penetrate deeper; in other cases no water was added. A few of the older leaves turned yellow and dropped off a day or two after the treatment, but no further injury was noticeable. In two weeks the nematode root galls, containing living nematodes, were found to be almost as numerous as ever, although a good many of the galls on the roots nearest the surface were found to contain dead nematodes. These and other experiments lead the writer to believe that where the soil is rather deep and the liquids applied can drain through instead of remaining in the immediate vicinity of the roots this formaldehyde treatment is not likely to prove very effective.

Calcium carbid.—The use of calcium carbid was also recommended by Gándara.[1] His instructions were to mix 4 parts of it with 1,000 parts of water. After letting it stand half an hour this milky solution is to be injected into the soil in five holes per square meter, 10 grams to a hole. Through lack of other trees suitable to test it on, papaya trees were also used in testing this method. A modification was also made in that about an ounce of the calcium carbid, without previous treatment with water, was placed in the bottom of 8-inch holes, which were promptly plugged with earth, about eight or ten holes being made to the square yard. Afterwards the soil was thoroughly watered. In this case a strong odor of acetylene was noticeable for two days. No damage was done to the trees and the nematodes in the galls were not killed by either treatment.

Other chemicals.—Various other chemicals recommended have the disadvantage that they are poisonous to living plants or too expensive. It is still possible, however, that some easily volatilizing liquid may be found whose vapors while fatal to the nematodes will not seriously injure the plants harboring them. Of those already mentioned carbon bisulphid has many desirable qualities; but its poisonous effect on vegetation is against it. It is possible that by applying it only during the dormant season of the plant and carefully regulating the quantity applied it may prove as effective as it is claimed by some investigators to be against phylloxera in the vine. The writer's experiments were mainly carried on at Miami, Fla., where there is no dormant season; hence this point could not be well determined. It is also conceivable that after a period of dry weather the chemical might be less harmful, as the trees would then be in a less actively

[1] Gándara, 1906.

growing condition and perhaps, therefore, less injured when the root hairs were killed by the chemical. Further experiments on this line should be carried out.

FERTILIZERS.

It is the result of general observation that if trees affected by root-knot can be forced into rapid growth, especially in the early part of the season, so that the roots penetrate deeply into the ground and form a widely branching system, they will thenceforward usually develop normally and cease to show much injury from the nematode. This is particularly the case with the peach. Many growers now on setting out an orchard where the pest is present fertilize the trees very highly, so that they may start right into growth and keep ahead of the nematode injury. As shown on page 41, the nematodes are mostly confined to the upper 12 to 16 inches of soil, so that if the roots can be forced to grow rapidly and deeply enough they will escape much injury. To accomplish this, it is necessary that the soil be prepared to a good depth before setting out the trees and that an abundance of nitrogenous fertilizers be given. The various potassium salts, too, are apparently very beneficial in the Southeastern States, so much so that some people believe that they destroy the root-knot nematode. Perhaps in the naturally rather potash-poor soils of many of the Southern States the addition of potassium is simply another factor in bringing the plant to its normal resistant power. At any rate, in the writer's experiments plants given an excess of potash suffered less from root-knot than those not so fertilized. It has been found in Germany that the sugar-beet nematode removes the mineral salts from the roots about equally. If, however, the soil is not much overstocked with potash it would be exhausted in the plant sooner than the others, for, being less abundant in the soil, it would be taken up less rapidly by the roots. The same would be true of any other of the necessary minerals. This may explain the effect of potash in combating this disease.

FLOODING.

In view of the fact that root-knot injury never seems to be severe in soils that are flooded for a part of each year it seemed reasonable to suppose that flooding might have a beneficial effect when applied to affected trees. Unfortunately, however, through a misunderstanding of instructions the experiments arranged to be carried out on this line failed to be performed. It is certain, however, that great care must be taken, for many trees are killed by having their roots submerged even a few days.

CONTROL OF ROOT-KNOT IN THE FIELD WHEN NO CROP IS PRESENT.

Land known to contain the root-knot nematode and not occupied by a permanent crop like an orchard, grove, etc., may be freed from the pest far more readily than land so occupied. The methods are the same, whether the land is to be planted subsequently to annual crops or to trees. The only difference is that land destined for perennial crops must be more thoroughly cleared of the root-knot nematode than that destined for simply one-year crops.

CHEMICALS.

Carbon bisulphid.—Carbon bisulphid is undoubtedly the most efficient chemical for the destruction of the nematode in fields. Experiments were made by the writer at Monetta, S. C., in 1906 and repeated in 1907, which showed that when used as previously described at the rate of 4 ounces per square yard of surface the nematodes were practically exterminated, being found only at the edges of the plats, where they could have come in from the surrounding untreated land. Two ounces per square yard did not prove so effective, although the nematodes were largely destroyed by even this application. In view, however, of the quantity required and of the high price of this chemical it is very evidently out of the question to apply it on a large scale. Even in bulk the crude carbon bisulphid costs 10 to 15 cents a pound. At 4 ounces a square yard the cost for an acre, not including cost of the labor required, would be from $120 to $180. Nearly all the chemicals that have been suggested have the same fault. Yet for small patches when it is desired, perhaps, to destroy the nematode where a tree is to be set out, or in a small spot where the pest has appeared but has not spread badly, it would probably be found very effective.

Formaldehyde.—Formaldehyde was tested at Monetta, S. C., in both 1906 and 1907, and at Miami, Fla., as well, in 1906. It was applied as a solution of 1 part commercial formaldehyde (36 to 40 per cent) in 100 or 200 parts of water. The solution was either sprinkled directly on the surface or poured into deep furrows, which were leveled off after the solution had soaked in. From 1 to 2 gallons per square yard of surface were used. As a whole, the treatment did not recommend itself. In no case were the nematodes entirely destroyed, although they were considerably reduced in numbers. The plants grown on these plats after the treatment showed the presence of root-knot galls on their deeper roots, although most of the upper layer of soil seemed to be free from the pest. This would indicate that a larger quantity would perhaps penetrate deeply enough to kill all the nematodes in the soil. With formaldehyde at 20 cents a pound, wholesale, the cost of treating an acre with the stronger solution,

2 gallons per square yard, would be about $150 exclusive of labor, which would include the hauling of 5,000 to 10,000 gallons of water.

Calcium carbid.—At Monetta, S. C., experiments were made with calcium carbid. It was strewn in furrows which were then covered over so that the resulting acetylene gas should penetrate throughout the soil, or it was applied as a solution in water. The amount of root-knot was reduced, but in all cases where the reduction was great the injury to the crops, especially to tomatoes, was also great. Better results were obtained from the dry application in 2-inch furrows than from the solution. Planting was not undertaken for a week or two, but still the results were such that in spite of replanting a second and even a third time the test crops—okra, beans, tomatoes, and cowpeas—were badly killed out. The odor of acetylene was perceptible for several days. The fairly effective amounts were 1,500 pounds per acre, dry, in shallow furrows or a solution of 5 pounds per 100 gallons of water applied in deep furrows, 1 to 2 gallons per square yard. In view of the high cost of the treatment (at 10 cents a pound this would be $150 per acre exclusive of labor for the dry application and $25 to $50 for the solution) this method can not be recommended. The injury to vegetation is also against it.

Potassium sulphocarbonate.—This salt is obtained commercially as a concentrated dark-brown solution, smelling strongly of sulphureted hydrogen. Gándara [1] states that it has been tried against phylloxera in France and recommends it for root-knot, at a rate of 1 part of potassium sulphocarbonate to 5 parts of water. Accordingly, the following experiments were outlined. Plats of land were laid off as follows: (1) Check, no treatment; (2) 10 parts of the chemical to 90 parts of water, 2 quarts per square yard in holes which were quickly filled; (3) 1 part to 99 of water poured on the surface at a rate of 2 gallons per square yard, that being the quantity necessary to wet the surface thoroughly; (4) a similar quantity of a solution of 1 part to 199 of water; (5) check. After a few days beans, tomatoes, okra, and cowpeas (New Era) were planted. In all cases where the chemical was used, both weak and strong, the tomatoes, okra, and beans were to a large extent killed, but the cowpeas were not hurt. Root-knot was present, however, even where the solution was the strongest. As a fungicide, too, this chemical had little value, for Rhizoctonia was very abundant at the crowns of all the plants.

For field use, then, this chemical is not to be recommended as a means of combating the root-knot nematode.

Ammonium sulphate.—Van Breda de Haan [2] recommended against the nematode on tobacco in the Dutch East Indies the use of ammonium sulphate followed by quicklime. The latter sets free the

[1] Gándara, 1906.

[2] Breda de Haan, 1905.

ammonia, which that author supposed might have value in destroying the pest. The writer's experiments at Monetta, S. C., were as follows: Plats of nematode-infested land 10 feet by 70 feet and 10 by 140 feet were laid off, separated from one another by ditches 2 feet wide. The chemicals were scattered on the surface and worked in with a cultivator or hoe. The rate per acre of the applications is here given, not the actual quantity put on the particular plats. (1) Water-slaked lime (quicklime put in a hole in the damp earth and left several days until slaked to a powder) 2 tons per acre, ammonium sulphate 1 ton per acre; (2) quicklime 2 tons, ammonium sulphate 1 ton; (3) slaked lime 2 tons; (4) quicklime 2 tons; (5) check. Summer squashes were planted on one half of each plat and New Era cowpeas on the other half, both these crops being very susceptible to nematodes.

Plats 3 and 4, respectively, slaked lime and quicklime, showed a very great abundance of root-knot, even more than plat 5, the check. The plants were pale in color and weak. Evidently lime in the quantities used is not effective against root-knot. In plats 1 and 2, ammonium sulphate plus slaked lime and quicklime, respectively, the squash roots were fairly badly knotted, especially in plat 1, but not nearly so badly as in plats 3 and 4 or in the check plat (5). The cowpeas were very dark green in color and very vigorous, and only moderately affected with root-knot, far less than plats 3 or 4, perhaps about like the check. The two plats with ammonium sulphate ripened their seed earlier than any other of the experimental plats. The next year these plats were again planted, this time to cowpeas, okra, tomatoes, and beans. The chemicals were not added, but observations were made to determine whether any beneficial effect might show the second year. The ammonium-sulphate plats were distinctly better than the check or those with lime alone, and were only moderately affected with root-knot, although by no means free from it.

Experiments similar to these but on a very much smaller scale were made in Miami, Fla. Quicklime, even at the rate of 5 tons to the acre, did not suffice to prevent nematode injury, while root-knot was quite abundant in a plat treated with quicklime at the rate of 2 tons per acre with 2 tons per acre of ammonium sulphate dissolved and poured over the surface.

We must then conclude that these chemicals are not of special value for the combating of nematodes.

Abbey[1] recommends using silicofluorid of ammonium at the rate of 1 ounce to a square yard. It must not be applied to soil containing living plants, as it will kill them. It soon decomposes and then is

[1] Abbey, 1898 and 1899.

harmless. Abbey also recommends 3 ounces of Little's soluble phenyl in 3 gallons of water applied around affected roots. Dyke[1] and Iggulden[2] also tried the latter, but Dyke found it a failure, claiming, however, that kainit was effective.

FERTILIZERS.

Closely related to the use of chemicals may be considered the effect of various fertilizers on the development of root-knot. At Monetta, S. C., the following fertilizers were tested in 1906, mostly in one-twentieth acre plats separated by ditches (or rather very deep furrows) 2 feet wide, the numbers in parentheses referring to the field numbers of the plats: (12) Kainit, 1,000 pounds per acre; (13) ammonium sulphate, 667 pounds per acre; (14) kainit, 500 pounds per acre; (15) high-grade potassium sulphate, 1,000 pounds per acre; (16) check; (17) high-grade potassium sulphate, 500 pounds per acre; (18) 17 per cent acid phosphate, 1,000 pounds per acre; (19) 17 per cent acid phosphate, 1 ton per acre; (20) check. In 1907 the following tests were made: (1) Kainit, 1,000 pounds per acre; (2) kainit, 1,500 pounds per acre; (3) high-grade potassium sulphate, 667 pounds per acre; (4) high-grade potassium sulphate, 1,333 pounds per acre; (5) ammonium sulphate, 1,000 pounds per acre; (6) muriate of potash, 1,000 pounds per acre; (7) potassium magnesium carbonate, 667 pounds per acre; (8) potassium magnesium carbonate, 1,333 pounds per acre. The checks received no numbers in 1907. The plats of that year and the checks were planted to tomatoes, okra, beans, and New Era cowpeas, all of which are very susceptible to root-knot. The last year's plats (1906 experiments) were also replanted in 1907 with these four plants. In 1906 the fertilizer plats were planted with New Era cowpeas and summer squashes. To all of the fields was applied each year, at the rate of 500 pounds per acre, a special brand of commercial fertilizer in common use in that vicinity, the soil being so poor that without some complete fertilizer nothing would grow well. The experiments were intended to show the effect, if any, of an excess of some particular fertilizer over the normal quantity applied.

The 1906 plats showed plainly the beneficial effects of potash fertilizers on the sandy soil of the experimental field. All the plats treated with kainit and potassium sulphate were darker green and the plants were far more vigorous than on the other plats. In fact, plats 12 and 15, respectively, kainit and potassium sulphate, both 1,000 pounds to the acre, were, so far as the cowpeas were concerned, hard to excel anywhere. The squashes did not show much difference in any of the plats. They were badly infested with the squash bug,

[1] Dyke, 1897. [2] Iggulden, 1898.

which killed the plants out in some of the plats. The cowpeas in plat 12 showed no nematodes and but few were present in the squashes. Plat 14 had a fair amount of root-knot in the cowpeas and from few to many on the different squash plants. The rest of the plats did not differ materially from the check plats which were fairly badly affected, in spots very badly.

The plants grown on these same plats in 1907 without the addition of the fertilizers again were badly affected except in plat 12, and somewhat in plat 15, which remained fairly free, showing a residual effect.

In the 1907 fertilizer experiments the following results were obtained. The kainit applications were injurious to the germination of the seeds, both the 1,000 as well as the 1,500 pound application, but naturally the latter more markedly. The amount of root-knot, however, in these plats was slight. Potassium sulphate at 667 pounds per acre was not injurious, but at twice that amount it so injured the germination of the cowpeas and beans that they required replanting. Root-knot was fairly abundant and, strangely, more so in the more highly fertilized plat. In both plats the growth of the plants was very vigorous. The sulphate of ammonia at the rate used exerted a very harmful effect on germination, requiring several replantings. The plants that did grow, however, were very vigorous, dark green, and rather free from nematodes. The muriate of potash injured the germination of the beans and cowpeas, while the nematodes were fairly abundant. The potassium magnesium carbonate gave the best and most vigorous plants of all, without injury to germination. Root-knot was present in most of the plants, but not abundant.

Judging from these experiments, it is clear that fertilizers alone can not be depended upon to exterminate root-knot. On the other hand it is also plain that some fertilizers exert a beneficial effect upon the plant and enable it to make a good crop in spite of nematodes. Perhaps they may also increase the resisting power of the plant against the entrance of the nematodes into the roots. The potash fertilizers seem to be most favorable for this purpose, so far as the experiments at Monetta and observations elsewhere go. However, it will not be safe to conclude that they will be equally beneficial everywhere. In the sandy, rather potash-free soils of South Carolina and Florida the application of potash in amounts not too large seems to be followed by favorable results.

According to Stift,[1] Hollrung, in Germany, has shown that fertilizing highly with potash alone is not of much benefit to beets attacked by the sugar-beet nematode. Wimmer has shown that the nema-

[1] Stift, 1908.

todes remove the different minerals almost equally, so that only where one element is rather deficient will the addition of that alone be of benefit. The sugar-beet nematode removes large quantities of mineral food from the roots, so that unless these minerals are present in the soil in considerable excess over that naturally needed by the crop the plants will suffer from lack of that mineral which is not sufficiently superabundant. Thus, an amount of potash sufficient for a healthy crop may be insufficient if the sugar-beet nematode is present, and the symptoms of potash hunger can be averted only by applying an excess of potash. Probably this is also true of the root-knot nematode. The sandy soils of South Carolina are rather potash poor, so that a diseased plant will suffer from potash hunger, while the other elements may be in sufficient abundance. At any rate, the addition of potash in excess proved helpful. The nitrogen-containing fertilizers when not in too great excess also benefited the plants somewhat, but not so markedly as the potash. This is to be expected, as nitrogen is not any too abundant in those soils. The phosphatic fertilizers, however, showed no benefit at all.

Caution must be taken not to apply too much potash. In 1907, in fact, kainit at 1,000 pounds per acre was harmful in that many of the young seedlings were killed, necessitating replanting several times in order to get a fair stand. This quantity was not harmful in 1906 on another plat, showing that the danger limit is probably not far below that amount. Muriate of potash at the same rate was very harmful in 1907, as was also the same amount of ammonium sulphate. Potassium sulphate, 667 pounds to the acre, and potassium magnesium carbonate, 667 and 1,333 pounds to the acre, were absolutely harmless, while the latter amount of potassium sulphate was only slightly harmful.

In spite of the high fertilization a field continually planted to nematode-susceptible crops will, if the nematode is present, eventually become so infested with that parasite that it will be impossible to make paying crops. However, it can not be denied that for special occasions it is of value to reduce part of the evil effects of the nematode infestation by high fertilization.

FLOODING.

The objections to flooding the soil that would apply in the case of land occupied by permanent crops do not hold good in fields devoted to annual or short-period crops. In the former case the soil can not be kept submerged longer than a few days or the roots are killed. In the latter case, however, the fields can be flooded for as long a period as desired before the crops are planted. There is no doubt that under such conditions flooding has value. This has already

been mentioned, reference being made to the conditions in the Everglade islands, where the never submerged tops of the islands are full of root-knot and the annually submerged sides are free from it. The writer has records of fields in Georgia badly infested with the root-knot nematode that were free from the trouble after a spring freshet that kept the ground submerged several days.

Apparently flooding, unless possibly of long duration, will not kill the nematodes inclosed within the root galls, so that if such knotted roots of perennial plants are present the flooding must be continued much longer. In Yuma, Ariz., under the writer's directions a field was flooded. It had once been a vineyard of Old World grapes, but these had become unprofitable owing to the ravages of the root-knot, and the vines had been cut down or pulled up. Many of the roots, however, were left in the ground. The next year the field was planted to melons. When the writer saw the field in May, 1907, the young cucumber and melon plants were dying from root-knot and the pest was found in the old living grape roots. The field was flooded the following winter, but root-knot was again prevalent the following spring, although apparently not so abundant. It seems likely that the vine roots may have harbored and saved from destruction many nematodes, or perhaps the flooding was not continued long enough. That under some circumstances even three weeks is insufficient appears to be the conclusion to be drawn from an experiment performed at the writer's suggestion by a fruit grower and nurseryman in California. He kept submerged for three weeks his field of sandy alluvial soil which was badly infested by nematodes. Afterwards grape cuttings and peach seedlings were set out in it. The grapes (a resistant sort, Rupestris St. George) showed no root-knot, but the peaches became knotted. This period seems excessive in view of laboratory results, and is not entirely free from doubt as to other possible means of infection, yet, until disproved, three weeks should be regarded as not enough time to exterminate the nematode by flooding.

It is of interest that flooding the soil is claimed by Stift[1] to be of no value against the closely related sugar-beet nematode.

Flooding, then, can not be recommended as a certain means of exterminating root-knot under all circumstances. Probably the soil should be flooded at least 25 days; in the laboratory the nematode larvæ usually succumbed much sooner when isolated and placed in water. Furthermore, no roots of perennial susceptible plants must be present. When water is expensive or means of flooding are not at hand, or when the soil is too porous, it will be out of the question to try this method. The subject is one, however, that needs further investigation. It will be of interest to call attention to the phenom-

[1] Stift, 1903.

enon often observed that a sloping field may have nematodes at its upper or middle portion and be free from them at the lower end where the soil is water-soaked part of the year.

DRYING.

Laboratory experiments by the writer seem to show that the root-knot nematode can not withstand the drying out of the soil. Thus, two pots of badly infested earth, containing badly knotted plants, were allowed to remain without watering from June 4 to September 22, 1908. The soil became very dry and dusty. It was then watered and seeds of susceptible plants were sown. These remained entirely free from root-knot. It is certain that the adults are killed by drying out, they being, indeed, very susceptible to injury of that kind. The foregoing experiments led the writer to the conclusion that thorough drying was fatal to larvæ and eggs as well. This was strengthened by the observation that in his cross-inoculation work where carefully washed root-knot roots of various plants were planted in sterilized pots of soil and seeds of the desired plants sown in the pots, infection was obtained wherever the roots used were fresh, while whenever they were somewhat wilted, not even dry, no infection was obtainable. Frank[1] and Stone[2] were also of the opinion that drying out was fatal to these nematodes.

On the other hand, there are several recorded observations which would seem to indicate that the opposite is true, at least sometimes. Thus, Göldi[3] dried the roots of coffee affected with root-knot, both in the sun and in the shade. After two months he wet them up and soon found, with the aid of the microscope, numerous nematode larvæ, which he considered to be those of the root-knot nematode. A second case was as follows: Prof. P. H. Rolfs, of the Florida Agricultural Experiment Station,[4] kept some sandy soil in the laboratory for 10 months. It became dry long before the expiration of that period. The soil was watered and tomato seeds were sown. The radicles of the seedlings became swollen and œdematous in a manner resembling the work of the root-knot nematode. No nematodes were found within the roots, but clinging to the outside were found nematodes which he identified as *Heterodera radicicola*.

Göldi's conclusions may have been erroneous, for there are many nematodes, almost indistinguishable from *Heterodera radicicola* in the larval state, that endure drying out for long periods. If they were examined only with the microscope and not tested in connection with living plants on which they could be grown to maturity, it would be almost impossible to tell whether those seen by Göldi were the one or the other. Prof. Rolfs, on the other hand, is not likely to have made

[1] Frank, 1885. [2] Stone, 1899. [3] Göldi, 1892. [4] Rolfs, 1894.

a mistake of this nature, performing the experiment as he did. Still it is not certain that he had *Heterodera radicicola* unless he actually had the mature nematodes, but on this point he says nothing. There are some other nematodes besides this species that cause root galls, and it is barely possible that it may have been one of these, not the root-knot nematode that Prof. Rolfs had, since this latter species is rarely even partially external in the tomato. Yet with the confirmation of these reports by Dr. Cobb's observations, it can hardly be doubted that under some circumstances some of the root-knot nematodes may survive drying out of the soil.

Whether the drying out of the soil kills all the root-knot larvæ or not, there is no doubt that their activity ceases and there is no injury by them in fairly dry soils. In a letter to the writer, C. P. Lounsbury, entomologist of the Department of Agriculture of the Cape of Good Hope, states that the nematode occurs only in loose soils well supplied with moisture. Badly knotted grapevines set out in rather dry soil not only recovered, at least in part, but the nematodes did not spread to surrounding susceptible plants. Lavergne [1] in Chile, Gándara [2] in Mexico, and Huergo [3] in Argentina also point out that dry soils are unfavorable to the development of root-knot. The writer has repeatedly sought for these nematodes in susceptible plants in dry soil outside of but in close proximity to badly infested irrigated fields in the semiarid parts of the country, but without success.

In view of the foregoing facts, it is probable that deep plowing, so as to loosen up the soil quite deeply without harrowing to pulverize it, would permit it to dry out sufficiently in a dry season to reduce greatly the injury from the pest. Of course, this is possible only where the climate is dry and the rainfall slight. In irrigated districts it could probably be carried on, such fields not being irrigated for some months after plowing. Of course this will not have much effect if underground seepage or rains keep the soil moist. Unfortunately the writer was unable to test the efficacy of this proposed method by direct experiment. It is a method that should be tested at the earliest opportunity in those regions where it can be carried out.

TRAP CROPS.

After Kühn, the great German agriculturist, had demonstrated [4] that the so-called Rübenmüdigkeit (beet tiredness) of sugar-beet fields was due to a nematode, *Heterodera schachtii*, he devised [5] a method of reducing the injury based upon the principle of trapping the nematodes in some susceptible plant and destroying the latter before the larvæ which had entered the roots had reached maturity. For his trap crop he used a sort of summer rape. This was sown closely and

[1] Lavergne, 1901. [2] Gándara, 1906. [3] Huergo, 1902, 1906. [4] Kühn and Liebscher, 1880.
[5] Kühn, 1881, 1882, 1886–1, 1886–2, 1891.

when the plants had grown long enough so that the first nematodes that entered the roots were not yet mature but were in the nonmotile stage they were plowed up and either removed and destroyed or turned under with the tops down and roots up. The plants treated in the latter manner died quickly and the nematodes in the exposed roots died within a few hours. By repeating this process several times (three to five) in a season the number of nematodes was found to be so reduced that good crops could be grown again for several years. In using this method extreme care must be taken to plow under or remove the plants at the right time, for if left too long the nematodes will reach maturity in the roots and lay eggs, thus increasing instead of diminishing the number of nematodes in the soil.

Frank [1] and others have also recommended this method for combating the root-knot nematodes. The writer has found no record of any such experiment having been tried. He made experiments on this line two different years at Monetta, S. C., but with no success. A badly infested field was separated from adjacent plats by a shallow ditch, 2 feet wide. The plat was sown very thickly to Whippoorwill cowpeas, a variety susceptible to root-knot. Roots from numerous plants were examined microscopically at short intervals to determine the stage at which the nematodes first entering the roots had become motionless and were approaching sexual maturity. At that stage the plants were destroyed, on one plat by plowing them under, on another by loosening the roots and removing and destroying the plants, roots and all. The time necessary to reach that stage was found to be from 19 to 21 days after the sowing of the seed. As soon as the trap crop was removed or turned under, the soil was made ready and resown with cowpeas, the process being repeated. This was done until four or five crops of cowpeas had been removed in this manner. The next year through these plats and the check plat were planted rows of tomatoes, beans, okra, and New Era cowpeas. Some of these plants remained free, while some were slightly affected and some very badly affected by root-knot, no difference being noticeable between the trap-crop plats and the check plats. This was true both in the experiments of 1906–7 and of 1907–8, which were conducted on another field.

The cause of the failure of this method can not be that a sufficiently susceptible host plant was not chosen, for the variety of cowpea used is very susceptible. Furthermore, cowpeas had been grown frequently on that land, so that the nematodes were, so to say, accustomed to that crop. The period of growth allowed was carefully checked by microscopical examinations so as to avoid any chance of letting the development of the nematodes progress too far, for if that

[1] Frank, 1885.

were permitted and egg laying were started the number of nematodes would be increased instead of diminished. Probably such large numbers were present that only a part entered the trap plants and were destroyed, enough remaining in the soil to infest badly the next year's crop. It is possible that some other crop would have done better, but it could not have been clover, as Frank suggested, for that did not do well where the experiments were being carried on. The requisites of a good trap plant are fairly cheap seed, great susceptibility to nematode attacks, a wide-spreading root system, and rapid growth. All these are possessed by the cowpea to a greater or less extent.

STEAM.

It has been seriously proposed to use steam to destroy nematodes in the field in view of the success with its use in the greenhouse, cold frame, and seed bed. The writer has made no experiments along this line, owing to the expense of the undertaking. It is seriously to be doubted whether a large field, producing a crop selling at $25 to $50 or even $100 net per acre, could be profitably piped for steam sterilization. Small fields isolated from danger of reinfection by deep ditches, water, stiff soil, or other obstacles and devoted to the intensive culture of some very remunerative crop might be so treated with profit. For a large field a very large boiler and many hundred feet of perforated pipe would be necessary to steam the soil by the greenhouse method.

Several schemes for sterilizing the soil in a field by means of movable apparatus have been devised, some of which have proved effective under certain conditions. Thus, for combating the Thielavia root-rot of tobacco, Gilbert [1] recommends the inverted-pan method of steam sterilization. This was devised by Mr. A. D. Shamel, of the Bureau of Plant Industry, for sterilizing nematode-infested soils in Florida. The following description is taken from Gilbert's account:

> The apparatus consists of a galvanized-iron pan, 6 by 10 feet and 6 inches deep, which is inverted over the soil to be sterilized and the steam admitted under pressure. The pan is supplied with steam hose connections, has sharp edges, which are forced into the soil on all sides to prevent the escape of steam, and is fitted with handles for moving it from place to place, the weight of the entire pan being not over 400 pounds. The soil is prepared as in the greenhouse method, a few potatoes being buried at a depth of a foot to gauge the degree of heat attained. A soil thermometer may also be used if desired. The steam should be kept at as high a degree of pressure as possible, 80 to 100 pounds being best, and the treatment should continue for one to two hours, depending on the pressure maintained. In experiments conducted in the spring of 1907, one hour's steaming at 80° C. under 100 pounds pressure gave best results in killing both the fungus and the weed seeds. When one section of the bed is treated, the pan is lifted and carried to an unsterilized portion and the operation repeated until the entire bed is steamed.

[1] Gilbert, 1909, pp. 35–36.

The great objection to this method, and one that makes it impracticable except for use on small spots, is the smallness of the area that can be treated at one time. Even with a pan of twice the area of that described, and allowing only one hour's sterilization each time, it would require more than 15 days, working day and night, to sterilize the soil on one acre of land. Furthermore, for deep soils, where, as already explained, the nematode sometimes is present at a depth of more than a yard, it is extremely doubtful whether the steam would penetrate deeply enough to destroy all the nematodes. This last objection applies to all methods of sterilization where an attempt is made to kill the nematode by heat or poisons.

FALLOW.

It is self-evident that if a field be kept free from all vegetation for a long enough period all the plant-parasitic nematodes within the soil will die from starvation. This is the principle involved in the use of the bare fallow. The field is plowed and kept free from weeds and other plants by frequent cultivation. In those localities where the winter is cold enough to prevent the further development of the nematodes during that period, it does no harm if grass or weeds grow up after the weather has become decidedly cool. This date might safely be put at November 1 for North Carolina, South Carolina, northern Georgia, Alabama, Mississippi, northern Louisiana, and northern Texas. In central and southern Florida and probably the southern portion of Texas and Louisiana, however, the nematode is active the year around, so that it would be necessary to keep the ground bare the whole time until the nematodes had died. In the early spring, where vegetation was allowed to grow in the winter, the cultivating to keep down the weeds must be taken up again before the soil begins to warm up. The length of time necessary to remain in fallow is not certainly known. Mr. A. D. Jackson, of Denison, Tex., found that 15 months in fallow was not sufficient to rid a field of root-knot nematodes entirely, although the number was greatly diminished. On the other hand, two whole years seem to be amply sufficient.

This method has some objections which make it impossible to use in some localities. The land is idle and not only not productive, but requires the expenditure of time and labor to keep the vegetation down. Furthermore, the light soils where the nematodes abound are easily leached out when there is not a covering of vegetation. Then, such soils are subject to bad washing during heavy rains when they have no plant roots to bind them in place. A further objection is the destruction of humus in the soil exposed directly to the action of the fierce summer sun. The use of this method therefore can not be universal, although it is successful where it can be put into effect.

NONSUSCEPTIBLE CROPS.

The most promising method, and the one that has given the best results wherever carefully tried, is that of growing crops that are not subject to root-knot until the nematodes causing the disease are starved out. To carry out this method successfully several things are requisite: (1) The crops planted must be free from nematode attack, so that the larvæ in the soil may not be able to find any nourishment to sustain their life and enable them to undergo their development. (2) The crop grown should at least pay the expense of working the land, as well as the rent, taxes, etc. (3) At the same time, if possible, the crops should enrich the land, or at least not impoverish it. (4) The plants must make such a vigorous, dense growth as to choke out all weeds or other plants that might harbor nematodes and permit them to develop and produce their numerous eggs.

On referring to the list of susceptible plants it will be seen that with few exceptions none of the ordinary farm crops fulfill the first requirement. However, the following plants appear to be free from nematode attack, at least under most conditions: Cowpea (the Iron variety), all species tested of Stizolobium (the velvet bean and close relatives), Florida beggarweed (*Meibomia mollis*), peanut (*Arachis hypogaea*), rye (*Secale cereale*), most varieties of winter oats (*Avena sativa*), crab-grass (*Syntherisma sanguinalis*), and possibly a few others. Webber and Orton [1] first called attention to the nematode-resistant quality of the Iron cowpea and recommended its use in combating root-knot. The velvet bean and beggarweed have been recommended by Rolfs,[2] of the Florida Agricultural Experiment Station, who has also pointed out the value of crab-grass in a plan of rotation for reducing the number of nematodes. Thus, he found the nematodes far less abundant the next year after an infested field was allowed to grow up to crab-grass for one year.

The following rotations were planned by the writer for his work at Monetta, S. C., there being four plats measuring, respectively, 0.152, 0.217, 0.217, and 0.166 acre:

TABLE III.—*Rotation of crops planned for four experimental plats at Monetta, S. C.*

Season.	Plat 1.	Plat 2.	Plat 3.	Plat 4.
Winter	Abruzzes rye	Abruzzes rye	Virginia winter oats	Virginia winter oats.
Summer	Beggarweed	Velvet bean	Velvet bean	Beggarweed.

This experiment was planned for three years. It was begun in the fall of 1905. It was planned to keep careful records of all yields, etc., but in some cases the records are lacking. Unfortunately, the soil

[1] Webber and Orton, 1902. [2] Rolfs, 1898.

proved so very poor for the oats that for it was substituted Abruzzes rye in succeeding years. Once each year the land was fertilized with the special commercial fertilizer previously mentioned at the rate of 500 pounds per acre.

The grain was harvested when mature, thrashed, and measured. As soon as the land could be put into proper condition the beggarweed and velvet bean seed were sown. In October a measured part of each field was carefully mowed and the vines cured to hay and weighed, thus permitting an approximate estimate of the actual yield per acre. The grain was sown as soon as the hay crop was cut and the land prepared. Unfortunately it was impossible, in addition to the substitution of rye for oats, to carry out the rotation just as planned, for in 1907 the beggarweed seed obtained germinated so poorly that those plats were resown to velvet beans, as it was then impossible to get good beggarweed seed.

In the summer of 1908 across the south edge of the field rows of tomatoes, beans, okra, and New Era cowpeas were planted to test the degree to which the nematode infestation had been reduced by two years of these rotations. In the spring of 1909 another strip was sown to the same four kinds of plants, the remainder being planted with two varieties of cotton, viz, Triumph and Columbia. A similar area to the north of the rotation fields was also sown to the same sorts of cotton, while to the east was a field of Peterkin cotton belonging to a renter and not planted with reference to the experiment. The choice of the field to the north was made through an unfortunate misunderstanding. It was not discovered until the planting was done and the plants above the ground that that field too had undergone somewhat of a rotation, viz, 1906, cotton; summer of 1907, Iron cowpea; winter of 1907–8, rye; summer of 1908, Iron cowpea; winter of 1908–9, rye. The field to the east, which was sown to Peterkin cotton, was in cotton for the third successive season.

The experiments were further interfered with by torrential rains which were harmful in two particulars, viz, they washed out much of the cotton and brought soil from nematode-infested fields and deposited it on parts of the rotation plats.

The yields on the plats were as follows:

TABLE IV.— *Yield of crops on four experimental plats at Monetta, S. C.*

Season and year.	Crop.	Actual yield.	Yield per acre.
Spring of 1906	Oats.....bushels..	.....	.....
	Rye.....do....	2	5.42
Fall of 1906	Velvet bean hay.....pounds..	About 4,900	11,300
	Beggarweed hay.....do....	About 1,575	5,000
Spring of 1907	Rye.....bushels..	10¼	14
Fall of 1907	Velvet bean hay on own plat.....pounds..	About 1,600	3,700
	Velvet bean hay sown late on beggarweed plat...do....	About 730	2,300
Spring of 1908	Rye.....bushels[1]..	10¾	14
Fall of 1908	Velvet bean hay.....pounds..	About 3,840	8,850
	Beggarweed hay.....do....	About 560	1,770
Spring of 1909	Rye [2].....do....	.....	.....

[1] 20½ bushels on 1½ acres; therefore estimated at 10¼ bushels for that field, 0.752 acre.
[2] Cut before ripening to allow cotton to be planted.

At the prices current at Monetta, S. C., for hay (about $18 per ton) and grain ($3 per bushel in 1909 for seed, but here estimated at $1 per bushel) the value of the hay produced in the three years amounted to about $117 and that of the grain to $22.50, a total of $139.50, at the rate per acre of $156, $30, and $186, respectively, an average of $62 per acre per year. While these yields are probably considerably more than enough to pay for working the land and the rent of the land besides, as well as payment for the seed, velvet beans having cost about $4 per bushel, it must not be concluded that the experiment was a failure in that the yields were not greater, for the primary purpose of the rotation was to reduce the nematode infestation while improving the land, or at least keeping it from deteriorating, and yet to make enough money to pay for the labor and seed used.

To test to what extent, if any, the land was improved was the purpose of planting a plat of cotton at the north of the rotation plat. Unfortunately, so many plants in each section were washed out by the heavy rains that a very poor stand was obtained, with the result that the yield per acre on the rotation and check plats could not be determined. The yields of the unginned cotton on the rotation plat were at the rate of 1 pound of cotton for 6 plants of Triumph and 6.1 plants of Columbia, while on the control plat to the north it took 6.9 and 7.25 plants, respectively, to make a pound. The Peterkin plants to the east were not half as large and yielded even less.

The soil which at the beginning was very poor in humus, so poor in fact that the rye would scarcely grow and the oats did not pay for cutting, gave a much better appearing field of rye the following years. The foliage of the cotton on it had a good color, showing that the leguminous crops had increased the nitrogenous content of the soil.

From the standpoint of nematode extermination the results were very satisfactory. Both in 1908, after two years of this rotation, and in 1909, after three years, the susceptible plants on part of the plat remained free from root-knot except as specified below. These plants were, as in previous tests, tomatoes, okra, beans, and New Era cowpeas, all extremely susceptible to root-knot attacks. Several rows of each were planted in 1908 along the southern edge of the plat, and in 1909 on the part just adjacent to that on the southern part of that portion of the field which had had a rotation of three years. Every plant was carefully dug up and all its roots examined after freeing them from the adhering soil. Every such plant was recorded as free, slightly affected, or seriously affected, a separate record being kept of all the plants in each hill.

The field slopes very gradually toward the south from higher, somewhat nematode-infested land on the north. Two slight depressions lead somewhat diagonally from the northwest to the southeast. In the spring of 1908 and again in the early summer of 1909 Monetta was visited by torrential rains which flooded and very badly washed the fields. Considerable soil from the fields to the north, and especially the badly infested field to the west, was washed down these depressions, settling on them and in the lower (southern) edge of the rotation field. Where these deposits of dirt occurred, and confined to these areas, some of the plants showed more or less nematode injury, most near the middle and least along the edges of the depressions. Furthermore, a few plants at the edges of the field, i. e., at the east and west ends of the rows, showed nematodes where they were probably introduced from the adjoining land in cultivating, plowing, etc. All the rest of the plants remained nematode free, although this field was badly infested before the experiment began.

In accordance with suggestions of the writer, Mr. A. D. Jackson, of Denison, Tex., made some rather similar experiments, using Iron cowpeas and rye as his rotation. Certain fields were very badly infested, so badly, indeed, that the crops on them were almost a total failure. By growing the cowpeas two seasons with rye as the winter crop the nematodes were so reduced in number that only 20 hills of cantaloupes out of half an acre were affected with root-knot and the crop of melons was excellent. Under date of July 10, 1909, Mr. Jackson wrote as follows:

> I am well pleased with the Iron pea. While I have not eradicated the pest entirely by growing the pea two seasons, I have enriched my soil, have grown a large crop of feed, and the succeeding crop of vegetables has not in any case been materially affected (by nematodes).

In Mr. Jackson's fields the writer's and Mr. Jackson's conclusions were that the few nematodes surviving were those that were pro-

duced on the few weeds whose presence it was impossible absolutely to prevent in the cowpeas. Thus, the weed known as careless weed (*Amaranthus* sp.) was found to have root-knot in the field of Iron cowpeas the second season these were grown.

Mr. Jackson also made the experiment of using summer fallow in combination with winter rye, as follows: The preceding crop was taken off the summer of 1906, being badly knotted. The field was then kept in bare fallow from August, 1906, until the fall of 1907, when it was sown to rye. This was turned under when about mature, and in July, 1908, the field was sown to tomatoes (which are especially susceptible to root-knot). A fine crop of tomatoes resulted, the only nematodes present being in a small part of the field where Irish potatoes were badly attacked in 1906 and where volunteer potatoes came up in 1907. The remainder of the field remained free the succeeding year also (1909).

Prof. P. H. Rolfs [1] recommends letting the field grow up to crabgrass (*Syntherisma sanguinalis*) after the crops are removed, first taking up and burning or otherwise destroying the plants to avoid infection from them. According to him this method when used even for only one year greatly reduces the number of nematodes present. Dr. Neal [2] recommended the use of beggarweed, Japan clover, or Mexican clover. Regarding the latter the present writer knows nothing, but the first two are practically, if not entirely, immune and so ought to be valuable for this purpose.

This method was used with complete success by Schroeder [3] in Germany against the stem nematode (*Tylenchus dipsaci*) after all other practicable methods had failed. He planted infected fields for a series of years with crops not susceptible to the nematode. After this period the fields gave again their normal yields of susceptible plants.

RECOMMENDATIONS FOR FREEING A FIELD FROM ROOT-KNOT.

In view of the results of the experiments described, the writer would make the following recommendations for freeing a field from root-knot. If the situation is one where the winters are cold and cool weather sets in in October, it will not be necessary to give attention to the subject during the fall and winter or in the spring before the ground begins to warm up. Under such conditions it would probably suffice to plow the land in the autumn, so as to have it in good condition for as early planting as possible in the spring. In the spring the field should be kept free from vegetation by cultivation or harrowing until the ground is warm enough to plant cowpeas. The field should then be planted thickly with Iron cowpeas, this

[1] Rolfs, 1898. [2] Neal, 1889. [3] Schroeder, 1902.

variety being usually sufficiently resistant to the root-knot to permit its use for this purpose. In the fall this can be cut for seed or hay. The ground should then be plowed up and the process repeated the next season. Except in exceedingly bad infestations, two seasons devoted to Iron cowpeas should be sufficient to free the land from the pest. If desired, some winter grain, preferably rye or perhaps wheat, may be sown in the fall, the cowpeas not being planted until the crop is harvested early the next summer, following them by grain again. Where the weather remains warm rather late in the fall it would be desirable always to do this and so prevent the growth of weeds which might harbor the nematode in the fall and winter. Where the summer is long enough, velvet beans or Florida beggarweed are perhaps preferable to cowpeas, as they give a denser growth that more completely smothers out all weeds. Special care must be taken that in the summer time no weeds are allowed to grow in the field, as it will be seen by reference to the list of susceptible plants that many of the common weeds harbor the nematode. Their presence in the field, therefore, would serve to perpetuate rather than kill the nematode.

Where practicable, the surest results can be attained by keeping the ground absolutely bare of all vegetation for two years. This can not be done on some soils, owing to the danger of the destruction of humus by the hot sun or of washing by heavy rain.

Where the field is free from roots of perennial plants which might shelter the pest and is so situated that it can be submerged easily for long periods, it may pay to flood the land for three or four weeks, or perhaps during the winter. This would be impracticable except in a few locations. Furthermore, in many soils it would leach out all the plant food and make the soil poor, but where an impermeable layer will hold the water and keep it from leaching out it is conceivable that this method might be found very satisfactory. A short period of flooding or attempting to do this while the soil contains perennial roots containing the nematode will hardly prove successful.

In the irrigated districts of the West, special care should be taken to avoid the introduction of this nematode into lands devoted to potato raising. To this end only perfectly sound, clean potatoes should be used; no potatoes from suspected regions should be planted, even should the individual potatoes appear perfectly healthy, without a preliminary sterilization with formaldehyde solution to destroy any nematodes present in the adhering soil.

Should none of the foregoing methods be feasible, high fertilization, especially with that element (potassium calcium or phosphorus) which is most nearly deficient in the soil, will prove helpful, although it will not kill the nematodes. When, as is often the case in

the sandy soils of the southern United States, the soils are already deficient in potash, rather strong applications of some of the potash fertilizers—for example, kainit, potassium magnesium carbonate, sulphate of potash, etc.—are very helpful. Care should be taken not to apply enough to prevent the germination of the seed.

BREEDING STRAINS RESISTANT TO ROOT-KNOT.

As already mentioned, Webber and Orton have shown [1] that the Iron variety of cowpea is practically immune to root-knot and wilt (*Neocosmospora vasinfecta*), while most other sorts are exceedingly susceptible to both diseases. The latter investigator has continued his breeding experiments, using the Iron cowpea as one of the parents, and has produced several varieties more prolific than that sort in which the resistant characteristics are present. Similarly in the breeding of tobacco, Shamel and Cobey [2] obtained a strain resistant to nematodes. Certain sorts of figs—for example, Celeste and Poulette—are said to be less subject to injury by nematodes than other kinds. Among grapes, so far as the writer's observations go, the Old World species (*Vitis vinifera*) seems to be especially liable to injury by root-knot, although the different sorts vary greatly in their susceptibility. Thus, Zinfandel and Muscat appear very subject to this trouble, while Sultanina (erroneously called Thompson Seedless) is apparently not so easily injured. Some of the phylloxera-resistant hybrids and pure American sorts are practically immune to root-knot as well as to phylloxera, although some American sorts are quite badly affected by the nematode. These observations of the writer are confirmed by Lavergne, who states [3] that the European varieties are very susceptible to *Anguillula vialae*, as he calls the root-knot nematode, while those of American origin that are resistant to phylloxera are also resistant to root-knot. Of the watermelon-citron hybrids bred by Mr. Orton with resistance to wilt as the main aim, it was found by the writer that of one strain only 4 out of 333 plants showed root-knot, i. e., 1.2 per cent, while in two other strains 28 and 51.9 per cent, respectively, showed root-knot. The presence of such marked differences shows that it would be entirely feasible to breed a watermelon variety that would be practically immune to root-knot as well as to wilt. Bouquet de la Grye [4] points out that *Coffea liberica* is less susceptible to root-knot than *C. arabica* and recommends grafting the latter upon the former. To obtain a firm union, this must be done by an approach graft with seedlings.

Simple selection can be and ought to be practiced by everyone who grows his own seed; more complicated breeding work, unless per-

[1] Webber and Orton, 1902. [2] Shamel and Cobey, 1907. [3] Lavergne, 1901. [4] Bouquet de la Grye, 1899.

formed by men who can devote considerable time to it, hardly pays for the time and expense required.

In carrying out simple selection we must remember that no new characters are originated by this method. We simply select and strive to fix in one strain certain characters that are present as variations in the plants we are working with. Thus, if we find in a field badly infested with nematodes that a certain proportion of the plants are free from root-knot while the rest succumb, it would probably pay to begin selecting seed from the unaffected plants. It is better still if we can inbreed or intercross similar resistant plants. On the other hand, resistance to nematodes seems sometimes not to be one of the variations occurring in a plant. Such a plant can not be selected, as there is no foundation on which to build. However, by crossing it with some nearly related nonsusceptible sorts, some of the progeny may possibly show desirable qualities of resistance while at the same time preserving the best qualities of the parent sorts.

In all such breeding it must be borne in mind as a very important principle that this work should be done in badly infested fields. If naturally infested fields are not available, provision should be made to do this work where the disease is abundant.

No attempt will be made here to describe the methods of selection or hybridization. These are known to all seed growers and breeders. They can be found described in detail in many publications.[1]

Every farmer ought to be able at least to carry on this simple selection: When any plants in an infested field show special vigor and freedom from root-knot they should be marked and the seed collected before the main crop is gathered. This should only be done, however, if these resistant plants are also up to standard in all other features.

SUMMARY.

(1) The disease known as root-knot, characterized by enlargements of the roots and often leading to the death of the plant affected, is caused by a nematode (*Heterodera radicicola* (Greef) Müll.). This was probably originally native in the Tropics (of the Old World?), but has spread into nearly every part of both Temperate Zones.

(2) The plants recorded as more or less subject to attack number almost 480 species and varieties, including nearly all of the larger families of flowering plants. Probably many more are actually susceptible, but have not been reported yet as hosts. Most of the important field and garden crops and ornamental plants are more or less subject to root-knot.

[1] Hays, 1901; Bailey, 1906; Orton, 1909; Reed, 1909; Salmon, 1907; Spillman, 1909; Wilcox, 1903; Oliver, 1910.

(3) The life cycle of this nematode, from egg to egg, may take place in four weeks, or longer, depending upon the temperature of the soil. The larval stage is that in which entry into the host takes place. It then becomes motionless and soon enlarges and undergoes a sort of metamorphosis, the males eventually recovering the original worm shape, while the females become pear or flask shaped and very much enlarged in their transverse dimensions. Each female lays 500 or more eggs. The winter is passed probably most frequently in the larval stage in the soil, but in the case of galls on perennial roots the nematodes may overwinter in these in a more advanced stage, even as practically mature and perhaps already fertilized females.

(4) For the rapid multiplication of the root-knot nematode the following conditions are necessary: (*a*) A certain degree of warmth of the soil. Thus, in southern Florida this nematode is active the year round, in part of South Carolina the active season is from April 20 or May 1 to the middle or end of October, while farther north the period is still shorter. (*b*) Loose-textured soil. Only sandy or at least light soil is favorable to its spread. (*c*) Moisture. The drying out of the soil is frequently fatal to the nematode and in any case prevents it from doing any harm. Apparently the moister the soil as long as it is well supplied with air, the more favorable it is to the nematode's development. However, wet soil, i. e., soil in which the air spaces are filled with water, is at length fatal to the nematode. (*d*) Food supply. The larvæ are able to exist in the soil for more than one year, but apparently not for two years, without the presence of living plants into which to enter. They are apparently unable to develop beyond the larval stage unless they enter a suitable host plant.

(5) The nematode is distributed in several ways: (*a*) The larvæ move through the soil by their own motion, but the distance traversed thus is probably not more than 6 feet or so a season. (*b*) They are carried from field to field in the earth clinging to implements, the hoofs of animals, the shoes of laborers, wagon wheels, etc. (*c*) They are conveyed in the soil that is washed from one field to another by heavy rains, a very common mode of distribution of this pest. (*d*) It is possible that heavy winds may carry larvæ or eggs with the soil blown from one field to another, but probably most would be so dried out in the process that this is not much to be feared. (*e*) They are introduced into new places in the roots or in the dirt adhering to the roots of nursery stock, in rooted cuttings, potted plants, etc., especially those of the peach, grape, fig, mulberry, potato, ginseng, etc.; also in the dirt in which some seeds are packed. (*f*) They are

sometimes brought to a field in manure if the manure pile has stood on infested soil.

(6) The following methods of control in greenhouses and seed beds may be used: (*a*) The most efficient method is the use of live steam at fairly high pressure. The steam is forced through a system of perforated pipes laid at the bottom of the bed or bench. (*b*) The old infested soil may be entirely removed and the benches thoroughly cleaned out. Then noninfected soil may be put in its place. This method is not advisable in regions where the nematode occurs out of doors in the vicinity. (*c*) Infected soil, when it is desired to save it and steaming is impracticable, may be freed by allowing it to lie through the winter in a place where it will be exposed to alternate freezing and thawing, and especially to drying. (*d*) Soil containing perennial plants can be nearly if not quite freed from nematodes by the use of an abundance of a solution of formaldehyde (1 part of commercial formaldehyde to 100 parts of water). This solution is fatal to many plants and can be used only with great caution.

(7) For the control of the nematode in the field where the land is occupied by perennial crops no entirely satisfactory chemical application can be recommended. Places where trees are to be reset should be freed from nematodes by the use of carbon bisulphid at a rate of 3 or 4 ounces per square yard placed in about nine holes per square yard, these holes being about 6 to 12 inches deep and to be filled with dirt as soon as the chemical is placed in them. Carbon bisulphid can not be used with safety around living trees. Flooding the land seems to be unsatisfactory, as flooding long enough to kill the nematodes is usually fatal to the trees. High fertilization and constant cultivation to induce growth often so help the trees that they are able, as it seems, to outgrow the trouble, the roots either penetrating to levels where the nematodes are less abundant or being formed faster than the galls can be produced. Avoid growing susceptible cover crops, like the ordinary nonresistant varieties of cowpeas, for example, for these multiply the nematodes in the soil manyfold. In preparing the land for setting out a perennial crop the soil should be freed from nematodes by the use of the methods suggested below.

(8) For land infested with nematodes and not bearing a perennial crop, the following methods may be recommended: (*a*) Keeping the land free from vegetation of all kinds for two years. This is the most effective method, but it is not practicable in many cases. (*b*) Planting the land to nonsusceptible crops for at least two (perhaps better three) years, using in the winter small grains, such as wheat, rye, or oats, and in the summer the velvet bean, Florida beggarweed, the Iron cowpea, or even peanuts, scrupulously destroying all weeds that might harbor the nematodes. (*c*) Making heavy applications of

fertilizers, especially those containing potash, except where the soil already contains this in abundance. This treatment often reduces nematode injury greatly. (*d*) Flooding the land for a period of some weeks. (*e*) Where rain is not likely to interfere, plowing and allowing the soil to dry out for several months. (*f*) Preventing, by the use of embankments, ditches, etc., the washing of soil from infested fields to the field which it is desired to free from the pest. The introduction of the pest by tools, wagons, farm animals, etc., should be avoided. The trap-crop methods and the use of various chemicals have not proved practicable as tested by the writer. The former needs, perhaps, further trial.

(9) The ideal procedure is to develop nonsusceptible strains of plants, so that the expense and trouble of exterminating the pest may be avoided. Such strains may be obtained by the selection of more resistant plants or by crossing with resistant strains followed by the careful selection and breeding of the progeny.

NOTE.—While this bulletin was in press, there appeared a note in Science,[1] by L. N. Hawkins, describing the occurrence of *Heterodera radicicola* in the roots of *Typha latifolia* near Ithaca, N. Y.

The writer has just received from Mr. G. L. Fawcett, plant pathologist of the Porto Rico Experiment Station, Mayaguez, P. R., specimens of the bark near the base of a 15-year-old coffee tree. Mr. Fawcett writes: "The disease is characterized by a roughening of the bark at the base of the coffee tree, extending from the surface of the soil upward for a foot or two. No doubt it injures the tree, but such injury must be slight. I have seen no sick tree the bad condition of which could clearly be ascribed to this nematode; only a small percentage of the trees in any plantation are infested. It is perhaps more common in moister and more shady places. Older trees, say, those of 15 years or more, are the only ones noticed with this disease." The living portion of the cortex was found to be very densely infested with mature females of *Heterodera radicicola*. It seems probable that these nematodes must have passed upward through the soft tissue of the cortex from some original infection in the root. It is worthy of note that sometimes in herbaceous plants, such as tomato, the writer has found nematodes 6 inches or more above the level of the ground within the cortical tissue of the stem.

[1] Science, n. s., vol. 34, no. 865, July 28, 1911, p. 127.

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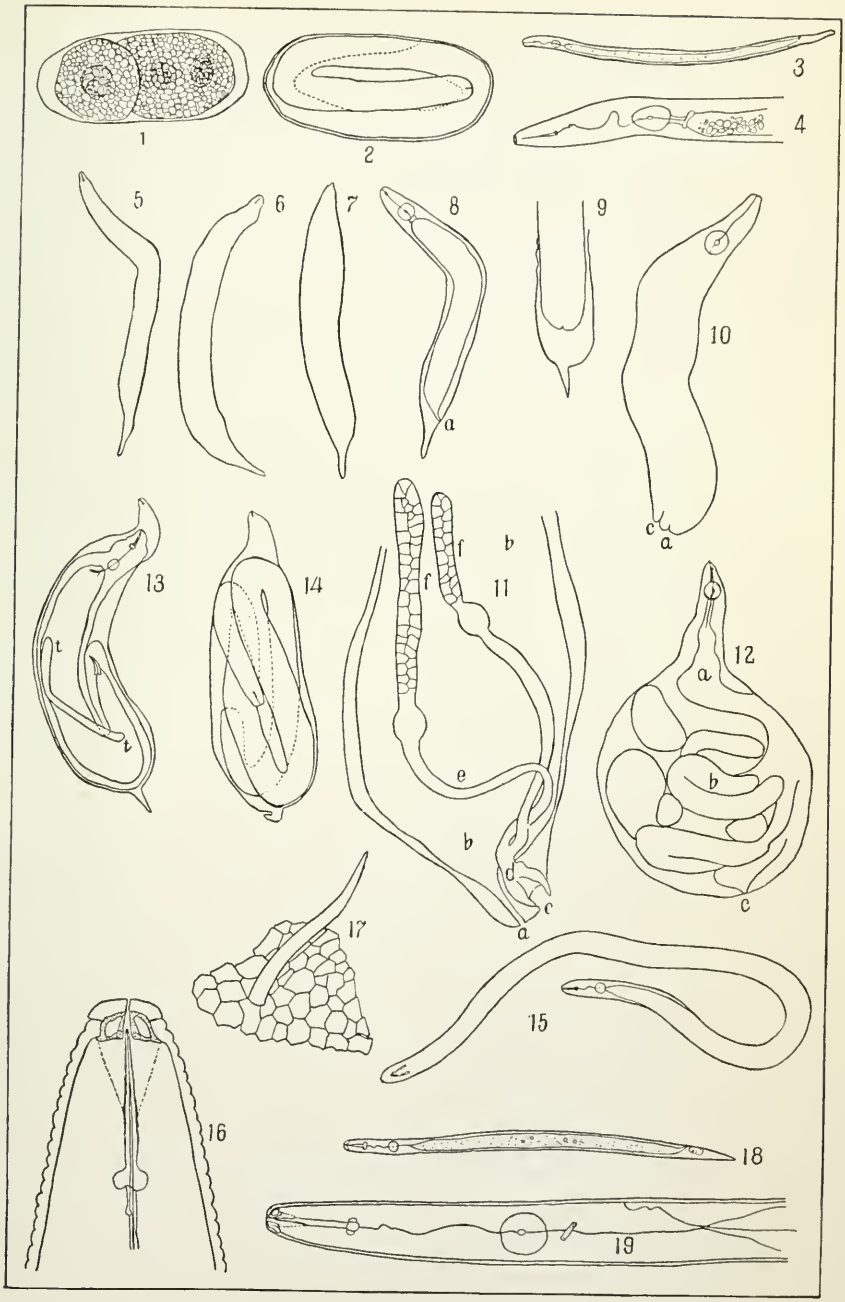
DESCRIPTION OF PLATES.

PLATE I. Stages in the development of *Heterodera radicicola* (Greef) Müll., etc. Figs. 1 and 2.—Eggs in two different stages of development, × 350. Fig. 3.—Larva immediately after escaping from egg, × 105. Fig. 4.—Anterior portion of same, × 410. Figs. 5 to 8.—Developmental stages of larvæ before sexual differentiation is apparent, × 105. Fig. 9.—Molt in which sexual differentiation first becomes apparent, female nematodes approaching sexual maturity, × 105. Fig. 10.—Sexually mature female nematode, a somewhat more advanced stage than shown in figure 9, × 105. Fig. 11.—Posterior portion of sexually mature female nematode somewhat compressed, × 220: *a*, Anal opening; *b*, alimentary canal; *c*, genital opening; *d*, vagina; *e*, *e*, uteri; *f*, *f*, ovaries. Fig. 12.—Egg-bearing female nematode, × 47: *a*, Alimentary canal; *b*, loop of uterus; *c*, genital opening. Fig. 13.—First visible stage in differentiation of the male nematode (compare with fig. 9), × 105: *t*, *t*, Testis. Fig. 14.—Mature male still within larval skin, × 85. Fig. 15.—Mature male, × 85. Fig. 16.—Anterior portion of adult male, showing spear and peculiar structure for guiding its movements, × 930. Fig. 17.—Larva entering root of clover, × 100. Fig. 18.—Larva of *Heterodera schachtii* Schmidt just escaped from egg (compare fig. 3), × 105. Fig. 19.—Anterior portion of same, × 435.

PLATE II. Fig. 1.—Root-knot on sugar beets grown at the Subtropical Laboratory, Miami, Fla. 1907. Photographed by E. A. Bessey. Fig. 2.—Root-knot on squash, from Beeville, Tex. 1904. Photographed by W. A. Orton.

PLATE III. Fig. 1.—Root-knot on carrot, from Morrison, Ill. 1908. Photographed by W. W. Gilbert. Fig. 2.—Root-knot on red clover grown in a pot of sterilized soil inoculated with affected roots of *Ipomoea syringaefolia*, Subtropical Laboratory, Miami, Fla., 1908. Photographed by E. A. Bessey.



STAGES IN THE DEVELOPMENT OF HETERODERA RADICICOLA (GREEF) MÜLL., ETC.

FIG. 1.—ROOT-KNOT ON SUGAR BEET.

FIG. 2.—ROOT-KNOT ON SQUASH.

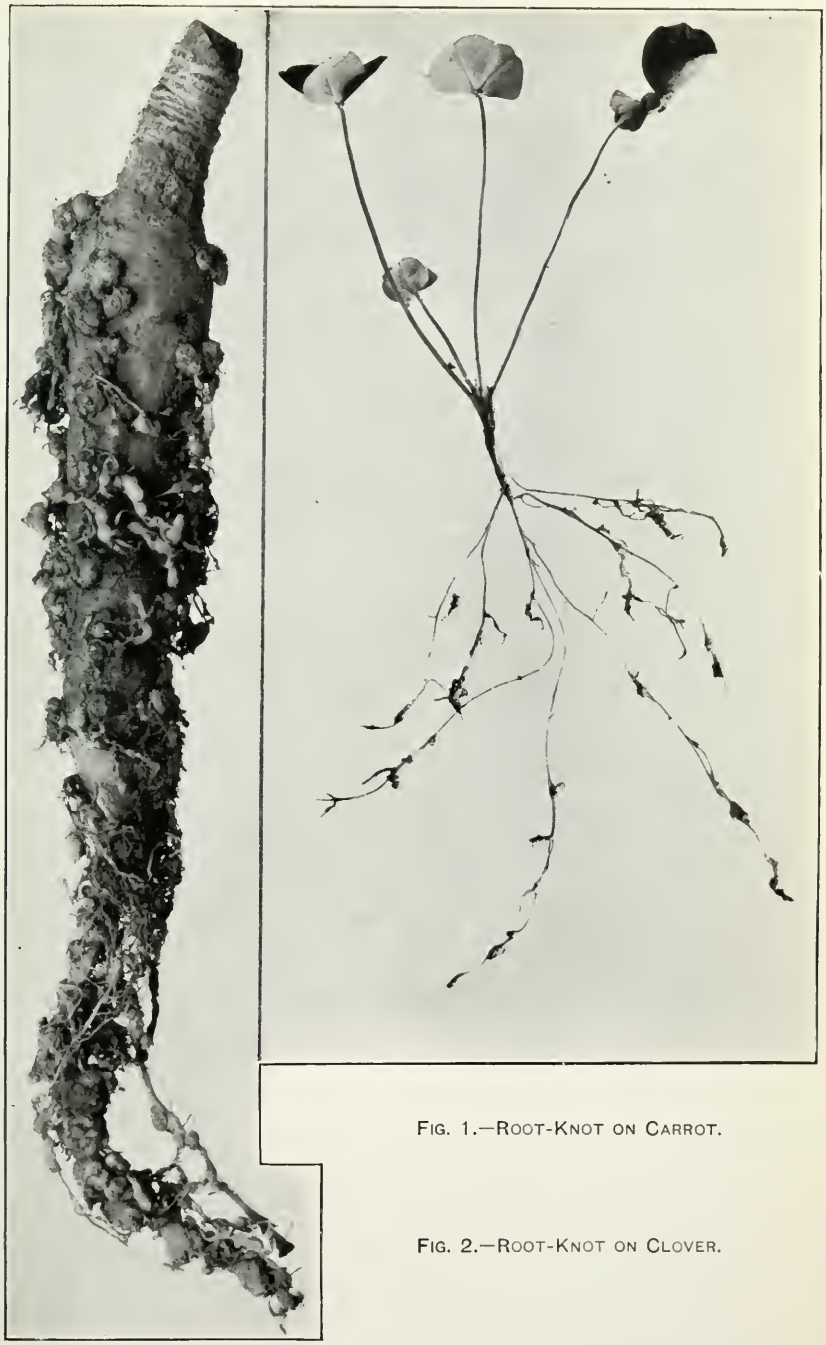


FIG. 1.—ROOT-KNOT ON CARROT.

FIG. 2.—ROOT-KNOT ON CLOVER.

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