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Corn Root Rot Studies

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The experimental data reported in this paper show that most of the kernels on practically every ear of corn grown in Missouri are internally infected with one or more of the following organisms: *Diplodia zeae*, *Fusarium moniliforme*, and *Cephalosporium acremonium*. Yield tests comparing heavily infected and lightly infected seed show that reduction in yield from planting heavily infected seed is due to reduced field stands caused by seedling blight and not to corn root rot. By increasing the planting rate of heavily infected seed over lightly infected seed so that nearly equal stands were obtained, the resultant yields from both lots of seed were made nearly equal. The employment of certain physical ear characters in selecting lightly infected seed corn was found to be more practical than the germinator method. Inoculation trials carried on in the field and in the greenhouse with *Diplodia zeae*, *Fusarium moniliforme*, *Cephalosporium acremonium* and *Gibberella saubinetii* showed these organisms were capable of producing a certain amount of seedling blight but not corn root rot, which develops as the corn plant nears maturity. A Pythium-like organism isolated from diseased corn roots was used to inoculate disease-free seedlings grown in uninfected soil in 1927. Typical corn root rot resulted only in the early plantings. From these diseased corn roots the organism used for inoculation was reisolated in pure culture. Corn root rot in Missouri is probably caused by a soil-borne Pythium-like fungus.

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Corn Root Rot Studies

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Corn root rot is a term used loosely for a number of more or less well known parasitic and non-parasitic diseases of corn which include corn root, stalk and ear rot diseases. Holbert and his co-workers, in a recent publication, stated: "For brevity, these diseases are sometimes called 'corn rot diseases' and frequently simply 'corn root rot'. However, at the outset, it must be realized that 'corn root rot' is not one disease, but several diseases, some of which do not result in any rotting of either roots or stalks." Thus one realizes that the corn root rot problem at the present time is a complicated one and sufficient attempts have not been made to separate the different diseases and to evaluate properly each of them.

In the earlier work on the problem, particularly by Hoffer and Holbert[43,55] at Indiana and Illinois respectively, the selection of good seed corn was emphasized as the most important factor in controlling and preventing corn root rot. It was suddenly realized that nearly every ear of corn was infected with one or more fungous organisms and by special methods of germination the least infected ears, which could be planted with no harmful effects from that source, could be separated from the others. The connection between infected seed corn and corn root rot was taken for granted, and, at first sight, was quite apparent. But confusion arose when seedling blight and rotting of full grown plant roots were considered to be due to the same causes. So much attention was given the seed-borne pathogene phase of the problem that the organisms directly responsible for and associated with the rotting of roots on full grown plants were studied very little. Seedling blights either killed the plant in the seedling stage or stunted it so that in many cases it never fully recovered, and thus produced an inferior plant with a small ear or no ear at all. So much stress was given to this idea that in much of the earlier literature delayed development after seedling blight injury was considered to be typical corn root rot. Valleau[118] rightly points out, in this connection, that; "If these effects of seedling blight are to be considered corn root rot, the fact should be recognized. If however, there is a true corn root rot other than seedling blight, the two diseases should be recognized clearly and a distinction should be made between them in future literature."

There is unquestionably a disease, or group of diseases, that causes seedling blight of corn. This disease should be called corn seedling blight

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since no one has confirmed his original work. Burrill himself never named the organism which he considered the cause of the corn disease he observed, but according to Rosen[96] the name *Bacillus zeae* Burrill was given it in 1892 by Russell[97] who probably for convenience adopted a name and used for authority the name of the man from whom he had obtained his cultures. Rosen[94,95], while working on another bacterial disease of corn, examined the specimens left by Burrill at the University of Illinois and found them to show various blade and sheath spots, quite comparable to the spots described by Durrell[12] and attributed to *Diplodia zeae*.

Rosen[94] described a bacterial root rot of field corn in 1919 which he considered at the time to be identical with the disease reported by Burrill in 1889. In a more recent paper, however, Rosen[96] described the disease as a stalk rot only and named the causal organism *Phytomonas dissolvens* (comb. nov.). It was in this latter publication that he mentions having examined Burrill's specimens and considers the disease described by Burrill to be in no wise similar to the disease produced by *Phytomonas dissolvens*. The disease has been observed in Arkansas and many other central states for a number of years. The disease is considered to be serious in Arkansas only during those periods of the corn growing season when the temperature, rainfall, and humidity are above normal. In the absence of high temperatures and humidity, the disease is found to be entirely absent or of minor importance. It is described as "primarily a disease affecting the stalk and leaves exhibiting the following symptoms: (1) a light or dark brown rotting of bases of leaves, particularly those at the base of the stalks; (2) a rotting of the lower portion of the stalk, the affected parts being dark brown, soft, putrid, and sunken in fresh infections. These may extend through the entire width; or, as more frequently found, remain localized as dark, rotted spots with margins appearing water-soaked. The disease consists of a localized necrosis of parenchymatous tissue."

The first mention made in the literature of organisms now considered to be connected with the corn root, stalk, and ear rot problem was by Sheldon[101] in 1904. He described a new species of fungus, *Fusarium moniliforme*, found on ears of corn from many farms in Nebraska. At that time the pink growth formed by *Fusarium moniliforme* on the ears of corn was thought to be the cause of certain diseases of livestock, particularly "staggers" in cattle, but Sheldon attempted to do no more than describe the organism and its appearance on corn ears.

The first suggestion made in the literature that *Diplodia zeae* is a parasite on corn was made by Heald[40] in 1906. He stated in a paper entitled: "New or Little-known Plant Diseases in Nebraska," that: "Moldy corn is often due to a fungus provisionally referred to

attributed to a species of fusarium, apparently became serious in Iowa for the first time in 1914 when it caused an estimated loss of $15,000,000 to the corn crop. One of the authors observed the disease in Missouri and Illinois that year, and its occurrence was reported from Nebraska and Minnesota. The fusarium disease was described as attacking the roots, the stalks, and the ears of corn, at least in some seasons; however, it was not determined whether or not all these symptoms were caused by the same organism. It was thought that the disease spread largely with the seed corn. The following recommendation as a preventive measure was made: "Careful seed selection is a good measure of precaution; in no event use seed corn that comes from a diseased field. The most feasible line of preventive work will be the development of resistant varieties."

Hewitt[42] reports that in Arkansas "ear molds caused by Fusarium are serious," constituting "the limiting factor in the production of some varieties." The affection is believed often to follow ear-worm injury but may be quite serious independently.

Selby[100] called attention to the fact that corn root rot was widespread in Ohio in 1918. In describing the disease he stated: "The diseased plants may be purplish-colored, dwarfed or stunted, and unproductive. The plants may show dying at the top or general lack of deep green color. The disease causes a rotting of the roots, the fungus extending upward into the stem of the corn plant, showing as a darkening of the joints in the stem; in severe cases the fungus extends upward, entering the ear of corn through the ear shank and making a final possible development as a pink mold of the ear. Growing corn is studied by lifting and cutting open the stem from the main root upward." He considered the causal organism to be one or probably more soil-borne species of fusarium. Since the soil was thought to be quite generally infected with these fusaria, he suggested crop rotation and development of resistant strains of corn as the best means of control.

About the time Selby found corn root rot prevalent in all parts of Ohio, the disease became generally recognized by plant pathologists and farmers alike as being more or less serious all over the corn belt. As a result the office of Cereal Investigations of the Bureau of Plant Industry, U. S. Department of Agriculture, organized in 1917 the "Corn Root, Stalk, and Ear Rot"project with G. N. Hoffer, at LaFayette, Indiana, in charge of laboratory and field investigations. In the first project publication, Hoffer and Holbert[43] considered species of Gibberella, Fusarium, Verticillium, Rhizopus, and Pseudomonas to be the harmful organisms responsible for the root, stalk, and ear rots. They state: "The planting of seed infected with these organisms is, in a great measure, responsible for missing hills, slow-growing stalks, barren stalks, downstalks, nubbins, and early blighting of plants in the field with the large

"the corn stalk tests" were extremely variable, "and therefore impossible to interpret, frequently conflicting and often misleading and unreliable."

Valleau[119] has recently described corn root rot as a soil-borne disease produced by a Pythium-like organism that he was unable to isolate in pure culture. From artificial inoculation studies he found that *Gibberella saubinetii* and *Fusarium succisae* could produce seedling blight, but that if the plants escaped seedling injury no root rotting occurred and the plants developed normally like the check plants. He considers *Fusarium moniliforme* and other species of the elegans section to be concerned only as secondary invading organisms. He concludes that the organisms heretofore considered as etiological in the corn root rot problem are probably able to produce seedling blight only, but that true corn root rot which occurs comparatively late in the development of corn plants, is a distinctly different disease produced by an altogether different fungus.

Johann, Holbert, and Dickson[64] reported in an abstract in Phytopathology that an undetermined species of Pythium caused considerable seedling blight of corn in Illinois and Wisconsin under conditions of comparatively low soil temperatures and high soil moistures. They found that if the infection was not severe enough to kill the seedlings, they were more or less retarded in size and vigor by the soft rot of the feeding roots.

In the review of the literature just given, it is seen that ear rot diseases, caused especially by Diplodia and Fusarium species, have been well known for more than twenty years, while the knowledge of the disease of corn roots is comparatively recent in origin. In 1918 when corn root rot first became recognized as a serious disease all over the corn belt, the most important manner of transmitting it was thought to be through planting seed corn infected with organisms as *Diplodia zeae*, *Gibberella saubinetii*, *Fusarium moniliforme*, and others. Accordingly, plant pathologists who were familiar with the problem recommended the most effective means of control to be the planting of seed corn comparatively free from fungous infection. Later it was found in Tennessee, Kentucky, and Nebraska that lightly infected seed corn selected by the approved germinator method gave no better yields than heavily infected seed, thus showing the unimportance of infected seed corn in producing corn root rot in those states. Other investigators found the organisms ordinarily present in seed corn to be responsible for seedling blights, but no direct evidence was given to show their capabilities of producing corn root rot. One type of root rot was found to occur generally when corn was grown on soils deficient in lime and available phosphate and potash. More recently results from experiments carried on in Kentucky suggest that only seedling blight diseases result from the more common seed-borne

readily penetrated by fungi. Many kernels (31 per cent)showed infection only in the tip one-fifth; the fungi evidently did not penetrate the remainder of the kernel. Selby[100] assumed that the fungi attacking corn

TABLE 1.—NUMBER AND KIND OF ORGANISMS IN VARIOUS KERNEL SECTIONS
Legend: D, *Diplodia zeae*; F, *Fusarium spp*; C, *Cephalosporium acremonium*; A, *Aspergillus spp.*; P, *Penicillium spp.*; and R, *Rhizopus spp.*

Ear number	1	2,3	4,5	Ear number	1	2,3	4,5
1	F	0	0	51	2C	2C,P	0
2	2F	2F	0	52	F,C	3A	0
3	0	0	0	53	2F	A	0
4	2F	0	0	54	2F	3F, A	2F,A
5	2D	2D,C	D,C	55	2C	4A	C
6	F,C	D,R	0	56	2F	2A	4A
7	2F	0	0	57	2F	4A	A
8	2F	4F	0	58	2F	4F	0
9	F	C	0	59	F,D	2D,2F	2F,A,P
10	0	0	0	60	C,F	C,A	2A
11	2F	0	0	61	F,C	2D,P,A	3A
12	2F	0	0	62	C,F	0	2P
13	2F	4F	F	63	2A	4A	3A
14	2F	0	0	64	2F	2F,2A	4A
15	F,D	4D	2F	56	2F	3A	3A,P
16	2F	2F	0	66	2C	2F	F
17	2F	C,F	D	67	2F	3F,P	A
18	2F	2F	F	68	C,F	2A,2F	0
19	2F	4F	0	69	F,C	2D,A	2D,P
20	2F	3F	F	70	F,C	2F,P	D
21	2F	0	0	71	2F	2F	2F,A
22	F,C	0	0	72	2D	4D	2F,A
23	2F	4F	2F	73	2C	2C	3P
24	2F	2C	0	74	2P	4P	4P
25	F,C	0	2F	75	C,F	2C,P	3P
26	2F	0	0	76	2C	C	A
27	2F	F	2F	77	2F	3F	3P,F
28	2F	2F	0	78	2D	4D	2F,2D
29	2F	0	0	79	C,F	2F,C	2F,P
30	2F	0	0	80	2P	4P	2P
31	2F	0	0	81	0	0	0
32	2C	0	0	82	2C	0	0
33	C	0	0	83	2C	0	0
34	2C	0	0	84	2C	0	0
35	2C	0	0	85	2C	P	0
36	2C	0	0	86	2F	0	0
37	2D	4D	D	87	2F	0	0
38	2C	C	0	88	F,C	2F	0
39	2F	0	0	89	2F	3F	4F
40	2C	C	0	90	2C	0	0
41	2F	0	0	91	2F	4F	3F,P
42	2F	2F,D	0	92	2F	3D	0
43	2F	2F	0	93	F,C	3F	P
44	2F	3F,D	2F,P	94	2F	4F	2F
45	2F	2F	0	95	2D	4F	2P
46	2F	3F	0	96	2F	F,C	2C,2P
47	2F	0	0	97	2F	4F	F
48	2C	P	0	98	2F	4F	3P
49	2F	3F	0	99	C,D	3F,C	2F,D
50	2F	0	0	100	2P	4P	4P

grew up the stalk from the roots, entered the shank, and penetrated the kernels by way of the pith in the cob. That such a path of infection is followed has been proven for one organism only, *Cephalosporium acremonium*, by Reddy and Holbert[91]. The writer and others[18,42] consider the principal source of kernel infection is by way of wind-borne spores odging in the husks at the tip or butt of the ear since corn stalks containing no organisms whatever in the internodal tissue may and usually do bear an infected ear. Manns and Adams[76] found infection to be concentrated in the tip end of kernels for they reported that when the tip cap was removed a greater number of seedlings free from severe infection were obtained. This was thought to be the result of eliminating the greater part of the internal fungous infection. It was further established that by disinfecting kernels after removal of the cap, germination unaccompanied by developing fungi in many instances was secured. But whether the fungus enters the kernel tip by way of the cob pith and cob proper after having gained entrance at either end, or whether it enters after having developed somewhat between the rows of kernels without entering the interior of the cob is a question yet to be determined.

The Prevalence of Ear Infection as Shown by a Disease Survey of Seed Corn.—During the winter of 1921-22 representative seed ears of corn from all sections of Missouri were collected. Every county agent and many farmers were asked to send in four ordinary seed ears; two with clean, white butts and sound tips, and two with black, brown, or reddish discolorations in the butt end of the cob and with discolored and slightly molded tips. Samples were received from 49 counties, including all the principal corn-growing counties in the various sections of the State. As each sample arrived the two apparently infected ears were numbered one and two. The two remaining ears, though in most cases not free from apparent infection in the writer's judgment, usually showed disease symptoms to a less marked degree, and were numbered three and four. The source of each sample is shown in Table 2.

Eight representative kernels, located spirally from butt to tip, were removed from each ear. The tip one-fifth only from each kernel was isolated because it could be manipulated easier in sterilizing and placing in the agar in the petri dish, and also because the elimination of seedling growth was decidedly advantageous. This method in no way affected the accuracy of the results since the previous experiment showed that the tip was sure to be infected if any other part of the kernel was infected. The technique followed in making the isolations was the same as described in the previous experiment. Four kernel tips were put in each petri dish. The plates were incubated 20 to 30 days at room temperature of about 25°C.

standards is evident because the kernel type of the leading varieties of corn, such as Reid's Yellow Dent, Boone County White, and Johnson County White, has not changed appreciably. It is noticeable, however, that fewer practical corn breeders are holding to extreme types of rough indentation in the strains they are growing and developing.

In the writer's judgment Kiesselbach[70] offers the most reasonable explanation for the discovery by some investigators that ears with horny kernels outyield ears with very starchy kernels. He states in conclusion from his investigations under Nebraska conditions that "Whenever corn types are being grown which tend to be somewhat too large and late maturing for their environmental conditions, selection of this smooth type of ear, whether because of root rot disease considerations or otherwise, is likely to result in increased production because of the better adaption of plant type represented in this type of ear. These type considerations apply where the various types are selected from the same general variety of corn."

Dungan[26] has recently found that the so-called starchy corn contains no more total starch than horny corn, and frequently not so much. He suggests that the term "floury" be applied to corn having a large amount of soft starch. Seedlings from horny kernels are more vigorous than seedlings from floury kernels, he states, because there is often a greater food supply in the horny kernel and because the horny starch is more readily hydrolyzed to a soluble condition than the soft starch.

Recently, Jehle, Oldenberg, and Temple[63] reported yield tests in Maryland showing a relation of internal cob discoloration to yield in corn. They conclude from the results secured in ninety tests that the yield from seed corn on cobs free from internal discolorations is greater than the yield from seed corn on cobs with internal discolorations; and that the greater the internal discoloration, the smaller the yield. In determining the internal cob discolorations, they found that chopping off about two inches of the butt and tip of the ear before making the observations was more accurate than simply examining the two extremities of the cob. It was found that some cobs showed externally discolored butts but were perfectly free from discolorations at all points more than one inch from each extremity. This would indicate that the Maryland method of selecting seed ears on the basis of physical characters would secure more ears from a lot of corn than the method recommended by the writer, since in the latter case all ears with discolored butts would be thrown out. The writer questions, however, whether the possibility of securing a few more seed ears would compensate for the added inconvenience and waste involved in chopping off the buts and tips of all ears examined.

THE RELATION BETWEEN KERNEL INFECTION AND CORN ROOT ROT IN THE FIELD

The fact that Missouri seed corn grown in 1921 showed practically no infection with *Gibberella saubinetii* seemed surprising, especially in view of the fact that Hoffer and Holbert considered it at that time to be the principal organism infecting seed corn and causing root rot in Indiana and Illinois. It seemed desirable then to determine whether or not Missouri seed corn heavily infected with fungous organisms actually produced more root rot in the field than slightly infected seed. If *Gibberella saubinetii* carried in the seed was the pathogene largely responsible for corn root rot, comparatively little disease could be expected from planting Missouri seed corn.

Field Experiment in 1922.—Methods Used.—Plans were made during the early months of 1922 to compare in field experiments yields from heavily infected seed with yields from slightly infected seed. These experiments were conducted on the outlying experiment fields of the Missouri Experiment Station at Maryville, Stark City, Cuba, Warrensburg, and Kirksville. Seed corn was obtained from farmers in the vicinity of each of the experiment fields so that the seed used in planting each test would be adapted to that particular locality. About twenty times as much seed as needed for planting was obtained for each test so that there might be plenty of corn from which to select the kinds of seed needed. This corn was tested by means of the modified rag doll germinator described by Hoffer and Holbert[43]. Eight kernels were removed spirally from butt to tip of each ear and grown eight to ten days in the germinator. Only those ears having seven or all eight of the seedlings badly rotted were placed in the "diseased seed" lot. Much care was taken to avoid ears that did not give 100 per cent germination. Only those ears that showed no seedling rot on the germinator were used for "clean seed". Hereafter in this paper seed corn that shows badly rotted seedlings in the germinator will be referred to as "diseased" seed and seed corn that shows little or no rotting in the germinator will be referred to as "clean" seed. By "disease-free" seed is meant that coming from those ears from which 40 representative kernels have shown no internal infection when germinated on agar under sterile conditions. In each test the diseased and clean seed was planted in alternating replicated plots.

Table 5 shows the average yields of clean and diseased seed in five different tests. The differences are significant in only two cases, at Maryville and Warrensburg, and the writer is of the opinion that the Maryville data should be discarded because of the inaccurate manner in which the yields were determined by the man in charge of the harvesting. Some member of the Experiment Station staff had charge of harvest-

ing the corn at the other fields. This leaves only one test in which the difference in favor of clean seed was significant. However, only one test showed the diseased seed actually yielded more, and in this case the difference was not significant.

TABLE 5.—YIELDS FROM CLEAN AND DISEASED SEED IN 1922

Outlying field	Variety seed used	Average yield in bushels per acre[1]		Difference in favor of clean seed[2]
		Clean	Diseased	
Maryville	Reid's Yellow Dent	63.0±1.5	55.8±1.6	7.2±2.2
Stark City	Commercial White	40.1±3.4	41.7±2.3	−1.6±4.1
Cuba	White Pearl	8.9±0.7	8.0±0.9	0.9±1.1
Warrensburg	Boone County White	38.9±1.1	33.5±0.8	5.4±1.4
Kirksville	Reid's Yellow Dent	50.5±3.5	49.0±3.4	1.5±3.7

1. The probable errors were calculated by Peter's formula, thus:

$$E = \pm 0.8453 \times \frac{V}{N\sqrt{N-1}}$$

where V is the sum of the variations from the mean, and N the number of variates.

2. The difference between two values (mean yields) in this case is considered statistically significant when its value is 3.2 (or more) times its probable error. The probable error of a difference in two values, each having a probable error, is determined by the formula—

$$E \text{ of difference } \sqrt{E^2_a\ E^2_b}$$

where E_a is the probable error of one of the values under comparison and E_b is the probable error of the other.

The only conclusion that can be drawn from these data is that under the conditions of the experiment in 1922 there was practically no difference in yield between diseased seed corn and clean (comparatively non-diseased) seed corn. The large differences in yield usually obtained by other investigators[56,57,55,63] in comparing diseased and clean seed led the writer to believe that differences in stand in diseased and clean plots must be an important factor. Although no information concerning the comparative stands in the clean and diseased seed plots was obtained in the Missouri experiments in 1922, it was assumed that where nearly equal yields were secured approximately equal stands existed inasmuch as both lots of seed germinated 100 per cent. Therefore, plans for repetition of the experiment in 1923 were made to take into account the stand of corn in the clean and diseased seed plots.

Field Experiments in 1923.—These tests were conducted in much the same manner as those in 1922 except that in certain cases the stand of corn was determined. In addition to comparing clean and diseased seed of an adapted variety of corn, similar tests were made with unadapted Missouri corn and with Illinois seed corn. Adapted seed corn was

obtained from farmers in the vicinity of each outlying field: unadapted seed was obtained from a farmer in extreme southeast Missouri. The Illinois corn was furnished by the Illinois Experiment Station and came divided into two lots designated as disease-free and moderately diseased seed. The adapted and unadapted clean and diseased seed was selected as before from ears that germinated 100 per cent. Composite tests made on the seed received from Illinois gave 100 per cent germination for the clean seed and 90 per cent for the diseased seed.

The diseased and clean seed from each of the three lots was planted in alternating replicated plots at Maryville, Stark City, Cuba, and Warrensburg. The corn was planted in each outlying field at the usual time for planting corn in that locality. All the tests were observed in the fall just before the corn matured, but counts of plants living through to maturity were made only at Maryville and Stark City. At none of the fields was one able to distinguish with the eye any difference in appearance between diseased and clean seed plots.

Table 6 shows that the yield of Illinois diseased seed is less than the yield of clean seed in just about the same proportion that the stand is less.

TABLE 6.—YIELDS FROM ILLINOIS CLEAN AND DISEASED SEED

	Average number of plants per row	Yield in bushels per care
Maryville Experiment		
Clean seed	174	68.1±3.2
Diseased seed	164	66.4±1.4
Difference in favor of clean seed		1.7±3.5
Stark City Experiment		
Clean seed	131	45.5±2.3
Diseased seed	118	41.2±2.6
Difference in favor of clean seed		4.3±3.5

Thus at Maryville where the stand in diseased plots was 6 per cent less than in the clean plots the yields were about 3 per cent less; and at Stark City where the stand was 10 per cent less in diseased plots the yield was also 10 per cent less. It would seem from these results that the decrease in yield from planting Illinois diseased seed was not necessarily due to more corn root rot but to more seedling blight which materially reduced the stand. The difference in yield between plots planted to diseased seed and plots planted to clean seed, however, were not great enough in either case to be significant.

Counts were also made on the stand in plots planted with adapted seed, both clean and diseased, at Maryville and Stark City. Table 7 shows that the stand from clean and diseased seed was almost exactly the same in both tests, but that the diseased seed outyielded the clean seed at Maryville and the clean seed outyielded the diseased seed at Stark City.

An examination of Table 8 shows that under the conditions of the experiment there was no difference in yield between clean and diseased Illinois seed corn. The probable error of the difference in mean yield of the two series of plots is greater than the difference of 1.2 bushels. The lower average yield of the clean seed plots is to be expected on account of a slightly less stand. Although the stands were perfect after the second cultivation when all plots were thinned to 120 plants, a few more plants were destroyed during subsequent cultivations and by other causes in the clean seed plots than in the others. Furthermore, the yield in plot 38B was low compared with the adjacent plots presumably because of damage to many ears that fell to the ground when a part of the plants went down with root rot. The results from this test seem to confirm the suggestion that the superiority of Illinois clean seed over diseased seed at Maryville and Stark City was because the clean seed gave a better stand and not because less corn root rot resulted.

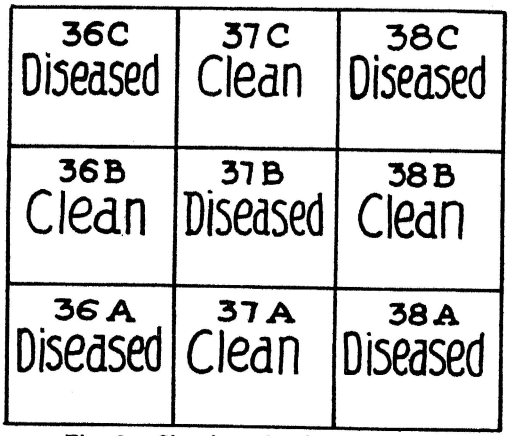

Fig. 2.—Showing planting plan of experiment with Illinois seed at Columbia in 1923.

All of the yield tests from the outlying fields are brought together and summarized in Table 9.

TABLE 9.—SUMMARY OF YIELD TESTS WITH CLEAN AND DISEASED SEED ON OUTLYING FIELDS IN 1923

Field	Clean seed, bu. per acre	Diseased seed, bu. per acre	Difference in favor of clean seed
Illinois corn			
Maryville	68.1±3.2	66.4±1.4	1.7±3.5
Stark City	45.5±2.3	41.2±2.6	4.3±3.5
Cuba	7.4±0.8	7.4±0.3	0.0±0.9
Warrensburg	12.7±1.9	10.1±0.4	2.6±1.9
Adapted Corn			
Maryville	71.7±3.6	77.9±2.7	−8.2±4.5
Stark City	33.7±0.8	25.2±1.8	8.5±1.8
Cuba	10.3±1.3	6.9±1.3	3.4±1.8
Warrensburg	37.7±1.1	39.6±1.3	−1.9±1.7
Unadapted Corn			
Maryville	70.0±0.7	61.9±1.7	8.1±1.8
Stark City	26.6±1.7	25.4±1.5	1.2±2.3
Cuba	3.8±0.3	3.6±0.4	0.2±0.5
Warrensburg	36.5±1.2	40.2±3.5	−3.7±3.6

1A	2A	3A C	4A D	5A D3	6A C	7A D
1B	2B	3B D3	4B C	5B D	6B D	7B C
1C	2C	3C D	4C D3	5C C	6C C	7C D
1D	2D	3D C	4D D	5D D3	6D D	7D C
1E	2E	3E D3	4E C	5E D	6E C	7E D
1F	2F	3D D	4F D3	5F C	6F D	7F C
1G	2G	3G C	4G D	5G D3	6G C	7G D
1H	2H	3H D3	4H C	5H D	6H D	7H C
1I	2I	3I D	4I D3	5I C	6I C	7I D
1J	2J	3J C	4J D	5J D3	6J D	7J C

Fig. 3.—Showing general plan of field Experiments in 1924. Plots in each series are numbered A to J. Legend: C, clean seed; D, diseased seed; D3, diseased seed planted three-rate.

On such soil a comparatively slight increase in optimum stand gives no difference in yield, but a large increase to twice or more optimum stand actually decreases the yield. Thus we may assume that the plots planted to clean seed which had 134 plants per plot in one case and 121.1 in the other, had just about the optimum stand for that season.

At Cuba the plots planted to clean seed with 10 per cent greater stand outyielded the plots planted to diseased seed by 11 per cent, but the plots seeded three-rate to diseased corn yielded less than the plots

planted to diseased seed, although the stand was 57 per cent greater. On the basis of the Stark City tests we would expect these plots planted three-rate to diseased seed to outyield the plots planted to diseased seed and even equal or better the yield from plots planted to clean seed. However, close scrutiny of Table 12 will reveal a marked difference in the productivity of the soil at Cuba compared with that at Stark City. Here only in the most favorable years, when corn yields as much as 20

TABLE 10.—YIELDS FROM CLEAN, DISEASED, AND UNTESTED SEED ON BLOCK B AT STARK CITY, 1924

Kind of seed	Number of plots	Average number of plants per plot	Yield in bushels per acre
Adapted Commercial White Seed Corn			
Untested	20	106	45.0±1.1
Clean	10	121.1	37.3±1.3
Diseased	10	108	31.3±1.6
Diseased (3-rate)	10	158.8	38.3±1.0
Difference in favor of diseased seed 3-rate compared with diseased seed			7.0±1.9
Unadapted St. Charles White seed corn			
Clean	10	131.9	38.2±1.2
Diseased	10	123.3	34.2±1.3
Difference in favor of clean seed			4.0±1.8

TABLE 11.—YIELDS FROM CLEAN, DISEASED, AND UNTESTED SEED ON BLOCK G AT STARK CITY, 1924

Kind of seed	Number of plots	Average number of plants per plot	Yield in bushels per acre
Adapted Commercial White seed corn			
Untested	20	71.4	21.6±1.3
Clean	10	134	26.1±1.1
Diseased	10	107	22.7±1.3
Diseased (3 rate)	10	156	27.7±1.2
Difference in favor of diseased seed 3-rate compared with diseased seed.			5.0±1.4
Unadapted St. Charles White seed corn			
Clean	10	125	34.8±1.7
Diseased	10	108	31.3±1.9
Difference in favor or clean seed			3.5±2.7

TABLE 12.—YIELDS FROM CLEAN, DISEASED, AND UNTESTED SEED AT CUBA, 1924.

Kind of seed	Number of plots	Average number of plants	Number of ears	Yield in bushe s per acre
Adapted White Pearl seed corn				
Untested	20	43.3	46.5	17.0±0.8
Clean	10	108.8	110.8	19.5±0.9
Diseased	10	99.4	43.5	17.4±0.9
Diseased (3-rate)	10	122.8	40.0	16.0±1.0
Unadapted St. Charles White seed corn				
Clean	10	102.3	55.3	22.1±0.7
Diseased	10	95.9	55.8	22.3±0.9

lot and the seedlings were germinated for nine days before observations were made.

The results given in Table 15 show practically no difference in the effectiveness of the one- and two-hour treatments. Probably because the disinfectant penetrated the kernels more deeply and killed the embryo in some cases, more dead kernels resulted from the longer treatment than from the one-hour treatment.

TABLE 15.—EFFECTS ON SEEDLING ROT FROM TREATING DISEASED SEED FOR DIFFERENT PERIODS OF TIME WITH MERCURIC CHLORIDE

Treatment	Dead kernels	Uninfected seedlings	Infected seedlings
None	20	19	11
Alcohol and mercuric chloride, one hour	14	34	2
Alcohol and mercuric chloride, two hours	17	33	0

As a result of these preliminary trials, it was decided to test under field conditions the effectiveness of the one-hour alcohol and mercuric chloride treatment in preventing seedling rot from infected seed.

Experiment 3.—Before describing the field experiments, however, it is proper to mention here the effect of long and short periods of disinfection and of temperature on the outgrowth of the organisms from an infected kernel tip placed on agar. In this test 48 kernels were used that came from a severely infected ear, all the tested kernels of which produced seedling rot on the germinator and showed in potato dextrose agar culture plates internal infection with *Diplodia zeae*, *Fusarium moniliforme* or both. None of the kernels were dead, however. The tips of the kernels were removed and the remainder discarded. Half of the kernel tips were immersed for one minute in 95 per cent alcohol and then soaked one hour in mercuric chloride, and the remainder were soaked in water for one hour. The tips were then rinsed with distilled water and dried at room temperature for one week. After the usual method of surface disinfection two potato dextrose agar plates with four kernel tips each of the mercuric chloride treated seed and two similar plates of the water treated seed were incubated at each of three different temperatures, 30°C, 25°C, and 12°C. The latter temperature was secured in an icebox which varied from 10°C to 14°C during the course of the experiment.

Infected corn kernels soaked one hour in mercuric chloride still retain the fungus in a viable form as Table 16 clearly shows. But the treatment retards the fungus in growing out of the kernel into the agar; for about three days at temperatures of 25°C to 30°C and for eight days at a temperature of 12°C. The kernels soaked for one hour and disinfected with mercuric chloride for one minute gave 100 per cent infection at all three temperatures, showing clearly that the temperature limits

for the growth of *Diplodia zeae* and *Fusarium moniliforme* are above 30°C and below 12°C. At the end of ten days, in the case of the plates containing kernels treated with mercuric chloride, the fungous colonies that appeared first had overgrown the other kernels so that one could not be sure whether or not these kernels were sterile from the treatment received. Subsequent tests, however, make the writer feel certain that the kernels were not sterile, but simply became overgrown before the fungus had time to grow out into the medium. The growth was so slow in the icebox that the plates containing kernels treated with mercuric chloride

TABLE 16.—EFFECTS OF TEMPERATURE AND SEED TREATMENT ON DEVELOPMENT OF FUNGI FROM INFECTED KERNELS

Legend: D, *Diplodia zeae;* F, *Fusarium moniliforme;* and ctn, contamination

Kind of seed	Temperature	Number and kind of colonies after:			
		Five days	Seven days	Ten days	Thirty days
Treated	30°C	1D, 1F	1D, 1F	2D, 2F	------
Untreated	30°C	7F, 1Ctn.	7F, 1Ctn.	7F, 1Ctn.	
Treated	25°C	1D	3D, 3F	3D, 3F	
Untreated	25°C	4D, 2F	5D, 3F	5D, 3F	--------
Treated	12°C	0	0	1D, 1F	----
Untreated	12°C	0	7F	8F	1D, 4F
					8F

for one hour did not become overgrown for 30 days, at which time three of the eight kernels had not yet produced any visible fungous growth.

It may be significant that only one Diplodia colony appeared in the 16 kernels incubated at 12°C. At the other temperatures *Diplodia zeae* grew just as often as *Fusarium moniliforme* from the infected kernels. Holbert[58] found that Diplodia infected seed yielded 46.7 per cent as much as good seed when both were planted early but the yield of the diseased seed decreased to 20 per cent of the yield from good seed when planted late in the season.

Experiment 4.—This experiment was designed to determine the effect of seed treatment on the occurrence of corn root rot in the field. Corn grown in 1920 was gathered from various sources in Missouri and ten representative kernels from each ear were germinated eight days on the table germinator. Certain ears were designated as being heavily infected, moderately infected, and slightly infected according to whether the seedlings showed a high, moderate, or slight per cent, respectively, of seedling rot while on the germinator. Six rows 400 feet long with hills three feet apart were planted to each of these three groups of seed, but the seed in three rows of each group was first disinfected by immersing momentarily in alcohol and then in a 1:1000 mercuric chloride solution for one hour.

No notes were taken on this test until September when most of the plants were just beginning to lose their natural green color and dry

up. At this time each plant was inspected for symptoms of root and stalk rot. Plants that had fallen to the ground or that were leaning at an angle of 45 degrees or more and when pulled gently, exhibited a badly diseased and rotted root system were considered to be affected with root and stalk rot.

TABLE 17.—EFFECTS FROM SEED TREATMENT ON DEVELOPMENT OF ROOT AND STALK ROT IN THE FIELD

Type of infection	Percentage of diseased plants per row:	
	Treated seed	Untreated seed
Heavy	15.5	27.4
Moderate	12.5	16.4
Light	8.9	14.4

An examination of Table 17 shows that more diseased plants resulted from planting more heavily infected seed and that disinfection of the seed as described above materially reduces the amount of root and stalk rot in the field. Unfortunately the yields from the different lots of treated and untreated seed were not determined. These results, obtained in 1921, were considered suggestive enough to justify a similar and more extensive experiment in 1922.

Experiment 5.—This experiment was planned to compare yields from disinfected diseased seed corn with yields from similar seed untreated. The seed used was secured from ears that had been found in previous tests with both the agar plate isolation and the table germinator methods to have 100 per cent kernel infection with *Fusarium moniliforme* or *Diplodia zeae* or both. This seed germinated 100 per cent. Part of the seed was "treated" by disinfecting with alcohol and mercuric chloride for one hour. The "untreated" seed, or check, was soaked in water for one hour. The treated seed was rinsed in distilled water and both lots were allowed to dry quickly. The corn was planted one week later on May 5, 1922 on Block B of the Station field. There were five replicated plots each containing three rows of treated seed and three rows of untreated seed. Three grains were dropped in hills a little less than three feet apart in rows 132 feet long.

From the first the treated seed did not appear as vigorous as the untreated seed. It was easily noticeable that the untreated seed came up more quickly and more uniformly, the plants grew off better and stayed a little ahead of those from the treated seed all summer long. When the corn was about eight inches high the hills were thinned to two stalks per hill. No counts were made at that time, but it was quite evident that there were fewer plants from the treated seed than from the untreated.

Aside from the slight tendency of the plants from treated seed to be stunted, all the corn plants developed normally with no evidence of

disease until about August 1st. A heavy wind without rain came on July 29 and another with rain came on August 6. Both these winds blew down many stalks, apparently all with greatly weakened root systems. Most of the plants that blew over did not have broken stalks, the rotted roots on the windward side simply broke off within three or four inches of the top of the ground and were left exposed as the plants fell to the ground. The total down stalks, some of which were already dead, were counted on August 9th.

TABLE 18.—SHOWING EFFECTS OF DISINFECTING DISEASED SEED WITH MERCURIC CHLORIDE

Kind of seed	Total down stalks	Average per row	Total dead stalks	Average per row
Treated	387	25.8	56	3.7
Untreated	351	23.4	64.1	4.3

It may be seen in Table 18 that more down stalks occurred in the treated seed plots, but a few less of these stalks were dead than in the check plots. Though the differences are not great, there is certainly nothing in the results to indicate that treatment of diseased seed with mercuric chloride lessened the development of corn root rot in the field.

Another observation that later proved to be quite significant was made at the time the diseased stalks were counted. There was an oval area spreading across most of the plots in which all of the plants went down and most of them died soon after. The root systems of many of these plants were badly rotted. It was also noticed that a majority of these plants bore small ears or no ears at all. This area was about 80 feet east and west by 50 feet north and south and had fairly distinct boundaries. The plots in this experiment ran north and south across the spot in which all the plants blew down. Examinations of soil in the spot revealed no difference from that outside where the corn plants had apparently healthy root systems. Examinations were made of the soil texture, structure, organic matter content, depth of surface soil, depth and character of sub-surface soil, character of subsoil to the depth of four feet, and acidity. With the brom-cresol purple indicator the hydrogen-ion concentration of a composite sample of soil in the infected area was p_H 6.4 while a similar sample outside the area where the corn plants were healthy gave a p_H of 6.0. This difference did not appear to be significant.

Table 19 gives the yield of treated and untreated seed in each plot. The higher yields in Plot 1 may be accounted for by the fact that it was just outside the spot to which attention was directed above. The inferior yields from diseased seed disinfected with alcohol and mercuric

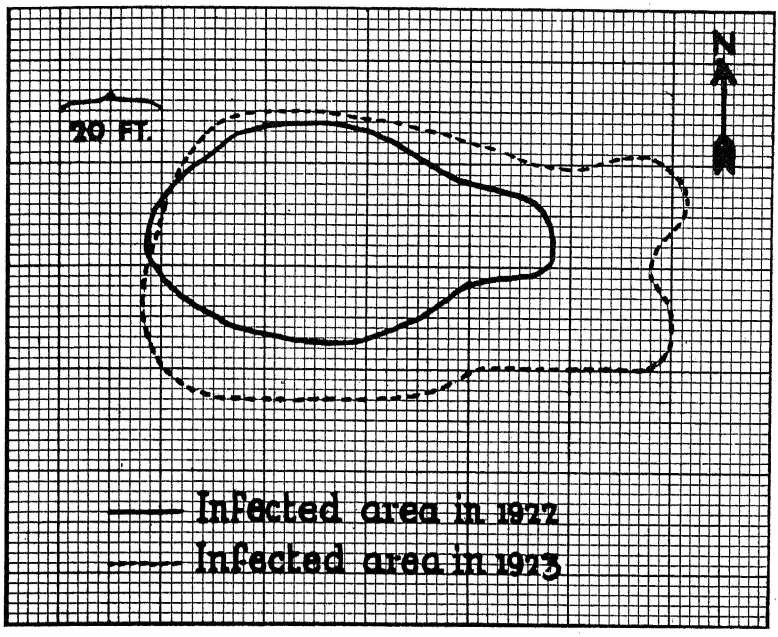

Fig. 4.—Showing approximate outline of infected area in Missouri Experiment station field in 1922 and 1923.

TABLE 20.—SHOWING KIND AND SOURCE OF SEED CORN PLANTED IN EACH OF 20 ROWS THROUGH INFECTED AREA IN 1923

1.	Bad ear	Furnished by Illinois Experiment Station.
2.	Good ear	Furnished by Ilinois Experiment Station
3.	Ear 40.1	Disease-free* ears grown in 1921 from disease-free St. Charles Yellow ear No. 2.4.
4.	Ear 40.6	Same as Ear 40.1.
5.	Ear 2.4	Disease-free St. Charles Yellow ear.
6.	Ear 3.3	Clean ear of Reid's Yellow Dent from Bates county.
7.	Ear 51.3	Clean ear of Reid's Yellow Dent from Randolph county.
8.	Ear 14.4	Clean ear of Reid's Yellow Dent from Cole county.
9.	Ear 69.3	Clean ear of Commercial White from Howell county.
10.		St. Charles Yellow diseased composite.
11.	Ear 18.3	Clean ear of Commercial White from Greene county.
12.	Ear 32.4	Clean ear of Reid's Yellow Dent from Linn county.
13.		Illinois Champion White Pearl diseased composite.
14.		Illinois Champion White Pearl nearly disease-free composite.
15.		St. Charles Yellow nearly disease-free composite from Boone county.
16.		St. Charles Yellow diseased composite from Boone county.
17.	Ear 40.3	Same as Ear 40.1.
18.	Ear 40.2	Same as Ear 40.1.
19.	Ear 40.5	Same as Ear 40.1.
20.		Clean seed composite of Boone County White from J. R. Shelton in Johnson county.

*A disease-free ear is one from which at least 40 representative kernels have been removed and when grown aseptically on agar found to be free from internal fungous infection.

five hills within the boundaries of the spot as it occurred in 1922. This row, however, was calculated to be quite near the boundary of the spot, but might easily have been outside it on account of the inexact measurements made of the spot in 1922. The spot evidently increased in size somewhat in 1923 as shown in Figure 4. The boundaries were not as distinct as in 1922; that is, the plants in hills near the border were often neither all down nor all erect. Although scattered plants all over the whole plot showed typical root rot symptoms, the disease seemed to be much more virulent in a more or less definite area. The fact that plants from disease-free seed as well as from diseased seed were equally affected in the spot still further confirmed the idea that some organism, agent, or condition in the soil was responsible for the corn root rot disease.

The work of Hoffer and Carr[46], published in 1923, in which they stated that the most severe cases of root rots had been found in soils notable because of their deficiencies in lime and available phosphates, suggested to the writer to test the effects of lime and phosphate soil treatments on the appearance of corn root rot in the infected area in 1924. The field was plowed late in the fall of 1923 and during the following spring the soil was prepared in the usual way for planting corn. Two-row plots running north and south across the infected area were laid off and given the different treatments shown in Table 21.

TABLE 21.—SHOWING PLAN OF SEEDING AND SOIL TREATMENTS IN INFECTED AREA IN 1923

Plot	Rows	Seed	Soil treatment
1	2	Badly diseased	None
2	2	Clean	None
3	2	Clean	2500 lbs. hydrated lime per A.
4	2	Clean	2500 lbs. hydrated lime plus 200 lbs. 16% phosphate per A.
5	2	Clean	200 lbs 16% phosphate per A.
6	2	Clean	None
7	2	Clean	None
8	2	Clean	2500 lbs. hydrated lime per A.
9	2	Clean	2500 lbs. hydrated lime plus 200 lbs. 16% phosphate per A.
10	2	Clean	200 lbs. 16% phosphate per A.
11	2	Clean	None
12	2	Clean	None
13	2	Clean	None
14	2	Badly diseased	None

The lime and phosphate were applied on the surface by hand, care being taken not to let the treatments overlap the adjoining plots. Immediately after application the material was raked in the top two or three inches of soil by hand. After a period of ten days the corn was planted by hand at the rate of two grains per hill on June 6, 1924. The soil was warm and the corn came up quickly and uniformly. From the

TABLE 22.—SHOWING EFFECTS OF DIFFERENT SOILS AND SOIL TREATMENTS ON THE DEVELOPMENT OF CORN ROOT ROT

Pot Number	Kind of soil	Condition of roots at maturity
1	Infected	Badly rotted
2	Infected	Badly rotted
3	Virgin pasture	Healthy
4	Virgin pasture	Healthy
5	Virgin excavated (check)	Healthy
6	Virgin excavated (inoculated with Diplodia)	Badly rotted
7	Virgin excavated (inoculated with Gibberella)	Healthy—a few roots slightly rotted
8	Virgin excavated (inoculated with Cephalosporium)	Badly rotted
9	Virgin excavated (inoculated with Fusarium)	Healthy

Drawings of the roots from plant 1 and 3 respectively are reproduced in Plate II. This plate shows the characteristic nature of the root rot disease in a zone beginning one or two inches below the surface of the soil. This feature of the diseased roots being healthy for a distance of an inch or so under the soil is often noticeable on plants growing in the field. Furthermore, the roots in the bottom of the diseased pots also appeared healthy, but all the root tissue in the interior of the soil zone was quite brown to brownish black and about half of it was partially disintegrated. The top dressing of manure on the soil offered an explanation for the healthy condition of the roots near the surface, but subsequent observations in other experiments have shown that this view is untenable.

Many isolations were made from bits of affected root tissue from all the diseased plants. The method used was to wash thoroughly in sterile water a piece of root tissue about one-half inch long, then surface disinfect it by momentary immersion in 95 per cent alcohol, and one minute in 1:1000 mercuric chloride, after which it was washed again in sterile water and placed on a sterile slide. A sterile scalpel was used to cut the root portion into small bits, one or more of which were placed on potato dextrose agar in petri dishes. In most cases a Fusarium either identical with or resembling *Fusarium moniliforme* grew out of the isolated root tissue. Often a Penicillium or a bacterial colony appeared. Neither Diplodia, Gibberella, nor Cephalosporium were isolated from root tissue. However, typical pycnidia of Diplodia formed on the base of the stalk growing in the Diplodia inoculated soil and isolations from stalk tissue gave pure cultures of *Diplodia zeae*. Isolations from the base of the stalk growing in Cephalosporium inoculated soil invariably yielded a Fusarium, usually resembling *Fusarium moniliforme*.

These results quite convincingly indicated the presence in the infected soil of some causal agent producing corn root rot and the absence of such a causal agent in the virgin or uncropped soil. The evidence for the pathogenicity of Diplodia and Cephalosporium seemed inconclusive in view of the fact that neither organism was re-isolated from the rotted roots. No evidence for the pathogenicity of *Fusarium moniliforme* was secured. Inoculation with *Gibberella saubinetii* apparently produced seedling blight in the first planting but no seedling blight and little or no root rot in the second planting. The inoculation results as a whole seemed so inconclusive that plans were made to repeat the experiment but in a much larger way.

Experiment 2.—For this experiment, soil was taken from both inside and outside the infected area in the corn root rot experimental plot on the Experiment Station field. Infected soil was obtained in October, 1923 by removing the central block of soil, about 12 inches square and 5 or 6 inches deep, from a hill of corn containing plants with badly rotted root systems. "Clean" soil was secured in like manner from nearby hills containing plants with apparently healthy root systems. The root systems were deemed healthy if the selected well developed, erect plants were difficult to pull out of the ground and when pulled out had 25 or 30 pounds of soil, if fairly moist, attached to them. None of this clean soil was more than ten yards distant from the infected area of soil in which all the plants succumbed to root rot. Both lots of soil were shoveled into grain bags and removed at once to the greenhouse.

TABLE 23.—SHOWING PLAN OF GREENHOUSE EXPERIMENT NO. 2

Pot number	Kind of soil	Treatment of soil
1A, 1B	Clean	None (check)
2A, 2B	Infected	None (check
3A, 3B	Infected	Sterilized at 15 lbs. pressure for 4 hours.
4A, 4B	Infected	Artificially aerated
5A, 5B	Clean	Inoculated with 200 grams infected soil.
6A, 6B	Clean	Inoculated with 25 grams of the 4 organisms
7A, 7B	Clean	Inoculated with 50 grams rice culture of Gibberella
8A, 8B	Clean	Inoculated with 50 grams rice culture of Diplodia
9A, 9B	Clean	Inoculated with 50 grams rice culture of Fusarium
10A,10B	Clean	Inoculated with 50 grams rice culture of Cephalosporium
11A,11B	Infected	Infected soil half, sand half
12A,12B	Infected	Infected soil three-fourths, sand one-fourth
13A,13B	Infected	Limed—2 tons per A
14A,14B	Infected	Manganese sulphate 50 lbs. per A.

The kinds of seed corn, fungi, inoculation cultures, and pots used in this experiment were the same as in the previous experiment. All pots were set up in duplicate and the kinds of soil and treatments used in each pair of pots are given in Table 23.

The pots to be aerated, those containing manganese sulphate and those containing sand, were included to determine if additional oxygen is an advantage to corn roots growing in infected soil. That the lack of oxygen might be a factor in producing root rot was suggested in the previous experiment when the corn roots in the extreme upper and lower soil zones did not rot appreciably. Additional oxygen was to be given the aerated pots by forcing air into the soil with an air pump. Sand was mixed with infected soil to make it more porous and thus admit more air. Manganese sulphate was added to infected soil in order to increase the oxidative power of the soil. The lime treatments were given in order to change the slightly acid reaction of the soil to slightly alkaline and to supply more available calcium for plant growth.

The corn was planted in all pots on February 2, 1923. Four disease-free kernels were planted in each pot and as the plants came up all but one per pot were removed. On the seventh day after planting plants appeared in all pots except 6B, 10B, 12A, 13A, and 14A and B. These pots were replanted on February 9. Observations made at that time revealed two very sickly appearing plants in pot 6A, which was inoculated with all four organisms. Both plants in sterilized soil were below average in size and were light green in color. All other plants seemed normal and thrifty. By March 4 all the replanted pots had plants up and growing nicely except 10B, which was replanted the second time. One of the two sickly plants left in pot 6A had died by this time and the remaining plant appeared in a wilting condition. Within another ten days, however, this wilted plant seemed to recuperate and began to grow vigorously. Also at this time, March 14, the plants in sterilized soil had acquired a normal dark green color and appeared to be thriving as well as any of the plants. From this time on to maturity there was practically no apparent difference in the stalk growth of any of the plants except those in pots 4A and B, which were artificially aerated.

Aeration of pots 4A and B was attempted by pumping air through a tube extending from near the middle of the pot when filled with soil out through a hole in the bottom. A bicycle pump was used to force air into each pot for five minutes every other day. The manipulation or technique was not considered satisfactory because considerable amounts of soil were lost through the tubes when the pots were watered. The plants showed the effects of the treatment by requiring supports to hold them upright.

All the pots were emptied 130 days after planting and the dirt carefully washed from the roots of the plants as before. The condition of each root system with regard to comparative growth and diseased condition is tabulated in Table 24.

soil with *Gibberella saubinetii* were contradictory and may be explained in one of three ways: first, the organism may have been dead in the inoculum used in the soil producing a healthy root system; second, the soil producing the diseased root system may have become accidentally contaminated with infected soil by splashing while watering or by other means; and third, the condition of the root system may have been due to after affects of seedling blight due to *Gibberella saubinetii* from which the plant never fully recovered. Either of the last two views seems to the writer more plausible than the first one. It was at this time that the writer was obliged to give up all work on the corn root rot problem; thus no opportunity was afforded for making isolations of the diseased roots to determine the various organisms involved.

At this point in the investigations, although too early to draw any conclusions, it seemed quite obvious that corn root rot, at least as it occurred on the experimental plots, was caused by some unknown soil-borne organism hitherto unassociated with diseased corn roots, stalks or seeds. But two years intervened before further experiments could be performed to determine whether or not this was actually the situation, and if so, to study the nature of the organism involved.

It is significant to note here that before the corn root rot investigational work was resumed in the botany laboratory of the University of Missouri in the fall of 1926, Valleau[119] published a paper in which he reported soil sterilization and inoculation experiments conducted in 1925 which were nearly identical with those just discussed. In greenhouse experiments Valleau used infected soil from a field in which most of the corn plants invariably went down just before maturity. For clean soil he used either virgin soil or sterilized soil. He found no rotting of the corn roots when disease-free seedlings were planted in virgin soil, sterilized infected soil, or in clean soil inoculated with *Diplodia zeae*, *Giberella saubinetii*, *Fusarium moniliforme*, and other species of Fusaria. He did, however, obtain badly rotted root systems from healthy seedlings planted in infected soil and in clean soil inoculated with diseased corn roots. The methods used by Valleau were so very similar to those employed in this investigation that it is possible to compare roughly certain of the results obtained. Table 25 is such a comparison.

These results obtained by Valleau in 1925 are almost identical with those obtained in this laboratory in 1924. The only two exceptions occurred in the soil inoculated with *Diplodia zeae* in one case and with *Gibberella saubinetii* in the other. Valleau believes that the Diplodia inoculated soil in which corn root rot developed was contaminated with infected soil. A similar explanation has been offered by the writer for the root rot occurring in the soil inoculated with *Gibberella saubinetii* in

TABLE 26.—SHOWING PLAN OF GREENHOUSE EXPERIMENT No. 3

Pot number	Kind of soil	Treatment
9A, 9B	Clean	None (check)
10A, 10B	Clean	Inoculated with Gibberella, Fusarium, and Diplodia
11A, 11B	Clean	Inoculated with Diplodia
12A, 12B	Clean	Inoculated with Gibberella
13A, 13B	Clean	Inoculated with Fusarium
14A, 14B	Clean	Inoculated with 200 grams infected soil
17A, 17B	Clean	Inoculated with 10 grams diseased roots
18A, 18B	Clean	Inoculated with sterilized roots
40A, 40B	Clean	Sterilized—planted with diseased seed
1A, 1B	Infected	None (check)
35A, 35B	Infected	Sterilized
36A, 36B	Infected	Sterilized—inoculated with 10 grams diseased roots
37A, 37B	Infected	Sterilized—inoculated with 10 grams sterilized diseased roots
38A, 38B	Infected	Sterilized—inoculated with unidentified Fusarium
39A, 39B	Infected	Sterilized—planted with diseased seed
41A, 41B	Infected	None—planted with diseased seed

tions of thin razor sections of diseased corn roots. The diseased roots used for inoculation were secured from diseased corn plants and invariably contained oospore-like bodies apparently identical with those described by Valleau in diseased corn roots in Kentucky. Ten grams of such dried diseased root material were used to each inoculated pot. The pots were planted November 22 with kernels from a disease-free ear of Reid's Yellow Dent corn. The seedlings came up fairly uniformly in all the pots, but those in the pots inoculated with rice cultures grew a little more slowly for the first two or three weeks. A month after planting, two plants were dead and the pots were replanted immediately. These plants were, 36A, grown in sterilized infected soil inoculated with diseased roots, and 11A, grown in clean soil inoculated with Diplodia. Plant 11A was killed by ants and a second planting in this soil was also killed by ants which were not found infesting any of the other pots. Plant 36A showed no insect injury whatever, but examinations of the roots revealed a non-septate fungus and occasional oospore-like bodies in the diseased tissue of the cortex. Dozens of attempts to isolate a non-septate fungus from bits of this root tissue failed. Many different fungi were isolated, but a large per cent of the isolations gave Fusarium species. There seemed to be no question but that this plant had been killed by a fungous organism, the identity of which could not be determined.

The remaining plants including the second planting in pot 36A produced tassels and ear shoots, and many produced small ears. It was noticeable that the plants growing in inoculated sterilized soil were just as large as the plants growing in sterilized soil. Notes on the comparative size of the plants taken at maturity showed that only those plants growing in untreated clean soil and in the single pot inoculated with Diplodia were noticeably smaller than the others. The experiment

was terminated 135 days after planting. On April 5, the pots were emptied on a screen, and the soil carefully washed from the roots.

As shown in Table 27, only those plants growing in sterilized soil not inoculated with diseased roots had normal and healthy root systems (see Plate III). The fact that the untreated clean soil produced root rot the same as the untreated infected soil indicated that it was infected with the root rot disease. This was doubtless caused by the natural

TABLE 27.—SHOWING CONDITION AND WEIGHT OF ROOTS OF PLANTS GROWN IN EXPERIMENT 3

Pot number	Kind of soil	Condition of roots	Avg. wt. per root system in grams
9A, 9B	Clean (check)	Both badly rotted	2.2
10A, 10B	Clean (3 organisms)	Both badly rotted	4.7
11A, 11B	Clean (Diplodia)	Both badly rotted	2.2
12A, 12B	Clean (Gibberella)	Both badly rotted	4.1
13A, 13B	Clean (Fusarium)	Both badly rotted	8.1
14A, 14B	Clean (Inoc. inf. soil)	Both badly rotted	4.2
17A, 17B	Clean (Inoc. dis. roots)	Both badly rotted	6.3
18A, 18B	Clean (Inoc. ster. roots)	Both badly rotted	3.6
40A, 40B	Clean (Ster. dis. seed)	Both healthy	37.8
1A, 1B	Infected (check)	Both badly rotted	6.0
35A, 35B	Infected (Sterilized)	Both healthy	71.6
36A, 36B	Infected (Sterilized-inoc. dis. roots)	Both badly rotted	8.8
37A, 37B	Infected (Sterilized-inoc. ster. diseased roots)	Both healthy	22.4
38A, 34B	Infected (Fusarium)	Both healthy	30.7
39A, 39B	Infected (Sterilized dis. seed)	Both healthy	46.7
41A, 41B	Infected (Dis. seed)	Both badly rotted	3.9

spreading of the disease in the field during 1924, 1925, and 1926. This might easily have occurred when the field was plowed and harrowed in 1925 and again in the spring of 1926. It will be remembered that the so-called clean soil for this experiment was secured from a place that produced corn plants with healthy roots in 1923 which was only ten yards from the edge of the infected soil area. If it is assumed that the root rot in 1923 was produced by a soil-borne fungus, it would seem strange indeed if all soil within ten yards of the boundary of the infected area had not become infected during tillage operations by October, 1926. In fact, one of the reasons for using the "clean" soil was to determine whether or not it had become infected since 1923.

The root systems of the corn plants grown in "clean" soil inoculated with Diplodia, Gibberella, and Fusarium showed no more root rot than the root systems of plants grown in uninoculated "clean" soil, showing that the effect of these organisms was negligible compared with infected soil in causing corn root rot. Likewise the unidentified species of Fusarium produced no rotting of the roots in the sterilized infected soil. But

the plants growing in sterilized infected soil inoculated with 10 grams of diseased corn roots had badly rotted root systems, identical in appearance with those in infected soil (see plates IV and V). Neither of the sterilized soils inoculated with sterilized roots or planted with diseased seed produced plants showing any sign of root rot. This demonstrates the presence of the root rot organism, or agent if not an organism, in diseased corn roots; and further, that this organism or agent may be destroyed by sterilization.*

Experiment 4.—A similar experiment was started December 1, 1926 when five pairs of pots were set up as follows: two pots with sod soil, used as check; two with sod soil inoculated with 200 grams infected soil; two with sod soil inoculated with 10 grams of diseased corn roots; two with sod soil containing 10 grams of sterilized diseased roots; and two with infected soil used as check. All these plants grew normally, but at maturity the plants in the sod soil inoculated with diseased roots were approximately 12 inches shorter and ¼ inch less in diameter of stalk than the other plants. The results giving the weight and condition of the roots are given in Table 28.

TABLE 28.—SHOWING CONDITION AND WEIGHT OF ROOTS OF CORN PLANTS GROWN IN UNTREATED SOD SOIL AND INOCULATED SOD SOIL—GREENHOUSE EXPERIMENT 4

Pot number	Kind of soil	Condition of roots	Avg. wt. per root system in grams
19A, 19B	Sod (check)	Both healthy	19.1
20A, 20B	Sod (inf. soil)	Both badly rotted	5.25
22A, 22B	Sod (inf. roots)	Both badly rotted	4.45
23A, 23B	Sod (ster. inf. roots)	Both badly rotted	6.2
2A, 2B	Infected (check)	Both badly rotted	5.9

All the root systems showed root rot except those growing in the untreated sod soil. One of the latter plants did not produce as extensive a root system as the duplicate plant, but there were no visible lesions on the roots. The root systems of plants 23A and B, grown in sod soil containing sterilized infected roots, showed nearly as much root rot as the plants grown in sod soil inoculated with infected roots. This result was probably caused by accidental contaminations, inasmuch as the root systems grown in sterilized soil containing sterilized infected roots in Experiment 3 were quite healthy. These results indicate that the sod soil does not contain the root rot producing organism or agent.

Experiment 5.—In this experiment some of the same infected soil was used as in Experiments 3 and 4. Duplicate pots of soil were given different fertilizer and lime treatments and planted to disease-free seed.

*The sterilized soil in this experiment was autoclaved for 15 minutes at 15 pounds pressure, then spread out in a layer about 2 inches thick on tables for a week before putting it in sterilized pots. Every precaution was taken to prevent the soil becoming contaminated with corn disease organisms or with infected soil during the process.

1923 and 1924. It may be that corn root rot failed to occur in this infected area because of the unusual weather conditions since corn planted at the same time in inoculated sod soil did not develop root rot symptoms. If this assumption is correct we would have to further assume that the seedlings employed in the first planting developed under more favorable conditions for infection with the corn root rot organisms.

It is well to point out here that further work on the problem is needed to determine whether typical corn root rot may be produced by growing disease-free seedlings to maturity under field conditions in uninfected soil inoculated with the Pythium-like organism. Furthermore, it will be necessary to isolate the Pythium-like organism from diseased corn roots grown in many other parts of Missouri in order to show that corn root rot, as it occurs on the Station field at Columbia, is typical of corn root rot that occurs elsewhere in the State.

From the results of investigations reported in this paper showing that reductions in yield from planting heavily infected seed corn are due to seedling blight and not to root rot; that root rot does not develop in corn plants grown in uninfected soil inoculated with either of the four most common seed-borne organisms; that corn root rot does develop in plants grown in uninfected soil inoculated with diseased corn root containing spores and mycelium of a Pythium-like fungus; and that this Pythium-like organism may be easily re-isolated from young corn plants after being inoculated and becoming infected with a pure culture of the fungus, it is believed that corn root rot in Missouri is caused by a Pythium-like fungus similar to the one found by Carpenter to be the cause of root rot of sugar cane in Hawaii.

SUMMARY

1. Corn root rot is a disease primarily affecting roots of the corn plant causing them to rot before the plant matures. Seedling blight is a disease affecting seedlings only, causing them to die or become stunted in their development. Corn ear rot and corn stalk rot are diseases resulting from the attacks of the fungi that produce seedling blight and probably some that do not produce seedling blight.

2. The tip one-fifth of every internally infected corn kernel contains one or more fungi, while the remaining parts of the kernel may or may not be infected.

3. Most of the kernels on nearly every ear of corn grown in Missouri are internally infected with either *Fusarium moniliforme*, *Diplodia zeae*, or *Cephalosporium acremonium*. Ears with kernels infected with *Gibberella saubinetii* are extremely rare.

4. Certain physical ear and kernel characters are reliable guides in selecting seed corn that is comparatively free from fungous infection.

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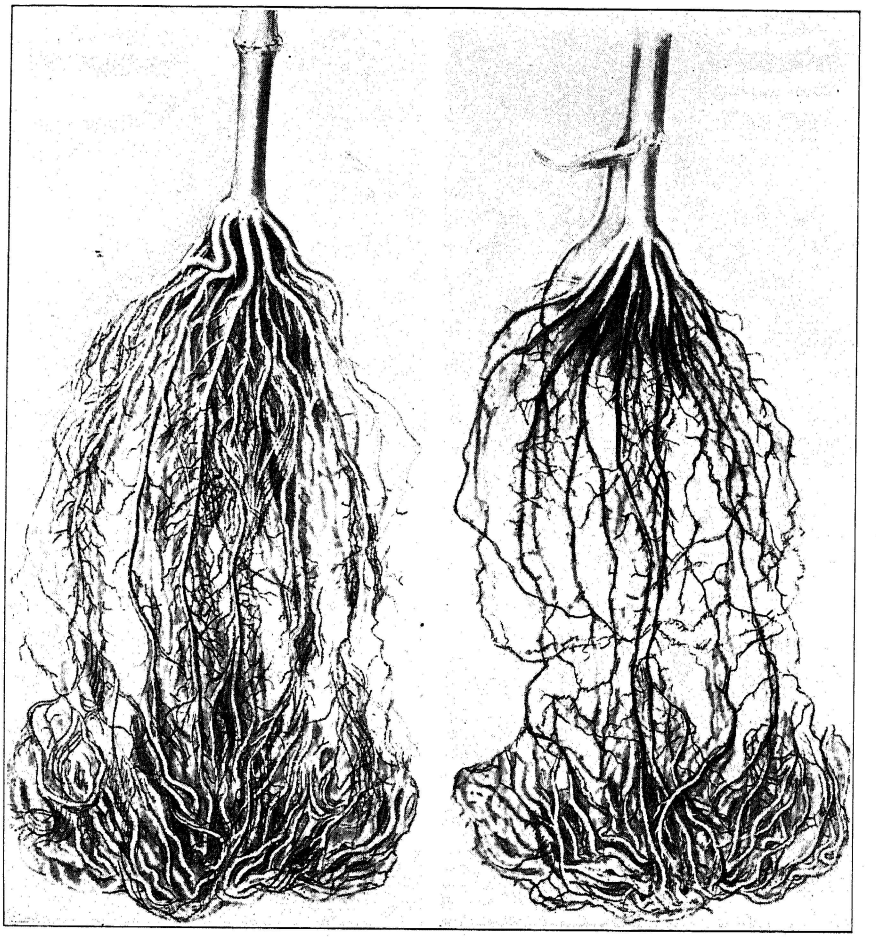


Plate II.—Reproduction of an artist's drawing showing (left) a healthy corn root system, and (right) a diseased corn root system.

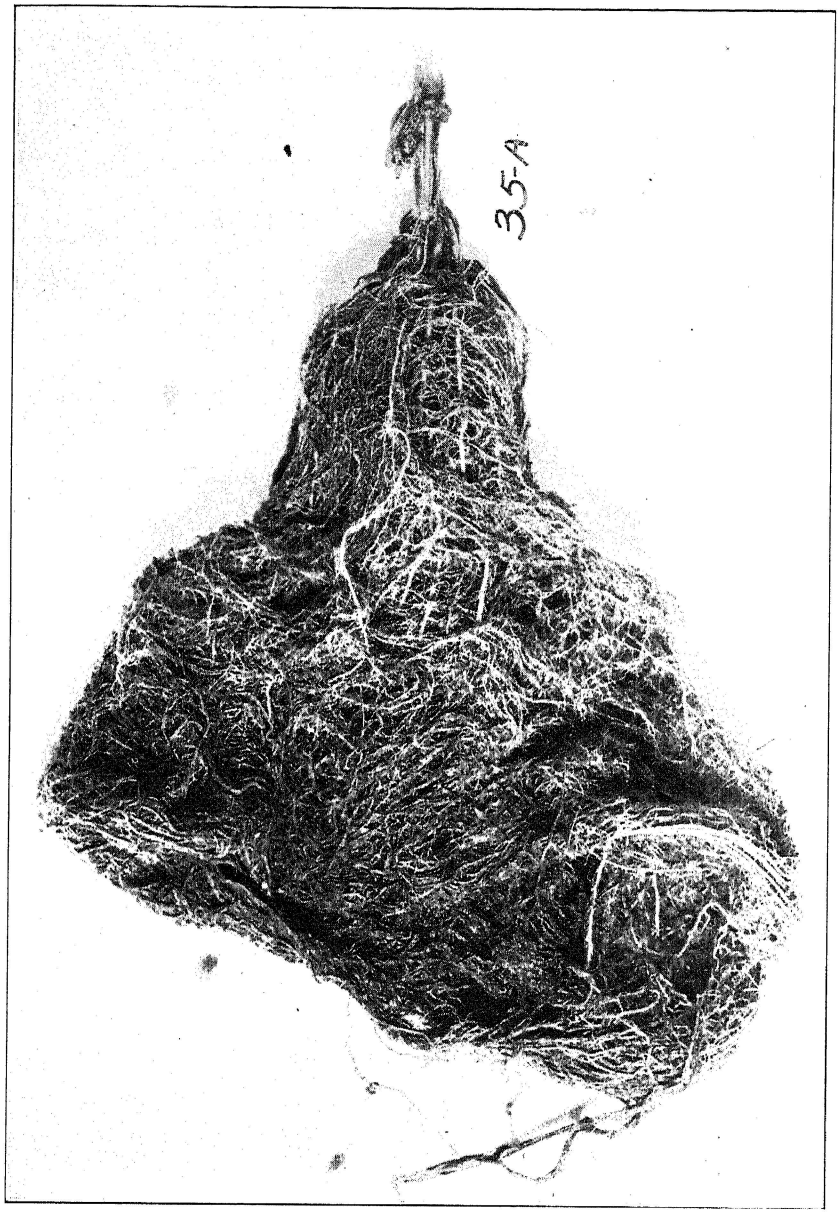


Plate III.—(35A) Root system of corn plant grown in sterilized infected soil (check). Weight 126 grams.

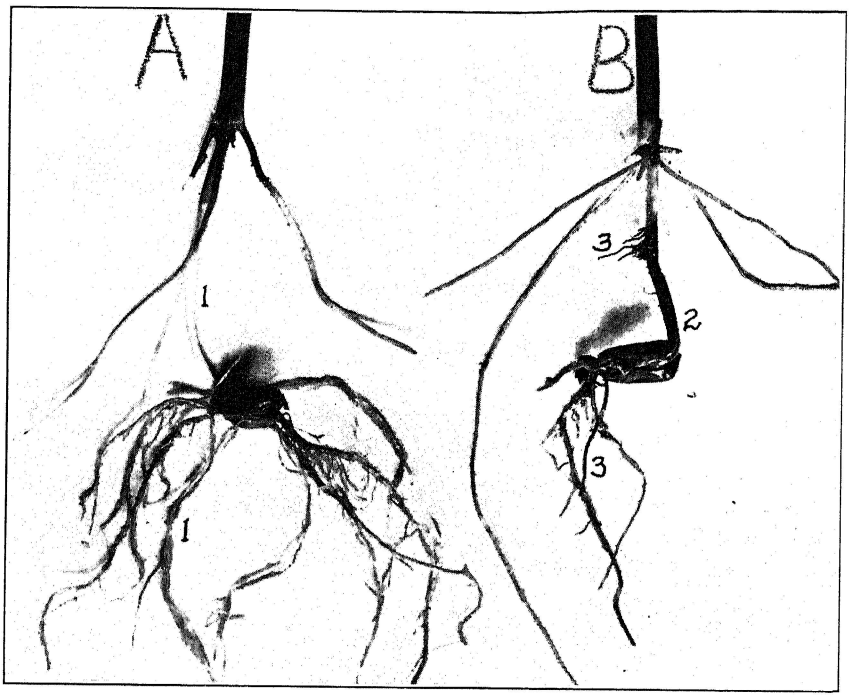
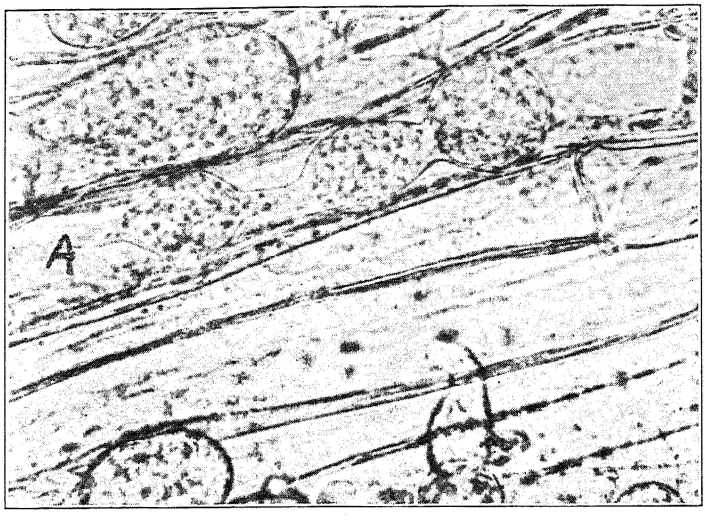


Plate VII.—A. Corn seedling grown in uninoculated sterilized sand. Note healthy mesocotyl (1) and roots.

B. Corn seedling grown in sterilized sand inoculated with the Pythium-like fungus. Note badly rotted mesocotyl (2), and roots. (3).



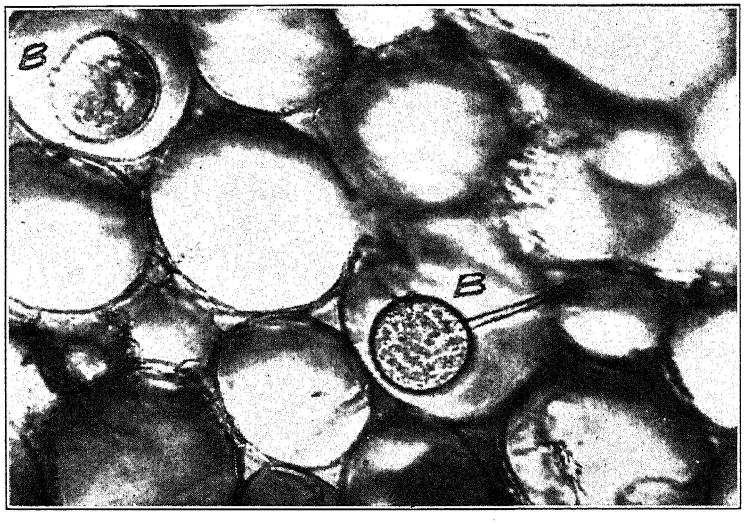


Plate VIII. A. Microphotograph of a crushed corn rootlet of an inoculated plant grown in sand. x 600.

B. Microphotograph of spores of Pythium-like fungus in diseased corn roots of mature plants grown on Station field, 1926. x 540.