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SEEDLING DISEASES OF SUGAR BEETS AND THEIR
RELATION TO ROOT-ROT AND CROWN-ROT

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serious losses. Many of the failures attributed to faulty germination are, in fact, the results of serious outbreaks of this disease, and in practically all the cases investigated where seedlings were "not growing well" the trouble has been found to be root sickness.

These or similar diseases have been known for a long time in Europe, where various and widely different theories have been advanced regarding their cause or causes. Refractory soil, cold ground, wet weather, poor cultivation, excessive rain, fungus infection, and the like have all had their advocates, who have based their opinions in many instances upon insufficient data.

Hesse (24)¹ reported the presence of *Pythium debaryanum* in diseased beet seedlings in 1874, but he seems to have done no experimental work with this host. Hellriegel (23), one of the first investigators who made a careful experimental study of this subject, showed that damping-off in his pot experiments appeared to be due to a parasite, and traced the source of infection to the seed. He did not, however, assign a specific organism as the cause. Eidam (13) produced artificial infection of beet seedlings with cultures of *Rhizoctonia betae* Kühn. Krüger (25, 26) found *Phoma betae* Fr. to be an active seedling parasite of the sugar beet and expressed the opinion that several different fungi are capable of producing diseases in seedlings of this plant so similar in appearance that they have been classed together under the name "Wurzelbrand." Several fungi, as well as bacteria, have since been added to this list of parasites, while other writers have denied the parasitic origin of the disease.

So much contradiction and uncertainty exists in the literature of the last 20 years regarding the nature and cause of Wurzelbrand of beets in Europe that Peters (31, 32, 33) and his associates (5, 6) found it necessary to go over the entire subject and submit to rigid experimental proofs the more worthy of the hypotheses that have been put forth.

From what appears to be a careful and trustworthy piece of work, Peters (33) concludes that under the conditions of his experiments *Pythium debaryanum* Hesse, *Phoma betae* Fr., and *Aphanomyces laevis* De By. are capable of producing damping-off of the sugar beet. He was unable to secure pure cultures of *Rhizoctonia violacea* Tul., but he infected soil with fragments of beets showing typical *Rhizoctonia* decay and failed to produce damping-off. The evidence of the parasitism of bacteria on this host seemed to him insufficient to justify serious consideration.

In America the only reported work on seedling diseases of beets, except the author's preliminary note (12), is by Duggar (9, 11), who conducted successful infection experiments with a species of *Rhizoctonia* that he secured from decayed beets and later designated as *Corticium vagum* B. and C., var. *solani* Burt. (10, p. 444-452). His experiments were carried out in sterilized soil and controlled by check plants

¹ Reference is made by number to "Literature cited," p. 165-168.

which remained healthy, but he makes no mention of treating the seed to insure the elimination of *Phoma betae*, which he regarded as absent (10, p. 344).

THE SECURING OF CULTURES

The first steps in the present work were naturally the securing of cultures, which were obtained in various ways. Beet seed was placed in sterilized filter papers in sterile moist chambers, and transfers were made from the colonies of fungi which developed during the progress of germination. This method is uncertain and yields a large number of harmless saprophytes. Seed was sown in soil which had been sterilized in an autoclave at 12 pounds' pressure for from four to six hours on two successive days. These were watered with sterile water and protected from outside sources of infection. Whenever damping-off appeared, the diseased seedlings were removed and treated for one minute with more or less shaking in a solution (1:1,000) of bichlorid of mercury in water or in similar bichlorid solutions containing either 1 gm. of ammonium chlorid or $\frac{1}{2}$ c. c. of concentrated hydrochloric acid per liter. They were then rinsed in sterilized water and dropped upon suitable nutrient-agar medium in Petri dishes (Pl. XVI and XVII). The agar most commonly used is sufficiently acid to materially check the development of bacteria and is at the same time a very satisfactory medium for the cultivation of most fungi. It has the following composition:

Dextrose.....	100	Dipotassium hydrogen	
Peptone.....	5	phosphate.....	2.5
Ammonium nitrate.....	10	Calcium chlorid.....	.1
Potassium nitrate.....	5	Water.....	1,000
Magnesium sulphate....	2.5	Agar.....	20

As soon as growth appeared from the seedlings, isolation transfers were made. Cultures obtained by these two methods were regarded as originating from the seed.

Cultures were made from the soil indirectly by means of seedlings in the following manner: Beet seed, treated by a method to be discussed later, so as to insure the absence of parasitic fungi, was sown in unsterilized soil in pots that were thoroughly sterilized before using. When damping-off occurred, isolations were made in the manner already described. Cultures were secured from decayed beets by cutting out with a sterile knife small portions of material on the border line between healthy and diseased tissue. These blocks were placed upon a suitable medium, and isolations were made from the developing colonies. Another method employed was to sow treated beet seed in sterilized soil, subsequently infected with fragments of decaying beets. Isolations were then made from the seedlings when disease developed. Large numbers of isolations were made from sugar-beet seedlings grown in commercial fields, and a considerable number of cultures were courteously contributed by various workers from time to time.

SEED TREATMENT

Sugar-beet seed is quite universally infected with parasitic fungi. It was therefore necessary to devise some method of freeing the seed from infection before inoculation experiments could be successfully conducted. Among the substances tried were hydrogen peroxid, hydrochloric acid, sulphuric acid, formalin solution, formaldehyde vapor, and hot water. Peroxid solution (6 per cent) was used for varying periods up to one hour. The seed was then sown in sterilized soil and watered with distilled water. The pots were protected from infection, but damping-off was in no degree checked, and *Phoma betae* was invariably isolated from the diseased seedlings (Pl. XVII).

Hydrochloric acid was employed in various concentrations up to a specific gravity of 20° B. for 15 minutes. The seed was then rinsed in sterile water, followed by lime water, which in turn was followed by sterile water. This treatment was without effect upon the vitality either of the seed or of the fungi.

Sulphuric acid was used in various strengths up to full concentration for one hour. The treated seed germinated strongly and from 24 to 48 hours earlier than the untreated control, but there was no decrease in the amount of damping-off.

Formalin solution was employed up to concentrations of 2 per cent of formaldehyde for various intervals up to one hour. This seriously injured the viability of the seed, but afforded no check to the disease. The results with formaldehyde vapor are inconclusive, since they lack uniformity.

The method finally settled upon for experimental work was one employed by Peters (7, p. 273-274), which consists of heating the seed in water at 60° C. for 10 minutes, promptly drying superficially upon filter papers, so as to prevent germination, and after an interval of 24 hours heating a second time for 10 minutes at 60° C. The seed treated in this way and sown in sterilized soil, watered with sterile water, and protected from outside infection remained practically free from disease. Not more than one seedling in three or four hundred was infected with *Phoma betae*. The percentage of germination is unquestionably lowered by this treatment, but it is the only method tried by which inoculation experiments could be controlled. It does not appear to be a method which could be applied on a commercial scale.

METHODS OF INOCULATION

Inoculation experiments were carried out in pots either in the laboratory or usually in the greenhouse, using seed treated in the manner just described, and with soil sterilized by heating three or four hours in the autoclave under a pressure of from 12 to 15 pounds on two, or usually three, consecutive days. Inoculations were made in the soil or upon the seed at the time of sowing, except when otherwise stated. Various

methods of inoculation were employed, but the results were uniformly the same. Either suspensions of spores or mycelial growth on various culture media, such as agar, corn meal, beet petioles, and sterilized beet blocks, were employed. The last method—that is, with beet blocks—was perhaps the most satisfactory and convenient. The corn-meal cultures appeared to exercise an unfavorable physiological action, possibly because of the bacterial growth which they fostered; so this method was discarded.

Inoculation experiments were invariably controlled by a considerable number of uninoculated pots. In a few instances disease occurred in the controls. In such cases the causal organism was determined, but the entire series was abandoned as an inoculation experiment, even though the presence of an intruder could be explained readily through the agency of insects and earthworms. It was the invariable custom to recover the fungus from the damping-off seedlings by the method already described (Pl. XVI and XVII) and to reinoculate and recover through from four to six generations of seedlings.

As reported in a former note (12), four fungi have been found to stand in causal relation to seedling-beet troubles. These are *Phoma betae* (Oud.) Fr., a species of *Rhizoctonia*, regarded as identical with the form described as *Corticium vagum* B. and C., var. *solani* Burt., *Pythium debaryanum* Hesse, and a fungus originally reported as *Aphanomyces laevis* De By., but which has since been found to be new.

PHOMA BETAE

TAXONOMY

Frank (16, 17, 18, 20) established the relation of *Phoma betae* to heart-rot of the sugar beet in 1892. The following year Krüger (25, 26), working in the same field, demonstrated its causal relation to damping-off. He found the fungus fruiting abundantly on all parts of diseased beets and held it to be identical with the fungus which had previously been observed on various portions of the cultivated varieties of *Beta vulgaris* L.

Oudemans (29, p. 181) had observed what appeared to be the same fungus fruiting upon leaf spots of old beets and applied the name "*Phyllosticta betae*." Prillieux (35, p. 19) observed the fungus on leaf blades and decaying heart leaves, as well as upon typical spots on the leaf, and applied the name "*Phyllosticta tabifica*." Saccardo recognizes the names "*Phyllosticta betae* Oud." and "*Phoma betae* Rostr." The latter name is given on the authority of the following paragraph from the pen of E. Rostrup (41, p. 323):

Eine zweite, an Runkelrüben auftretende *Phoma* habe ich zuerst in meinem Jahresbericht über Krankheiten der Kulturgewächse im Jahre 1888 (Tidsskrift for Landökonomi. R. 5, Bd. 8, S. 746) [40] unter dem Namen *Phoma sphaerosperma* beschrieben. Weil sich aber herausstellte, dass dieser Name schon im Jahre 1885 einer ganz anderen Art gegeben war, nannte ich später den Pilz *Phoma Betae*.

No reference to the publication in which this change of name was announced is given, and efforts to find it have failed. Moreover, Lind (28, p. 415), who had full access to Rostrup's specimens and publications, lists *Phoma betae* Rostr. under *Phoma betae* Fr. as a synonym. Frank suggested the name "*Phoma betae*" in the article previously cited (16), published in 1892, which contains a description of the fungus, accompanied by figures and a somewhat extended discussion of the root-rot produced by it on the sugar beet. In view of the established identity between *Phoma* and *Phyllosticta* on sugar beets it would appear that Oudemans' description in 1877 (29) has first claim to priority.

For these reasons it seems proper and convenient to retain the name which has persisted in most general use in literature, but with a correction to insure proper acknowledgment to Oudemans. The name "*Phoma betae* (Oud.) Fr." is therefore used in this paper. Pool and McKay, who agree in the justice of this usage, also employ it in a current paper (34).

Neither Frank (17) nor Krüger (25, 26) were able to find evidences of a perfect stage of this fungus in their cultural studies. Peters (31, 32, 33) also failed to find sexual fruits, and the same is true of the work of the writer. It should be pointed out, however, that Rostrup (40, p. 746) believed *Sporodesmium putrefaciens* Fuck. to be a perfect stage of *Phoma betae*, and Prillieux and Delacroix (37) regarded *Phoma betae* as the pycnidial form of *Sphaerella tabifica* Delacr.

IDENTITY OF PHOMA AND PHYLLOSTICTA ON THE SUGAR BEET

Hedgcock (22) has presented evidence by cross-inoculation of the identity of *Phyllosticta* and *Phoma* on the sugar beet. He grew beets from seed treated with concentrated sulphuric acid for 30 minutes, followed by an alkali, and successfully produced *Phyllosticta* spots by spraying upon the leaves spores of a *Phoma* culture isolated from decaying beets. Beets whose leaves were covered with *Phyllosticta* were placed in a dry cellar and held under observation for two months, during which time the characteristic black rot of *Phoma* passed from the leaf petioles to the crown of the beet.

While Hedgcock's conclusions are undoubtedly correct, the seed treatment he employed does not destroy the viability of *Phoma*, and it will be shown later that beets whose foliage was free from visible evidence of *Phyllosticta* decayed from *Phoma* when they were placed in a relatively dry environment. The case may be strengthened, therefore, by the presentation of the additional evidence now available. Cultures from *Phyllosticta* spots, as well as from decayed beets and from damped-off seedlings, have been found equally capable of producing damping-off of sugar beets, and a somewhat extended study of the morphology of the fungus from the three sources mentioned has revealed no differences

between them. The *Phyllosticta* cultures employed were supplied by Miss Venus W. Pool, of the Rocky Ford (Colo.) field station. Pool and McKay (34), who worked with *Phoma* cultures isolated by the writer from decayed sugar beets, found them capable of producing the characteristic leaf spots as readily as cultures isolated from the *Phyllosticta* pycnidia. The fungus, however, is not an aggressive leaf parasite, but does its greatest injury on the root.

SOURCES OF INFECTION

The source of original infection appears to be the seed. It has been generally recognized in Europe for years that seed infection with *Phoma betae* is universal. As American growers are using European seed almost exclusively, it follows that the disease is constantly being introduced into the United States on seed. A very large number of samples of both European- and American-grown beet seed have been examined for the presence of this and other pathogenic forms. With the exception of one single lot of seed, the examination of 100 seed balls by the seedling method has invariably demonstrated the presence of *Phoma betae*. Reexamination of this one lot, which was American-grown, revealed the presence of the fungus in it also when larger samples were tested.

Frank (18, p. 180, 272-293) believed the fungus capable of living over in the soil by means of its spores, but Peters (7, p. 278-286) holds that it can do so only when fragments of beets are present to support mycelial growth. A large number of trials of soil in America made by means of seedlings growing in it from pasteurized seed indicate that *Phoma* does not remain viable in the soil after the decomposition and disintegration of its host. Field soils containing decaying beet fragments occasionally yield cultures of the fungus in the spring of the first year following beets, but, as a rule, even seriously beet-sick soils fail to give them. Samples have been examined from Virginia, District of Columbia, Michigan, Wisconsin, Kansas, Colorado, Utah, and California.

MORPHOLOGY OF THE FUNGUS

Several hundred cultures of the fungus have been isolated and grown upon media (Pl. XVII). No constant differences in cultural characters of strains from the various sources have been observed. It is readily cultivated upon a great variety of media, although on many of these it develops mycelium only. It fruits abundantly upon string-bean agar (Pl. XVII, fig. 2) and this medium has been used for purposes of identification and for measurements of pycnidia and spores. It is evident that the curves which might be plotted from the following tabulated results of measurements would be irregular and consequently that they would probably be changed by increasing the number of pycnidia and spores

measured (Table I). Of 181 pycnidia measured the smallest was 125μ and the largest 635μ in diameter. The largest number fall between 225 and 325μ .

TABLE I.—Variation in size of 181 pycnidia of *Phoma betae*

Number measured.	Variation in diameter.	Number measured.	Variation in diameter.
	μ		μ
1	125	11	351 to 375
4	126 to 150	5	376 to 400
14	151 to 175	10	401 to 425
7	176 to 200	5	426 to 450
31	201 to 225	1	451 to 475
21	226 to 250	1	476 to 500
10	251 to 275	1	501 to 525
27	276 to 300	1	526 to 550
19	301 to 325	1	551 to 635
11	326 to 350		

The pycnospores showed quite wide variations in size. The shortest of 204 spores measured 3.8 and the longest 9.4μ . Practically all fell between 4.1 and 7μ , as shown in Table I.

The width of the pycnospores varied from 2.6 to 4.3μ . The single exception to this case is a spore which measured 4.9μ in width. Table II will show the distribution of numbers within the limits given.

TABLE II.—Variation in size of 204 pycnospores of *Phoma betae*

LENGTH

Number measured.	Variation.	Number measured.	Variation.
	μ		μ
1	3.8	14	6.1 to 6.2
4	4.1 to 4.2	11	6.3 to 6.4
3	4.3 to 4.4	6	6.5 to 6.6
2	4.5 to 4.6	6	6.7 to 6.8
14	4.7 to 4.8	7	6.9 to 7.0
13	4.9 to 5.0	1	7.1 to 7.2
22	5.1 to 5.2	1	7.3 to 7.4
21	5.3 to 5.4	2	7.5 to 7.6
24	5.5 to 5.6	2	7.7 to 7.8
22	5.7 to 5.8	1	7.9 to 8.0
25	5.9 to 6.0	2	9.3 to 9.4

WIDTH

5	2.6 to 2.7	22	3.6 to 3.7
15	2.8 to 2.9	17	3.8 to 3.9
43	3.0 to 3.1	7	4.0 to 4.1
49	3.2 to 3.3	3	4.2 to 4.3
42	3.4 to 3.5	1	4.9

VITALITY IN CULTURE

Phoma betae exhibits long vitality in culture, as is shown by the following tests.

On July 2, 1913, old cultures which had been apparently air-dry for months were opened under sterile conditions, and into each a portion of string-bean agar was introduced. The tubes were then placed to harden in such a position as to leave the old culture partly submerged. Table III gives the age of the cultures and the results after six days' incubation.

TABLE III.—Vitality of *Phoma betae* in culture

Phoma strain.	Number.	Age of culture.	Results.	Phoma strain.	Number.	Age of culture.	Results.
		<i>Days.</i>				<i>Days.</i>	
Strain 3...	39	413	Good growth and pycnidia.	Strain M...	1,304	315	Good growth and pycnidia.
Strain A...	423	372	Do.	Strain 3...	64	413	No growth.
Strain J...	564	358	Do.	Strain 3...	40	413	Do.
Strain J...	996	315	Do.	Strain 3...	281	404	Do.
Strain E...	1,296	315	Do.	Strain 3...	386	404	Do.
Strain J...	1,301	315	Do.				

INOCULATION EXPERIMENTS ON SEEDLINGS

The strains used in inoculation experiments are 24 in number, obtained from the following sources:

Direct isolation from sugar-beet seed by the moist-chamber method, 1.

Indirect isolation from sugar-beet seed through damping-off seedlings in sterilized soil, 2.

Direct isolation from *Phyllosticta* leaf-spot, 2.

Isolations from various sources such as beet leaves, beet seed, and beet-sick soil at Rocky Ford, Colo., 13.¹

Isolations from beets having heart-rot, from Colorado, 1; Wisconsin, 2; South Dakota, 1.

Isolations from seedlings grown in sterilized soil, inoculated with decayed beets, 2.

The inoculation experiments upon seedlings were carried out in the manner already described, invariably yielding positive results. There appeared to be no decrease in virulence from carrying the fungus in culture 14 months.

The first development of damping-off in the pots occurred usually about the third day after the seedlings broke the ground and continued till about the time they developed their third or fourth pair of leaves (Pl. XVIII, fig. 2). The method followed was to sow 100 seed balls per

¹ These cultures were kindly supplied by Miss Venus W. Pool, of the Bureau of Plant Industry, from the Rocky Ford (Colo.) field station.

pot and to examine daily for damping-off. Whenever disease occurred, the plants affected were removed and a record made of their number. The period of greatest susceptibility seemed to be passed by the time the third set of leaves appeared. After a sufficient period the remaining plants were harvested and examined for signs of infection on the roots.

The following record (Table IV) of a typical series illustrates the method and gives a good idea of the average results.

TABLE IV.—Results of inoculation experiment with *Phoma betae*

[Series of June 27, 1912]

Phoma strain.	Appeared above-ground on—	Number of seedlings diseased on July—									Number harvested on July 29.	
		3	4	5	6	7	10	12	15	17	Dis-eased.	Healthy.
Strain C.....	July 1			3	5	6	18	19	5	0	4	18
Strain E.....	do.....		7	10	7	8	9	7	3	2	5	3
Strain B.....	do.....		2	3	6	3	12	13	4	4	7	30
Strain A.....	July 2		1	2	8	12	27	17	6	2	7	13
Strain D.....	July 1	2	24	20	13	8	16	2	0	0	1	0
Strain F.....	do.....				5	6	9	9	10	1	5	14
Strain H.....	do.....		7	44	12	0	11	0	0	2		
Strain G.....	do.....				8	0	21	18	14	0	52	12
Do.....	do.....		5	11	22	45	5	0	0	0	5	7
Control.....	July 2										111	
Do.....	do.....											148

The plants described as diseased were typical root-sick beets. There was nothing about their appearance aboveground in the pots to indicate the presence of the fungus, but in the field such seedlings may often be detected from the fact that they appear to be suffering from lack of moisture. This type of disease is almost universal under field conditions and is far more common than damping-off. Histological studies upon such material, to be published in a subsequent paper, revealed the significant fact that it often carries the pycnidia and sometimes the vegetative mycelium of the fungus.

The number of seedlings developing in control pots was, as a rule, larger than the total number developing in inoculated ones. This is to be explained by the fact that some of the seedlings were attacked before they broke the ground and in consequence were unable to push through the soil. A careful examination of the surface soil in inoculated pots now and then revealed such seedlings in a state of more or less complete decay.

PHOMA FIELD-ROT AND STORAGE-ROT FOLLOWING SEEDLING INFECTION

When diseased plants were allowed to remain in the pots and were held under as favorable conditions as possible, a large proportion of them eventually survived and sent out new roots. This condition is

often observed in commercial fields, and it is probably safe to say that, under advantageous conditions of climate and cultivation, 75 per cent of the beets attacked by *Phoma betae* are not killed, but throw off the parasite sufficiently to make a good growth while continuing to carry the fungus in a dormant condition upon the crown. When the vitality of the host is sufficiently reduced, the parasite may become active again and develop either in the root, where it causes a black rot, or on the aerial portions of the plant, where it causes a spot disease, which, while it does not injure the host to an appreciable extent, enables the fungus to infect the seed growing on mother plants. The rot is, of course, more serious to the plants attacked. Cases of this so-called heart-rot in the field have been very destructive at times in certain sections of Europe and are not infrequent in America. Important instances of its occurrence in Michigan, Wisconsin, and Colorado, and a less serious case in New York, have come to the writer's attention. Material from the three States first mentioned was available for study. The fungus was easily secured in pure culture from the beets that were partially decayed by the black rot typical of the disease (Pl. XVIII, fig. 1). Many roots presented unmistakable evidence of having been diseased in the seedling stage. In some cases the original taproot had been destroyed, and only a knob of tissue just below the crown had survived.

Additional evidence that the fungus is capable of living upon its host for a long time in an inconspicuous and apparently harmless form until those conditions appear which are favorable to its development is readily found upon examination of beets in storage or those which have been kept over for seed purposes. Such examination in the spring has never failed to reveal the presence of the fungus. Sometimes the entire root is destroyed, but more frequently beets that are apparently healthy may be found to show black spots in certain sections in or near their vascular tissue, especially in the top of the crown. These areas usually develop longitudinally and are frequently confined to the vicinity of a single vascular bundle (Pl. XIX). When the conditions of storage have been unfavorable, the rot may assume an epidemic form and lead to the total destruction of roots which were apparently healthy and sound when placed in storage.

INOCULATION EXPERIMENTS ON GROWING BEETS

Evidence that the infection takes place only in the seedling stage or through the leaves in old age is found in the results of inoculation experiments made with cultures of *Phoma betae*. In August, 1912, 100 apparently healthy beets growing in the field at Madison, Wis., were inoculated. A portion of the infections were made directly upon the uninjured crown with vegetative cultures upon beet blocks. Other inoculations were made through wounds near the surface of the soil and

still others through wounds on the petiole or in the heart of the crown. In no case was there any evidence of rot in the field or at the time of harvest. It was at first believed that climatic conditions were responsible for the failure. However, the beets were placed in storage. The following spring they were examined for evidences of *Phoma betae* by culture methods. None of the beets had been destroyed; indeed, most of them appeared to be perfectly sound until they were opened, when the black lines previously mentioned appeared in the vascular regions. Cultures were obtained from each of the infected beets, except two. These results were at first interpreted as an indication that the inoculations made in August had produced infection which had persisted on the surface and penetrated the roots in storage, but subsequent developments throw doubt upon this conclusion and indicate that the infection occurred much earlier. About half a ton of beets grown as controls had been placed in storage beside the infected material, but in a lower rack. Evidences of rot were observed in only half a dozen of these beets, and they yielded cultures of *Phoma betae*. It was noted at the time of examination that the control beets, which had been kept nearer the surface of the soil, were all firmer than the inoculated material; but unfortunately no significance was attached to this fact at the time, and, with the exception of 12 of the control beets, the entire lot of material was sent to the feeding sheds.

A few weeks later a large quantity of mother beets grown in the vicinity of the experimental field from western seed was found to be seriously injured by the Phoma rot, practically every beet showing more or less evidence of it. These beets had been bored for analysis in the fall, so that any evidence of infection at that time would have been apparent. The decay did not originate from infection in the wound made by boring, as may be seen readily by reference to the illustration (Pl. XIX). In the large majority of cases the rot evidently started from the crown, although there were cases of pockets of decay on the sides or even at the tip of the beet. In some instances dark streaks could be traced from the crown to decayed areas in the lower portion. This led to the belief that the decay in beets inoculated with *Phoma betae* was due to infection occurring in the seedling stage and that its development was fostered by the less favorable, dryer conditions of storage to which they were submitted. Additional evidence for this belief is to be found in the results of the inoculation experiments with *Rhizoctonia* (p. 153), since the Phoma rot appeared upon this material also. Moreover, of the few control beets saved from the feeding sheds all but three developed Phoma decay during the late spring and early summer, and cultures of *Phoma betae* were secured from darkened bundles in the crowns of these three. It might be urged that the infection reached the crowns of these beets by way of the petioles from *Phyllosticta* upon the leaves. This seems a very probable means of infection, but appears unlikely in the instances above cited,

since *Phyllosticta* spots were at no time observed upon the foliage of the beets in question, which were grown in isolation and were under daily observation. The following season (1913) inoculations were made on beets grown outdoors at Madison, Wis., in pots filled with soil from Garden City, Kans., from Rocky Ford, Colo., and from Madison, Wis. Other beets grown in the usual way in the field at Madison, at Garden City, and at Rocky Ford were inoculated. In all cases this was done by placing a large fragment of actively growing culture on sterilized beet blocks upon the crown of the beet near the heart and a second portion in direct contact with an abrasion on the crown just beneath the surface of the soil. Through the courtesy of colleagues at Garden City and Rocky Ford it was possible to have the beets watched at these points as carefully as were those at Madison. At no time during the season did evidence of infection appear on any of the beets under observation.

Two lots of beets were grown in 1913 from pasteurized seed. One lot was isolated from other beets so as to be protected from infection. These beets were stored in the fall in a warm basement, which offered very favorable conditions for the development of *Phoma* storage-rot, as shown by previous experience. They were examined from time to time during the winter, spring, and summer for evidences of *Phoma betae*, with negative results.

The other lot of seed was sown in proximity to breeding stock and mother plants known to be infected with *Phoma betae*, and stored in a cellar with infected material. These beets developed *Phoma* rot during the winter. *Phyllosticta* appeared on seed plants grown from some of these roots during the summer of 1914, and the seed produced was found to carry the pycnidia and spores of *Phoma*.

PERPETUATION OF THE FUNGUS

The results of the various experiments coupled with field observations lead to the opinion that *Phoma betae* is capable of infecting *Beta vulgaris* L. only during periods of especial susceptibility, such as the early seedling stage, or, in the case of the leaf, during physiological old age. It may also be that the root is susceptible to infection during periods of low vitality induced by unfavorable environment, but experimental proof is lacking. It does appear, however, that this fungus is entirely capable of living for a long time in a hidden condition upon the crown of the beet, ready to take advantage of any diminished vitality in its host. After infection has once occurred, the parasite may remain present in a viable though inconspicuous condition, although the host appears to have completely overcome the disease. The *Phoma* field-rot in the summer and fall of 1913, in Wisconsin and Colorado, respectively, developing in the first instance under conditions of excessive moisture and in the second under severe drought, may be explained in this way. The crown

infections originating from the seed readily explain the source of the spores producing the outbreaks of *Phyllosticta*, which appear to be much more common in regions of low humidity, where, as Pool and McKay (34) have shown, viable spores of this fungus are common in the air. In this connection, attention should be especially called to observations during the seasons of 1912 and 1913 in Colorado, Idaho, and Wisconsin, since they indicate a direct connection between the occurrence of *Phyllosticta* on the leaves and seed stalks of mother beets and the presence of *Phoma* on the seed they produce. *Phyllosticta* was quite prevalent in the Idaho-Colorado region, but escaped detection by the same observer in Wisconsin. Tests upon seed produced in these regions during both seasons have shown that the more western seed was quite generally infected to an extent comparable to that from European sources, while that grown in Wisconsin showed only a very slight infection.

Pool and McKay, who kindly continued the observations at Madison during the absence of the author in the season of 1914, found the *Phyllosticta* form on the leaves and stalks of seed beets and also on first-year beets. In the absence of fruits the causal fungus was identified by cultural methods. In other instances pycnidia were produced. The spots were, as a rule, less clearly defined and less numerous than those in the Idaho-Colorado region, which are similar to those seen in Europe in 1914, where they were common on both field and mother beets. Since the leaf form of the disease occurred in Wisconsin in 1914, it is probably safe to assume that it existed there on seed beets, undetected, in the two preceding years. However, if the absence of *Phyllosticta* infection in Wisconsin in 1912 and 1913 be assumed, the presence of the small amount of *Phoma betae* on the seed produced there may still be accounted for in at least three different ways. The seed balls may have been infected at any time between blossom and maturity by air-borne spores developing in the crown. The fungus may have spread from the crown of the mother plants on the surface of the seed stalks to the racemes and infected the fruit, or it may have passed through the vascular system of the stem to the seed.

CONTROL OF THE FUNGUS

It is apparent that *Phoma betae* is one of the most serious obstacles which the growers of beet seed in America have to face. Its ravages on mother beets during the winter may be largely overcome by favorable conditions of storage, but in case the mother beet escapes destruction the infection remains to proceed to the leaves and seed stalks and to infect the seed. Since the fungus sometimes inhabits the vascular region of the root, it may possibly progress through the stem, as well as on the surface. In any event, it is an undoubted fact that it finds its way into the young seed ball, where it starts with the seed upon another 2-year cycle.

From what has been said regarding sources of infection, it appears that ordinary attention to rotation should eliminate danger from soil infection, but that all seed at present available is heavily infected. The only hope of control therefore lies in one or more of three alternatives: (1) The natural resistance of the beet to the attacks of the fungus, (2) seed treatments, (3) the production of disease-free seed.

From the facts that the period of infection with *Phoma betae* is normally confined to a relatively short period in the seedling stage and that infected plants frequently throw off the attack, proper attention to cultural conditions would seem to offer hopeful prospect of control. European experience has demonstrated the value of such methods. Indeed, some prominent agriculturists have denied the pathogenicity of *Phoma betae* because of their success in preventing damping-off and root sickness by proper cultivation and fertilization. From this they have argued that the cause lies in unfavorable cultural conditions rather than the presence of parasites. The truth undoubtedly is that unfavorable environment is a predisposing cause which so weakens the beet that it is unable to compete successfully with the fungus. When planted in good soil, which has been well prepared and suitably fertilized, the seed germinates promptly and the young plants pass rapidly through the period of danger. Early cultivation at this stage to insure proper aeration of the roots is beneficial. The studies upon control by means of cultural methods and fertilizers demonstrate the value of properly prepared soil and thorough cultivation promptly after the seedlings come through the ground. There is no doubt that frequently an infected stand may be saved in this way. In Europe the use of phosphoric acid and potash has given good results. The question of soil reaction has also been found very important. Applications of lime on certain types of acid soil result in almost complete control. This may also prove to be the case in America, but the point can be determined only by local experiment.

As has already been said, the only method of seed treatment which has given satisfactory results in pot experiments is seed pasteurization. Experimental work to test the efficiency of this method in field practice has been attempted. It is not believed that a method of treatment as difficult to carry out as pasteurization will prove useful except in the hands of experimentalists and for experimental purposes. It is apparent that seed must be treated in small lots and with extreme care in order to secure the desired result. If temperatures much above 60° C. are employed, the injury to the seed becomes serious. Temperatures below 60° are ineffective. The substitution of one treatment for two is likewise unsuccessful. Attempts to apply the treatment to samples of seed of even 5 pounds have not been altogether satisfactory, but appear to hold the fungus in check.

One experiment in seed treatment carried out under field conditions where epidemic development of root sickness has annually occurred for

several years will illustrate the point. A half-acre field was prepared. Five pounds of pasteurized seed were sown on one quarter-acre and five pounds of untreated seed on the other. As soon as the seedlings were well out of the ground, examination was made for root sickness and damping-off. No signs of damping-off were seen, but considerable root sickness was in evidence on both the treated and untreated sections. Cultures were started in the field by treating the seedlings for one minute in a triturate of citric acid and bichlorid of mercury in water of such strength as to give a 1 to 1,000 solution of sublimate. They were then transferred to test tubes of sterilized water and were brought to the laboratory, where they were plated. A period of 36 hours elapsed between the treatment with the bichlorid of mercury and the plating. From the untreated seed 95 seedlings yielded the following results: *Phoma*, 29; *Fusarium*, 19; *Macrosporium*, 2; *Mucor*, 6; miscellaneous, 18; no growth, 21. From the treated seed 69 seedlings yielded: *Fusarium*, 23; an unidentified ascomycete, 11; *Macrosporium*, 2; *Mucor*, 2; *Penicillium*, 1; miscellaneous, 8; no growth, 22. *Phoma betae* was not found in the treated lot. The long interval before plating undoubtedly accounts for the large amount of *Fusarium* and perhaps also for the failure to secure growth in many cases.

The average results secured from prompt plating may be seen from the following series, which was from the same locality but was made two weeks earlier. Many of these were plated immediately. Most of the *Fusarium* resulted from seedlings which were carried in the water blanks for half a day or more. Two hundred seedlings yielded cultures as follows: *Phoma*, 149; *Fusarium*, 29; *Pythium*, 3; miscellaneous, 11; no growth, 8. It therefore appears that, while seed pasteurization may be employed successfully to rid seed of *Phoma betae* for experimental purposes, it is not applicable on a commercial scale. Moreover, such treatment does not guarantee freedom from physiological root sickness associated with saprophytic fungi, since the vitality of the seedlings seems to be lowered by pasteurization.

A realization of these facts suggests the necessity of clean stock for breeding purposes. It would seem that if the growers of elite strains could rid their stock of this parasite it would thereafter remain clean, provided a reasonable rotation were observed and the seed fields were sufficiently isolated to escape reinfection with *Phyllosticta*. Danger from this source would diminish with the increase in the supply of clean seed. The experimental work already reported has shown the possibility of eliminating the fungus from plants grown in isolation from pasteurized seed. These plants would produce clean seed which could again be sown in isolation from infected stock and made the basis for a seed supply for an entire community, from which the fungus would be eliminated. This community could be employed as a breeding center where the entire seed supply of a factory could be grown, and one of the most

serious fungous pests of the sugar beet eliminated from its territory. Since sugar companies have absolute control over the sources of seed supply of their growers, it is quite possible for a company producing even a portion of its own seed to maintain an area of quarantine within any portion of its territory where it does not compete with other companies for acreage, provided table beets and mangel-wurzels are not allowed to bring in the infection.

RHIZOCTONIA

The genus *Rhizoctonia* has been used to include a group of sterile fungi, more or less closely related morphologically. Much confusion exists regarding the identity of the various forms, and there is likewise great diversity of opinion as to the pathogenic properties of the members of the group. To make clear, especially to foreign investigators, the identity of the fungus under consideration in this paper, a brief discussion of the literature seems essential. The name "*Rhizoctonia*" was first applied by De Candolle (8) in 1815 to a fungus on alfalfa. He eventually distinguished three species, *R. crocorum*, *R. medicaginis*, and *R. mali*. During the following 35 years various workers described a series of diseases caused by similar fungi, which were referred to this or other genera. In 1851 Tulasne (43, p. 188) united the known forms of *Rhizoctonia* into one species under the name "*Rhizoctonia violacea*." This classification has been followed by many workers.

In 1858 Kühn (27, p. 222-249) published an account of three species, *R. solani*, *R. medicaginis*, and *R. crocorum*. He distinguished between the two forms first mentioned by the difference in appearance of the sclerotia, those of *R. solani* being smooth, and those of *R. medicaginis*, woolly. He mentions *R. medicaginis* as being parasitic on the beet and carrot, as well as alfalfa, and states that the fungus produces a reddish brown or purplish color in the cells of the beet. This is the first mention of *Rhizoctonia* on the beet, and it is likewise the first mention of the fungus in Germany. Saccardo has included Kühn's species under *R. violacea*, while Güssow (21), who described a disease on potatoes and alfalfa in England due to *Rhizoctonia*, considers *R. solani* Kühn to be identical with *R. violacea* Tul. Eriksson (14) in 1903 published the results of inoculation work on various hosts, including the sugar beet. He designated his fungus as *R. violacea*, but later reclassified it as *Hyphomysces violaceae* (Tul.) Eriks. (15, p. 421-430). He believed there were biological forms of it, since the form on carrot attacked the beet with more virulence in the second generation than in the first in which it was carried on that host.

Atkinson (1) in 1892 described a damping-off of cotton due to a sterile fungus later classified as *Rhizoctonia*. Balls (2, 3) has found the same disease in Egypt, and Shaw (42) has more recently reported it from India. In the meantime Duggar (10, p. 344) described the same fungus as a damping-off parasite of the sugar beet, and Pammel (30)

reported a root-rot of beets in Iowa that he believed to be due to *Rhizoctonia betae* Kühn. The fungus, however, appears to be indistinguishable from the one which Atkinson and Duggar, respectively, had reported as a damping-off agent on cotton and sugar-beet seedlings and from forms of *Rhizoctonia* upon a variety of hosts throughout the United States, acting either as damping-off agents or as the causes of other forms of plant diseases. Rolfs (38, 39), working with the fungus on the potato in Colorado, found a fruiting stage which he designated as *Corticium vagum* B. and C., var. *solani* Burt. He was unable to produce the *Corticium* in culture, but the growth from spores yielded typical *Rhizoctonia* mycelium, and infections on living plants with *Rhizoctonia* gave rise to *Corticium*. Shaw observed the fruiting stage on the groundnut and later succeeded in producing it by artificial infection on that host. European workers have referred what appears to be the same basidial form to *Hypochnus solani*.

Shaw (42) found marked differences in the character of sclerotia produced by his strains. One which formed small black sclerotia more or less differentiated into cortex and medulla he designated as *R. solani* Kühn. The structure of the sclerotia of the other *Rhizoctonia* which he designated only as *Corticium vagum* B. and C. appears to correspond closely with that of those obtained in America in cultures and less frequently on the host. He believed this *Corticium* to be identical with the form common on potatoes in America, but was unable to see justification for referring it to *R. solani* Kühn. Furthermore, he believed *R. violacea* Tul. to be a compound species, possibly including *R. solani* Kühn and the *Corticium*, since Prillieux (36, t. 2, p. 144) described *R. violacea* Tul. as possessing two distinct forms of sclerotia, one of which, according to Shaw, is similar to those of *R. solani* Kühn and the other to *Corticium vagum*.

A form of crown rot on the sugar beet caused by *Rhizoctonia violacea* Tul. is well known in certain sections of Europe, but rot from *Rhizoctonia solani* is unknown there. This fact has led many students to question the identity of the fungus causing the rot of the beet in America. The organism here considered is distinctly different from the *Rhizoctonia violacea* Tul. type as the writer saw it in Europe on living or preserved material from a variety of hosts, including beets, carrots, potato tubers, alfalfa, and asparagus, but it appears to be identical with the *Rhizoctonia solani* type which forms the sclerotia on the potato in Europe and America. These two fungi when studied on the same host differ in the character of the disease produced, in their appearance on the plants, and in the histological relation of the parasite and host. The two are so distinct in these characters on both the beet and the potato that it seems impossible for one who has seen both types to confuse them. The cultural relations of the two are also distinctly different. *Rhizoctonia solani* is readily cultivated on a variety of media, but all attempts to put

R. violacea into artificial culture have thus far failed, though many different workers have undertaken it. So far as can be determined from the literature, the American fungus also appears to be identical with Shaw's (42) *Corticium vagum* B. and C., and it is indistinguishable in culture from a strain isolated in Ireland by Pethybridge from a single spore of *Hypochnus* on the potato, and kindly contributed by him under the name "*Hypochnus solani*."

The fungus is characterized by a septate mycelium the branches of which in young cultures are either parallel to or inclined at a more or less acute angle to the direction of growth of the parent branch. There is a constriction where the branch unites with the old hypha and a septum is formed a few microns from the point of origin. The threads are hyaline when young, becoming a yellowish brown with age. In mature cultures the branches are usually arranged very nearly at right angles to the parent thread at the point of origin. In culture and less frequently upon the host it forms sclerotia, which vary greatly in size (Pl. XX, fig. 2). One shown in the illustration of a sugar beet measured a full half-inch (Pl. XXIII). They are usually much smaller, from 1 to 3 mm., and those produced in cultures are likely to be quite irregular in outline. The sclerotia consist of interwoven branches, forming a loose pseudoparenchyma of uniform structure throughout. The sclerotial hyphae are broken up into short cells each of which may function as a spore when placed under favorable conditions for development. The *Corticium* stage has not been observed upon the sugar beet, but the fungus appears to be identical with the form on the potato and a variety of other plants upon which the *Corticium* is common, and the name "*Corticium vagum* B. and C., var. *solani* Burt.," which is the one most generally accepted in America, is being retained for the purposes of this paper.

The beet diseases produced by this fungus in America are unknown in Europe, and this fact has been used as an argument that they can not be correctly attributed here to the fungus which produces the sclerotia on potatoes there. This argument is fully met, however, when we approach a study of the environmental factors upon which the fungus is dependent for the production of disease, since the climatic and soil factors under which it becomes an active parasite in some portions of America are not found in Europe.

INOCULATION EXPERIMENTS ON BEET SEEDLINGS

The 34 cultures used in the inoculation experiments on seedlings were obtained from the following sources:

Sugar-beet seedlings grown in the field at Rocky Ford, Colo., 5; at Madison, Wis., in Rocky Ford soil, 5; at Madison in Garden City, Kans., soil, 2; at Washington in greenhouse soil, 3; in sterilized soil infected with decayed beets, 2; in Madison greenhouse soil, 1; at Madison in

field soil, 1; crown-rot sugar beets from Rocky Ford, Colo., 5; from Garden City, Kans., 2; from Chino, Cal., 1; from Kenosha, Wis., 1; potato tuber from Carbondale, Colo., 1; radish from Madison, 1; carrot from Madison, 1; pine seedlings grown at Garden City (contributed by Mr. Carl Hartley, of the Bureau of Plant Industry), 2; and decaying tomato grown on Potomac Flats, Washington, D. C. (contributed by Dr. H. W. Wollenweber (44), of the Bureau of Plant Industry, as *R. potomacensis* Wollenw.), 1.

The various strains in cultures exhibited no striking differences. Those which did appear are due largely to difference in vigor. The virulence is reduced temporarily by long continuance in artificial culture. Difference in the virulence of the several strains, both when freshly isolated and when rejuvenated, was sometimes noted, and this difference appeared to be quite constant, although it bore no relation to the host which furnished the original culture. For example, one of the two strains most virulent to beet seedlings was secured from the beet root and the other was the form from tomatoes received as *R. potomacensis* Wollenw. Certain of the strains that were least virulent were obtained originally from sugar-beet seedlings.

The inoculations were carried out with extreme care, following the methods already described. Every precaution was taken to insure the accuracy of the results, which were uniformly positive. Each strain was recovered and reinoculated into seedlings through from four to six generations.

The type of disease produced upon beet seedlings is similar to that caused by *Phoma betae*, but the plants are attacked at a younger stage, and the progress of decay is likely to be more rapid, so that it was necessary to exercise considerable care in making inoculations at the time of seeding. In the cases of heavy inoculation few or no seedlings broke through the soil. With lighter inoculation a milder form of damping-off developed, or the disease took the form of root sickness, in which case a relatively large number of plants eventually recovered. The fungus is capable of attacking its host at any time after germination. Inoculations upon older seedlings also gave positive results. Young beets 4 or 5 weeks old were readily killed by inoculations upon the crown when no wound was made.

DISTRIBUTION OF THE FUNGUS

The distribution of the fungus is very general, but under field conditions damping-off due to *Rhizoctonia* is far more general in the soils of the semiarid West. Soils brought from western Kansas and Colorado to Wisconsin and placed in pots in the pathological garden yielded a large percentage of damping-off from *Rhizoctonia* sp., while Wisconsin soils in control pots were practically free from the ravages of this parasite. The fungus has been isolated a few times from the Wisconsin beet fields, but it appears to be of little consequence as a beet parasite under Wisconsin conditions. The reverse is true in Colorado and Kansas, where a majority

of the diseased seedlings examined have yielded cultures of *Rhizoctonia*. The fungus is the cause of a very destructive crown-rot in the West (Pl. XX, XXI, and XXII), where it frequently becomes epidemic. It is not uncommon to see entire fields of 50 or 100 acres practically destroyed in August by root-rot, of which there is no evidence earlier in the season (Pl. XXI, fig. 1). This form of rot is seen only occasionally in the more eastern beet-growing districts, where it appears to be of no economic importance.

CONDITIONS INFLUENCING INFECTION

The controlling influences in the distribution of the *Rhizoctonia* diseases of the beet may conceivably be associated with the unequal distribution of the fungus or with differences in climate or in soil, or with any combination of these. Some light has been shed upon this point in the course of the inoculation experiments on growing beets. Field inoculations were first made in Wisconsin, using cultures obtained from Colorado and Kansas. The first series was made on August 21, 1912, by placing portions of mycelium upon agar among the heart leaves of beets in the field. The inoculations were made just at dark, and the beet leaves were moistened with water from a sprinkler, in imitation of a heavy dew. The morning of the 22d was cloudy, and a very little rain fell. The weather of the next few days was dry and hot. Examination a few days later showed that infection had occurred in all except one of the 29 beets inoculated. The disease, however, failed to make the progress typical of western conditions. At the time of harvest, October 23, one beet showed no evidence of infection even at the point where inoculation was made and where the original dried culture was clearly seen. Six showed no injury other than slight lesions on petioles such as shown in Plate XXI, figure 2. Five showed old lesions on the crown, but they had entirely recovered. Eleven beets showed so slight evidence of decay that it was observed only on close examination. Five showed clearly defined decayed spots, but even these were restricted in area. One had entirely lost its original crown of leaves, but had formed scar tissue and had developed new leaves from the meristem at the sides. (Pl. XXII, fig. 1.) This was the only beet which had been injured for the commercial market, unless it might be that the sugar content of the others had been lowered.

A second set of inoculations were made on August 28. In this instance 40 beets were inoculated by placing on the crown of each a portion of *Rhizoctonia* mycelium growing on a sterilized beet block. The results were very much like those of the first series. While the inoculations took in every case, most of the beets outgrew the infection. Five beets were sufficiently injured to be unfit for market, but only one was killed. In these cases of serious infection the progress of the disease corresponds closely to that seen in the West, although it was far

less rapid. The fungus was readily recovered in culture from them. A photograph of the most seriously injured beet taken at the time of harvest on October 23 is reproduced in Plate XXII, figure 2. At that time the scars where the original infection had been produced could be found on all the beets. Some showed small areas of decay, but most of them were practically sound.

A third series of inoculations were made on September 11 through knife wounds near the surface of the ground. These were made in imitation of cultivator injury and were infected by placing a rapidly growing culture of *Rhizoctonia* sp. on a beet block directly in contact with the injured surface. Thirty beets were inoculated; none of them was destroyed. About half of the number healed completely, so as to leave only a local scar at the point of inoculation. The others showed local decay, more or less characteristic of crown-rot. The fungus was readily recovered from several of these. The largest decayed area produced was about 4 inches in diameter. At least half of this beet was still sound.

At the time of harvest the beets which showed no decay were topped to remove the leaves, the crown being left uninjured. They were placed on racks in the vegetable cellar and examined for decay from time to time. On April 3 all but two of them showed evidence of rot, although in most cases a close examination was necessary to discover it. Eighteen were selected and submitted to cultural tests for *Rhizoctonia*. Out of 68 attempts to isolate the organism 54 yielded *Phoma betae*, 2 failed to develop, and the remaining 12 gave growths of various saprophytes. In no instance was it possible to secure a culture of *Rhizoctonia*. It was apparent that those beets which failed to develop decay in the fields had entirely thrown off the infection from *Rhizoctonia*.

In order to determine to what extent this resistance to attack is to be attributed to local conditions of climate or soil, two large lots of soil from seriously infected beet fields, one in Kansas and one in Colorado, were shipped to Madison, Wis. Both types of soil were quite heavily infected with *Rhizoctonia* sp. That from Kansas was a sandy loam deficient in organic matter. It had received generous applications of factory waste lime and was of good mechanical texture. The Colorado soil was of compact structure containing an admixture of clay and fine silt. It was very deficient in organic matter, so that it was quite impervious and lumped badly. These soils were placed in unperforated, unglazed, 12-inch crocks containing cinders at the bottom for drainage, and sunk into the ground out of doors to within 2 inches of the top. Soil from Madison was employed in similar crocks as a control. Six crocks of each soil were sterilized in an autoclave by heating for 12 consecutive hours under 15 pounds' pressure, and six were left untreated. Untreated beet seed which showed remarkably strong germination and less than 1 per cent of infection with *Phoma betae* was sown. Damping-off developed only in the unsterilized soil from Kansas and Colorado. Attempts to

isolate the causal organisms in 27 cases gave the following results: *Rhizoctonia*, 15; *Phoma*, 8; *Fusarium*, 2; *Mucor*, 1; undetermined, 1. Both of the parasitic forms were isolated from each of the two types of soil developing disease. A good stand, which was thinned to three, four, or, in a few cases, five plants per pot, was secured in each crock, however, in spite of damping-off. Early in July evidences of crown-rot developed in four of the six pots of unsterilized Colorado soil, but not in that from Kansas. In two of these pots the stand was entirely destroyed by July 21, and in a third there remained only one small seedling with four leaves, which appeared after the original stand had been killed. No disease appeared in the pots of sterilized soil, nor did root-rot develop in the unsterilized Kansas soil. On July 23 inoculations were made with a recently isolated *Rhizoctonia* strain in two pots of each of the six classes of soils. Two pots of each class were reserved as controls, and two were inoculated with *Phoma betae*, as previously reported. The inoculations were made on one beet only in each pot, by placing a piece of beet-block culture on the crown and a second fragment against a wound just below the surface of the soil. The other beets in the pots were not disturbed in any way. One beet in each control pot was wounded in a manner similar to that employed in the inoculations.

The results in inoculated and in uninoculated pots are given in Table V. It is worthy of note that as a result of inoculating 1 beet in each of 12 pots, 26 beets were killed and 7 more were so seriously diseased as to be made worthless, while only 3 resisted infection. One beet attacked in July by spontaneous rot recovered later. The fungus was recovered in culture from several of the diseased roots.

TABLE V.—Results of pot experiments with *Rhizoctonia rot*

Source of soil.	Pot No.	Condition on October 18.			
		Number of beets in pot on July 21.	Number dead.	Number infected but living.	Number sound.
Colorado.....	19	3	3		
Do.....	22	^a 1		1	
Colorado (sterilized).....	1	4	3		1
Do.....	4	4	2	2	
Kansas.....	25	3	2	1	
Do.....	28	4	2	1	1
Kansas (sterilized).....	31	2	2		
Do.....	34	4	4		
Wisconsin.....	13	2	1	1	
Do.....	16	3	1	1	1
Wisconsin (sterilized).....	7	3	3		
Do.....	10	3	3		
Total.....		36	26	7	3

^a The original stand of three beets had already been destroyed by spontaneous *Rhizoctonia rot*. The one plant living resulted from seed delayed in germination.

TABLE V.—Results of pot experiments with *Rhizoctonia rot*—Continued

UNINOCULATED POTS

Source of soil.	Pot No.	Number of beets in pot on July 21.	Condition on October 18.		
			Number dead.	Number infected but living.	Number sound.
Colorado.....	20				
Do.....	23	^a 5	I		^b 5
Colorado (sterilized).....	2	4			4
Do.....	5	4			5
Kansas.....	26	3			3
Do.....	29	3			3
Kansas (sterilized).....	32	2			2
Do.....	35	3			3
Wisconsin.....	14	4			4
Do.....	17	3			^b 4
Wisconsin (sterilized).....	8	3			3
Do.....	11	3			3
Total.....		37	I		39

^a Two of these beets were diseased with *Rhizoctonia rot*. One of them eventually recovered, and the other died.

^b The additional beet is known to have resulted from seed delayed in germination.

Other inoculations with *Rhizoctonia* were made on beets growing in the field at Madison, Wis., Garden City, Kans., and Rocky Ford, Colo.

Of 30 plants inoculated at Madison on July 23, 1 escaped infection, 2 were infected but recovered, and 27 were killed. The fungus was recovered in culture from several of them. The control plants, of which there were several hundred, remained healthy.

The inoculations at Garden City were made on July 27 on beets furnished by Dr. C. F. Clark, of the Bureau of Plant Industry, who kindly made the field observations. The field was known to be somewhat infected with *Rhizoctonia*. One row was inoculated, those adjacent on either side being reserved as controls. The same procedure was observed in the inoculations with *Phoma betae*, previously reported, but since these did not produce disease, five rows, or 150 beets, became available for controls. Of the 30 inoculated plants, 23 were killed, 2 others were so seriously injured at the crown as to become entirely defoliated and apparently dead but developed a few new leaves late in the fall (October), and 5 escaped infection. The rate of progress of the disease is shown in Table VI. Three control beets became infected during the season.

TABLE VI.—Results of inoculation experiments with *Rhizoctonia* sp. at Garden City, Kans.

Row No.	Treatment.	Number of dead sugar-beet plants on—						Total number of dead plants.	Number of healthy plants on Sept. 23.
		Aug. 7.	Aug. 13.	Aug. 23.	Sept. 3.	Sept. 17.	Sept. 23.		
1	Control.....	0	0	1	0	0	0	1	29
2	Rhizoctonia....	2	1	11	6	1	4	^a 25	5
3	Control.....	0	0	0	0	0	1	1	29
4	Do.....	1	0	0	0	0	0	1	29
5	<i>Phoma betae</i>	0	0	0	0	0	0	0	30
6	Control.....	0	0	0	0	0	0	0	30

^a Two of these made feeble effort at recovery in October, showing that a little parenchyma had survived.

The 30 beets inoculated at Rocky Ford were killed, while all the controls remained healthy (Pl. XX, fig. 1).

A consideration of the facts related indicates that soil properties are potent factors influencing the susceptibility of beets to attack by *Rhizoctonia*. It has long been maintained that clay soils and those which are seriously deficient in organic matter and of fine, compact texture, so as to bake readily, are most likely to develop *Rhizoctonia* diseases. Further indication of this is found in the development of spontaneous root-rot in the Colorado soils used in the pot experiments at Wisconsin. It did not develop in the other soils employed, although the amount of *Rhizoctonia* damping-off indicated that the Kansas soil was at least as heavily infected with *Rhizoctonia* as was the Colorado material. The experimental data lead to the conclusion, however, that, in the case of the *Rhizoctonia* root-rot of the beet, soil temperature is a more important factor than soil texture. The inoculations in the field at Madison in 1912 were made at the beginning of a very hot period which endured for several days. Infection was produced uniformly, but in practically every case the beets completely recovered during the cooler weather which set in a few days after the inoculations were made. In 1913 infection was attempted at an earlier date when it might be expected that a somewhat longer period of hot weather would ensue. This proved to be the case and, as already pointed out, the inoculations were very generally successful. It is also significant that the cases of partial or complete recovery which occur appear late in the season when the soil temperatures are considerably lowered.

PYTHIUM DEBARYANUM

Hesse (24) reported *Pythium debaryanum* Hesse as a damping-off parasite of beets in 1874, but his experimental work appears to have been done on other hosts, so that while no one has doubted the accuracy of Hesse's deductions, Peters (33, p. 221) appears to have been the first to demonstrate by culture methods that this fungus includes the sugar

beet among its hosts. The fungus is so well known that a description here is superfluous. It is very readily secured in pure culture and is easily carried upon media (Pl. XVI, fig. 1). It grows especially well with long-continued vitality upon string-bean agar. The sexual fruiting bodies are quite common in Petri-dish cultures upon this medium, but are rarely met with in tube cultures. The asexual conidia, as well as oospores, are formed abundantly when the fungus is grown in water upon sugar-beet seedlings in Petri dishes. The cultures obtained throughout the experiments were invariably identified by fruiting bodies, and the same method was applied in proving up the cultures recovered from artificial inoculation. Suspected seedlings were treated in bichlorid of mercury, rinsed in water, and plated upon the acid synthetic agar previously mentioned (p. 137). When growth developed, the mycelium was examined through the bottom of the Petri dish by inverting the plate upon the stage of the microscope. If no septa were visible, the seedling was transferred to a sterile Petri dish after a subculture had been made from the growth. Sterile water was added to the fresh plate containing the seedling. In case the growth was *Pythium debaryanum*, the characteristic conidia developed in great numbers in from 24 to 48 hours, to be followed during the next few days by oospores. Direct germination of conidia was often seen and could be very readily induced by adding a fresh beet seedling to the culture. Germination by zoospores was not observed, but no special effort was made to induce this type of development.

The cultures used in the inoculation experiments were all morphologically identical, so far as could be determined. They were secured from the following sources:

Damped-off beets grown at Washington in the greenhouse, 2; at Madison, Wis., in the greenhouse, 6; at Madison in the greenhouse in Utah soil, 3; at Madison in the greenhouse in Michigan soil, 2; damped-off seedlings grown in Utah in the field, 1; grown in Wisconsin in the field, 1; grown in Colorado in the field, 1; damped-off pine seedlings from Kansas (contributed by Mr. Carl Hartley, of the Bureau of Plant Industry), 1; decaying potatoes, isolated in 1909, 1.

Mr. Hartley reported this strain pathogenic to pine seedlings, having produced damping-off with it to the extent of 100 per cent in the seed bed.

Pythium debaryanum proved to be exceedingly destructive in the pot experiments. When infection was made at the time of seeding, even a temporary stand was seldom secured. Examination showed that the seed germinated, but that the plants were destroyed before they could come up. In many cases the embryo was killed while still within the seed. By delaying the inoculation until the seedlings were well started typical damping off was produced and the fungus recovered. It was a very common thing to find infection on the tips of the cotyledons.

This probably occurred while the leaves were still within the seed coat. The fungus was found to be capable of attacking the beet after it was 5 or 6 weeks old. Peters's statement (33, p. 228) that it is able to infect the side roots during the entire vegetative period is probably correct. When the taproot is once attacked by *P. debaryanum*, the ultimate death of the plant seems to be assured. Fortunately the soil relations in early seeding time are usually not sufficiently favorable to the rapid development of the fungus to make it an aggressive parasite under average field conditions. This fungus does not develop well in cold soil, but does its most serious work under seed-bed and greenhouse conditions or in the fields which have been seeded very late when the soil temperatures have begun to rise.

UNDESCRIBED SPECIES INJURIOUS TO SUGAR BEETS

In the author's preliminary note (12) it was reported that *Aphanomyces laevis* De By. had been found as a damping-off fungus of sugar beets in America, but subsequent detailed morphological studies of the fungus as it developed in artificial culture and on beet seedlings have shown that it differs in some important respects from the published descriptions of De Bary and others. *A. laevis* was first reported in a parasitic relation by Peters (31) in 1906. He found the fungus as a damping-off parasite of considerable importance upon sugar beets in Germany. Barrett (4) has reported its occurrence in America as the cause of a disease of radishes. The first cultures of the fungus temporarily mistaken for *A. laevis* were secured from damped-off beet seedlings grown in soil which had previously produced the black-root disease of the radish, like those shown in Plate XXIV, figure 2. It was later obtained from soils at Madison, and from Kenosha, Wis., as well as from seedlings damping-off in soil which had been infected with fragments of a diseased radish obtained from Illinois. The causal relation of the organism to the radish disease as well as to damping-off of sugar-beet seedlings was confirmed repeatedly by inoculation experiments, and it was at first thought possible that the discrepancies between this fungus and published descriptions of *A. laevis* might be the result of response to the changed environmental conditions of culture or to variations within the species, since it is well known from the study of many investigators that the Saprolegniaceae are exceedingly variable. Through the courtesy of the Kaiserliche Biologische Anstalt at Dahlem, Germany, and Dr. Leo Peters, of that institution, the author was permitted to isolate *Aphanomyces* from the experimental fields of the Anstalt. An organism was secured from damped-off seedlings, which Dr. Peters identified as the organism with which he had worked and which conformed in every respect to De Bary's description of *A. laevis*. It was secured in pure culture, and its pathogenicity to beet seedlings was confirmed by inoculation experiments. Unfortunately the culture was lost

before it had been tested upon the radish and thereafter could not be secured again. The morphological studies, however, prove that the American fungus with which we have been working is not *A. laevis*, but a hitherto undescribed organism. Morphological, physiological, and cytological studies will be presented in another paper.

In the work with sugar-beet seedlings five strains of the organism were employed which were obtained from the following sources:

Seedling sugar beets grown at Madison, Wis., 2; at Kenosha, Wis., 1; in soil originally infected by radishes showing black-root, 2.

The disease which it produces on the sugar beet is very similar to that caused by *Pythium debaryanum*. The fungus is even more aggressive as a parasite than *Pythium* (Pl. XXIV, fig. 1). When the inoculations were made at the time of seeding, it was unusual for plants to appear above-ground. The evidence obtained all goes to show that a seedling once attacked never recovers.

A disease of the side roots of growing beets was encountered during the course of the studies in soils which had been inoculated with artificial cultures of the fungus several months earlier. A photograph of a specimen is reproduced in Plate XXIII, figure 2. The fungus was readily isolated from diseased side roots of this beet and there appears to be no reason to doubt its causal relation to the trouble. Peters (33, p. 244) has quoted a similar disease of European beets caused by *Aphanomyces*.

Comparatively little is known regarding the range of distribution of the fungus. What appears to be the black-root of radish has been observed in the District of Columbia, Maryland, Virginia, Long Island, Illinois, and at several points in Wisconsin, and there is reason to believe that other workers have found it in various places, although no records of such observations have been published. The disease produced by this fungus is so similar to that reported by Barrett (4) that they are not readily distinguishable, and it may be that either of these organisms is responsible for the disease in any of the stations mentioned. The author does not consider that his results should be construed to throw doubt upon the accuracy of Barrett's observations, merely wishing to record a radish disease that is indistinguishable in external appearance from that produced by *Aphanomyces laevis*, but which is due to this hitherto undescribed parasite.

The fungus is readily isolated from beet seedlings by the method already described for *Pythium debaryanum*. In this case, however, the subcultures should be made to string-bean agar, upon which the organism produces a luxuriant growth. Sterilized beet seedlings in water in test tubes make an excellent medium for the cultivation of this fungus. The limit of vitality in culture has not been determined, but transfers made to string-bean agar on July 2 from beet-seedling water cultures made on February 12 developed a heavy growth overnight, showing not the least loss of vitality.

The vegetative stage of the fungus is strikingly like that of *P. debaryanum*, so that they can not be distinguished readily except by the fruiting bodies, which develop readily in water cultures in plates in from 24 to 48 hours. The zoospores develop first, to be followed somewhat later by the Pythium-like oospores. The asexual fruiting bodies are first noted as the swollen ends of hyphæ, which vary greatly in length and are characteristically somewhat branched. They average from 150 to 900 μ in length or even more. When mature, these bodies discharge their contents in a spherical mass which cleaves in the course of 20 or 30 minutes, giving rise to numerous zoospores.

OTHER FUNGI FOUND ON SUGAR BEETS

In the course of the isolation work various other fungi were secured. Some of these were known saprophytes, while others, like *Macrosporium*, *Mucor*, and *Botrytis*, have sometimes been reported in parasitic relations, but gave negative results in our trials. *Fusarium* and *Verticillium* cultures were secured frequently, but inoculation experiments with these genera were deferred pending the completion of taxonomic work by other investigators.

There remains to be discussed a peculiar type of decay of growing beets and a root sickness of seedlings associated with *Rhizopus nigricans* Ehr. Cultures of this fungus were frequently isolated in the course of experimental work from seedlings. Specimens of mature beets affected by a peculiar light-brown decay were received in the laboratory from California during the campaign of 1910. The interior portion of these beets yielded a very large proportion of cultures of *Rhizopus*. The decay was very characteristic and unlike anything before seen. In the early stages the material was almost normal in appearance, except for the discoloration. It later assumed a somewhat flabby texture and developed pockets in the interior which were filled with a nearly colorless fluid rich in acetic acid, as was determined by the odor and by chemical tests.

In 1912 a somewhat similar trouble was reported from Colorado, and a visit was made to the field (Pl. XXV, fig. 1). The beets at that time were dead over considerable areas. Those most recently attacked showed the same light-brown color previously referred to (Pl. XXV, fig. 2), while those in the more advanced stages of decay presented various symptoms between the early condition and almost complete dissolution of all except the vascular tissue. Some, however, were apparently pickled in acetic acid, formed probably from the fermentation of the carbohydrate content. A very large number of attempts to isolate an organism of known pathogenic properties was made. The trials yielded *Rhizopus nigricans* in pure cultures to the extent of almost 100 per cent. Inoculation work in the laboratory upon dormant beets in moist chambers resulted in the

reproduction of decay similar in appearance to that occurring in the two cases described. The acetic-acid development, however, did not occur except in a slight degree in a few instances, and there is no certainty that in these cases it did not result from contamination. Inoculation experiments made in the field upon living material invariably yielded negative results. Inoculation experiments upon seedlings made in the usual manner also failed to produce damping-off when reasonably good conditions of culture were maintained. It was possible, however, to produce a disease which showed the symptoms of root sickness when the soil was excessively wet and the temperature rather adverse. Naturally the fungus was easily isolated from such material. The control plants, however, were sickly or diseased on the roots, and it is highly probable that the results obtained in the inoculation experiments with seedlings are to be attributed to physiological injury, which opened the way for the *Rhizopus* to grow saprophytically upon the tissue.

Inquiry into the history of the fields that produced the peculiar rot with which this fungus was associated revealed the fact that at least one of them had been flooded for a time and that the other had been excessively moist for several consecutive days prior to the appearance of the disease. In view of these facts and the results of experimental work, it seems reasonable to conclude that the beets were originally killed or at least materially weakened by adverse physiological conditions and that *Rhizopus nigricans* followed as a saprophyte or weakling parasite producing a characteristic type of decay.

ALKALI INJURY TO SUGAR BEETS

During a field trip in Colorado late in August, 1912, the writer was called upon to visit a beet field in which a peculiar rot was developing. The beets had made a good growth, and most of them were above the average in size. The foliage was luxuriant, but was characterized by a bluish green color and a brittle texture. Little evidence of disease, aside from the abnormal appearance of the foliage, was evident until the plants were pulled, when it was seen that many beets were decayed at the lower portion of the root. Some agency had killed the taproot, following which a soft rot was destroying the tissue. A majority of the plants in the portions of the field most seriously affected showed a characteristic cracking and corroding of the root, like that shown in Plate XXVI.

Evidences of alkali could be seen on the surface of the soil here and there, and it seemed probable that the deeper branches of the taproot had been killed by alkaline waters. This probability was increased by the fact of the close proximity to the field of an irrigation reservoir the waters of which were evidently quite alkaline, as could be seen from the crust of salts on the ground at the edge of the lake. As a further test upon this opinion, several beets were taken to the laboratory and attempts were

made to isolate pathogenic organisms, but with negative results. Several of the plants upon which decay already had made considerable progress were placed in fresh soil in the greenhouse and held under observation to determine whether the disease would continue to develop. Without exception these plants healed and thereafter showed no evidences of disease. The foliage which they put forth was normal, giving no evidence of the brittleness or blue-green color noted in the field. The results seemed to justify the conclusion that the plants were suffering from excessive alkali brought in by seepage from the neighboring reservoir.

SUMMARY

The more important points brought out in this paper may be summarized as follows: Four fungi have been found to stand in a causal relation to damping-off of sugar beets in America. These are *Phoma betae* (Oud.) Fr.; *Rhizoctonia* sp. probably identical with *Corticium vagum* B. and C., var. *solani* Burt.; *Pythium debaryanum* Hesse; and an undescribed member of the Saprolegneaceae.

Under favorable conditions of culture, plants attacked by *Phoma betae* or *Rhizoctonia* may recover either temporarily or permanently. Attacks of the other two fungi upon the seedlings may be expected to prove fatal. *Phoma* and *Rhizoctonia* are capable of producing characteristic decay in mature beets. The former appears to infect the plants primarily in the seedling stage, and when recovery occurs it remains thereafter in a dormant condition upon the host. It occasionally develops a characteristic black rot on growing beets in the field and more frequently appears upon mother beets in storage. When it does not destroy the root, it may infect the seed stalk and appear upon the mature seed. Control measures are to be sought in proper cultural methods and seed treatment which looks forward to the production of seed free from infection. *Pythium debaryanum* is capable of attacking the feeding roots of the beet throughout its vegetative period, and the new fungus is also able to cause trouble on mature beets in a similar manner. *Rhizopus nigricans* Ehr., while unable to produce disease on normal plants in the field, is capable of attacking the tissue of dead or dormant sugar beets, producing a characteristic decay.

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PLATE XVI

Isolation cultures from sugar-beet seedlings.

Fig. 1.—*Rhizoctonia* sp.Fig. 2.—*Pythium debaryanum*.

PLATE XVII

Phoma betae.

Fig. 1.—Isolation culture from sugar-beet seedling.

Fig. 2.—Fruiting culture on string-bean agar.

PLATE XVIII

Fig. 1.—Half-grown sugar beets showing crown-rot caused by *Phoma betae*.

Fig. 2.—Sugar beet showing seedling injury caused by *Phoma betae*.

PLATE XIX

Mother beet showing storage decay caused by *Phoma betae*.

PLATE XX

Rhizoctonia sp.: Root-rot of sugar beet.

Fig. 1.—Result of artificial inoculation, control beet in center.

Fig. 2.—Result of natural field infection. Note sclerotia on specimens at right.

PLATE XXI

Fig. 1.—Sugar beet showing field-rot caused by *Rhizoctonia* sp. Natural infection.

Fig. 2.—Sugar beet showing artificial infection with *Rhizoctonia* sp. on the petiole.
The disease has been arrested.

PLATE XXII

Results of artificial inoculation with *Rhizoctonia* sp.

Fig. 1.—Sugar beet, photographed from above, showing original crown destroyed, and new leaves developing from the sides.

Fig. 2.—Section of sugar beet showing character of *Rhizoctonia* injury.

PLATE XXIII

Fig. 1.—Sugar beet showing large sclerotium ($\frac{1}{2}$ inch) resulting from artificial inoculation with *Rhizoctonia*. The beet has resisted infection.

Fig. 2.—Half-grown sugar beet showing injury to feeding roots due to an undescribed parasite.

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PLATE XXIV

Fig. 1.—Sugar beet showing damping-off due to an undescribed parasite; control pot at right.

Fig. 2.—Radish showing black-root caused by the same fungus.

PLATE XXV

Fig. 1.—Field in which Rhizopus rot developed.

Fig. 2.—Typical beets from the field shown in figure 1.

PLATE XXVI

Sugar beet showing alkali injury.

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