The effect of various soil amendments and preceding crops on seedling disease of sugar beets caused by Aphanomyces cochlioides Drechs

by Roscoe C Bellingham

A THESIS Submitted to the Graduate Committee in partial fulfillment of the requirements for the degree of Master of Science in Botany at Montana State College
Montana State University

Abstract:
A study was made of the effects of preceding crops and different soil amendments on the amount of sugar beet seedling disease caused by Aphanomyces cochlioides Drechs., which is suspected of being the main cause of sugar beet seedling disease in Montana. The antagonistic action of Trichoderma lignorum (Tode) Harz on A. cochlioides was also studied.

The pathogen under consideration was studied in non-disinfected soil, in which the competition of the soil microflora was preserved, and in soil disinfected with chloroplcrin, in which no such competition existed. Soil flats, in some of which preceding crops had been grown, and to others of which mineral fertilizers, manure, and Trichoderma lignorum had been added, were planted to sugar beets. Disease readings were taken at frequent intervals and also at harvest; the fresh weights of tops and roots were determined at harvest.

Preceding crops had very little beneficial effect in controlling seedling disease of beets in non-disinfacted and disinfected soils. Complete and balanced fertilizers of nitrogen, potassium, and phosphorus gave the best control of seedling disease. Trichoderma lignorum did not show any antagonistic action toward Aohanomyces cochlioides.

The use of balanced and complete fertilizers resulted in very good growth of tops and roots of sugar beet seedlings. Phosphate and nitrogen, and phosphate and manure, when applied to the soil together, also produced large tope and roots. Soils in which potatoes and alfalfa were grown as preceding crops produced beets with larger tops and roots than soils in which the other preceding crops were grown.

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AND PRECEDING CROPS ON SEEDLING
DISEASE OF SUGAR BEETS CAUSED
BY <u>APHANOMYCES</u> <u>COCHLIOIDES</u> DRECHS.

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INTRODUCTION

The incidence of seedling disease, or black root, of sugar beets (*Beta vulgaris* L.) is a factor of considerable economic importance in certain regions of Montana, resulting in reduced stands and beets of decreased vigor. Seedlings may be killed outright, or they may recover after a period of retarded growth. In either case, a loss to the grower results from decrease in the number of marketable beets and from beets of reduced size.

In the heavy soils typical of many of the beet growing areas of Montana, a number of organisms may be responsible for seedling disease, according to moisture, temperature, and other environmental conditions. However, Afanasiev (3) has concluded from isolations from diseased beet seedlings in the greenhouse and in the field that *Aphanomyces cochlioides* Drechs. is probably the most important pathogen concerned. Seedlings attacked by this organism generally show a browning of the hypocotyl first observable at the soil line. The brownish discoloration usually involves all of the hypocotyl and in severe cases may grade into a black color. The discoloration may extend down to the roots but the progress of the disease is more upward than downward, so that even the lower parts of the cotyledons may be somewhat discolored. All of the tissues of the hypocotyl except the vascular system apparently are affected. In very severe cases, nothing remains but a fine thread of tissue, the vascular system, and in this circumstance the seedling may topple over.

More often, however, the seedling remains erect. In both cases, the cotyledons remain green and turgid. Recovery usually occurs, except in very severe cases, but the disease results in retarded development of the seedling and subsequent reduced size of the mature beet.

The severity and amount of black root may be influenced by a number of factors. It has been shown by Coons et al. (15) and others (8, 20) that certain crops such as corn, soybeans, and potatoes influence the amount and severity of black root when they precede sugar beets. It was thought advisable to conduct further tests in this direction under controlled conditions. Both non-disinfected and disinfected soils were used to determine effects of previous crops and fertilizers in soil in which a complex of organisms existed and in soil to which *Aphanomyces cochlioides* had been added after disinfection. The literature (9, 13, 16, 19, 21, 23, 26, 30) presents evidence that the fungus *Trichoderma lignorum* (Tode) Harz and others may be antagonistic to some plant pathogens, and it was considered desirable to test the effect of *T. lignorum* on the pathogen under consideration.

REVIEW OF LITERATURE

The fact that seedling disease of sugar beets may be caused by several pathogens has been well established. Afanasiev (2, 3) isolated Phoma, Fusarium, Macrosporium, Rhizoctonia, Pythium, and Aphanomyces from diseased sugar beet seedlings. Of these, Fusarium, Macrosporium, and Rhizoctonia were found to be only mildly pathogenic, Pythium and Phoma moderately so, and Aphanomyces severely pathogenic. He indicated that some of the fungi are weak parasites and may act as secondary invaders. Stirrup (27) found Phoma betae Fr. to be the most important cause of "black leg" but stated that species of Pythium and Rhizoctonia were also concerned. Of 31 fungi tested in Washington, Campbell (12) found only Phoma betae pathogenic and all others saprophytic. Coons et al. (14, 15) determined that Pythium spp., Phoma betae, Rhizoctonia solani Kuhn and Aphanomyces cochlioides are the most important causes of black root. Aphanomyces spp. are considered by them as an important agent of damping off and of persistent rotting of the basal portion of the tap root. Drechsler (17, 18) found Aphanomyces cochlioides present with Corticium vagum B. and C. (Rhizoctonia solani), Phoma betae, and Pythium spp. but also made a number of isolations in which A. cochlioides was the only organism to which pathological significance could be attritubed. He found that while all four are favored by an abundance of moisture, A. cochlioides is especially so. Leach (22) attributed a species of Aphanomyces, probably A. cochlioides, to a high incidence of seedling disease in the San Joaquin delta region of California. He found little preemergence damping off and a high percentage of recovery under favorable conditions. Tilford and Young (28) stated that *A. cochlioides* is responsible for the major part of losses in Ohio from seedling disease, but that *Rhizoctonia* spp., although less common, may at times cause severe damage. *Phoma betae* and *Fusarium* were found to occur to a minor degree. Warren (29) also found *A. cochlioides* responsible for seedling disease to a greater degree than any other pathogen in Ohio, succeeded in importance by *Pythium debaryanum* Hesse and *Rhizoctonia solani* in that order. Buchholtz and Meredith (11) isolated *A. cochlioides* in 11 of 12 trials on one date and 23 of 24 on another from a random sampling of diseased tap roots. It would seem from these reports that while seedling diseases cannot be attributed definitely to any one organism, *A. cochlioides*, in many regions at least, is responsible for the major part of seedling disease.

Coons et al. (15) found that a legume crop residue favors the expression of seedling disease, while residues of corn, soybeans, and small grains to some extent inhibit it. Common pigweed (*Amaranthus retroflexus* L.) was found to depress beet stands as did some preceding crops. Hildebrand and Koch (20) found that the percentage of germination of sugar beets was greatest in steam treated soil, followed by corn, soybeans, alfalfa, check, and a 2-16-10 fertilizer. Incidence of black root was lowest after corn. Severity of the disease, after each of the following treatments, increased in the order in which they are named: soybeans, steam stérilization, alfalfa, 2-16-10, fertilizer, and the check. However, the vigor of the seedlings was greater after soybeans and also in steamed soil than after corn. In greenhouse experiments, Afanasiev et al. (8), using naturally infested soil, obtained the lowest amount of seedling disease after corn, followed in order of increasing disease by beets grown after potatoes, oats, alfalfa, beans, beets, and the check. Under field conditions, beets planted after potatoes had the smallest amount of disease. Those planted after beans, alfalfa, and beets had greater amounts of the disease in the order written.

Warren (29) reported that the amount of black root due to _A. cochlioides_ in Ohio tended to increase when soil temperatures rose from 18° to 24° C. In soils to which phosphate had been added, the amount of black root was much less at temperatures of 18°, 20°, and 24° C than in untreated soils at these temperatures. At 28° and 32° C, however, phosphate application did not control the amount of black root. Tilford and Young (28), by applying phosphate fertilizers at planting time, were able to reduce the amount of disease in fields which had received liberal applications of manure. In the greenhouse a row application of 20% phosphate or complete fertilizer reduced _Aphanomyces_ disease but had little or no effect on diseases caused by other organisms. Coons et al. (14, 15) found that whereas an untreated clay soil failed entirely due to black root, phosphate application proved very beneficial. They obtained promising results by incorporating phosphate with the seed. Young (31) reported that replacement of organic matter plus phosphate application, either alone or in conjunction with nitrogen and potash, decreased the amount of disease in worn out fields. The two latter were ineffectual by themselves. Leach (22) obtained almost complete control of A. cochlioides by applications of calcium hydroxide. Sergeant (25) reported 81% loss of beets because of black root in fields of low fertility. Morris and Afanasiev (24) observed small amounts of seedling disease in soils well fertilized with manure and phosphorus in the fall and supplemented by nitrogen in the spring as compared to large losses in soils poor in nutrients. Incomplete fertilizers in the form of nitrates, phosphorus, or manure alone did not control disease to any marked extent. Afanasiev and Morris (4, 5, 6) reported experiments verifying the above results with incomplete fertilizers. Although beets grown in soil to which manure alone had been added often suffered a high incidence of disease, their vigor was conducive to a high rate of recovery. Afanasiev (1) obtained better stands and lower amounts of disease with complete fertilizers than when a single fertilizer was used alone. He (7) obtained no beneficial effect from seed treatment with sodium nitrate and treble superphosphate.

It is a well established fact that certain fungi exert an antagonistic effect on other microorganisms. Weindling (30) reported that cultures of Trichoderma lignorum and T. koningi Oud. attack Rhizoctonia solani, apparently by the excretion of a toxic principle which is very unstable. R. bataticola (Taub) Butler and Armillaria mellea (Vahl.) Quel. were also attacked by Trichoderma. According to Daines (16) Actinomyces scabies (Thaxt.) Gussow is also attacked by the toxic principle of Trichoderma lignorum. Johnston and Greaney (21) found that T. lignorum and Pyronema confluens (Per.) Tul. noticeably lowered the pathogenicity of *Fusarium culmorum* (W. G. Sm.) Sacc. on wheat, especially at 10° C. Slagg and Fellows (26) found that *Trichoderma lignorum*, *Aspergillus niger* Van Tiegh, and *Gliocladium fimbriatum* G. and A. were antagonistic to *Ophiobolus graminis* Sacc., responsible for the take-all disease of wheat. They reported that *Trichoderma* parasitizes *Ophiobolus*; the action of the other two was unknown. *Rhizopus nigricans* Ehr. was observed to produce inhibitory substances at first, later stimulative ones. Allen and Haenseler (9) demonstrated that *Trichoderma* was antagonistic to *Pythium* and *Rhizoctonia* on cucumbers, and only to *Rhizoctonia* on peas. After a lapse of one month the same plots were replanted. The toxic effect had persisted and the antagonism had extended to *Pythium* on peas. Ledingham (23) studied the effects on emergence of wheat when *Helminthosporium sativum* P. K. and B. and *Fusarium culmorum* were inoculated together and when inoculated singly. Emergence was better when the two were inoculated together; this he attributed to antagonism between the two organisms. Growth on potato-dextrose agar showed that *H. sativum* was quite sensitive to other colonies, either of itself or of *F. culmorum*. Christensen (13) compared the germination of barley seed treated with Ceresan and *Trichoderma lignorum*. *Trichoderma* treated seed showed no improvement in germination and produced plants of no increased vigor while that treated with Ceresan showed improvement in both germination and vigor. However, the percentage of seedlings which were stunted or deformed was decreased by treatment with *T. lignorum* in plots infested by *Helminthosporium* sp. and *Fusarium* sp. Drechsler (19) observed considerable antagonism among species of *Pythium*. *Aphanomyces* weakly attacked several species of *Pythium*.

MATERIALS AND METHODS

For the purpose of this study naturally infested soil was obtained from the Huntley Experiment Station, Huntley, Montana. This is a heavy clay soil, characteristic of much of the irrigated land planted to sugar beets in this area. The particular plot, rotation 46, from which the soil was obtained had been planted to sugar beets in 1948. This rotation was begun in 1916 and consisted of two years of alfalfa, followed by one year each of sugar beets and oats in quarter acre plots. In the five years previous to 1941 the yield of beets in this rotation averaged less than 3 tons per acre. Replanting was often necessary because of large losses of seedlings from root rot. In 1941 the quarter acre plot bearing the second year alfalfa crop was subdivided into four parts. In one part the alfalfa stubble was shallow plowed after the second crop of hay was removed, and in another part the stubble was shallow plowed after removal of the third crop. Each west half of these sub-plots received manure and phosphorus in the fall. Each east half was left as a check. Both parts were deep plowed two to three weeks after being shallow plowed. Beets were planted the following spring. This procedure had been followed up to 1948 so that this soil had been planted to oats in 1941, alfalfa in 1942, alfalfa under the conditions described above in 1943, beets in 1944, and to beets again in 1948. Soil for this work was obtained from the unfertilized parts of the alfalfa plot. The beets grown after both subdivisions of alfalfa had a very high rate of seedling disease in 1948 and the stand was much reduced. The average seedling disease of beets planted on unfertilized soil from 1942 to 1945 was about 57% and the yield averaged approximately 5.1 tons per acre.

It was planned to test the effect of various preceding crops and soil amendments on seedling disease of sugar beets planted in this soil. In addition, it was thought desirable to determine if *Trichoderma lignorum* exerted any antagonism toward *Aphanomyces cochlioides*.

Seventeen treatments were used as follows:

1. Ch NI (Check non-inoculated)
2. Ch I (Check inoculated)
3. N (sodium nitrate)
4. P (treble superphosphate)
5. K (potassium chloride)
6. M (manure)
7. NP
8. PM
9. NPK
10. NPKM
11. Alfalfa (Grimm)
12. Potatoes (Bliss Triumph)
13. Sugar beets (Great Western 268)
14. Beans (Great Northern Montana No. 1)
15. Oats (Mission)
16. Corn (Rainbow Flint)
17. *Trichoderma lignorum*

Greenhouse flats, 20"x12"x3", containing about 24 pounds of the above mentioned soil were used for this work. Preceding crops were planted two months prior to planting of sugar beets and were harvested before planting the beets. Mineral fertilizers and manure were added in the following amounts per flat just before the beets were planted:

(1) Sodium nitrate - 15.7 g.

(2) Treble superphosphate - 1.8 g.

(3) Potassium chloride - 4.7 g.

(4) Manure - 1/5 soil volume.

Two flats of soil were used for each of the seventeen treatments. One flat of each treatment was disinfected with 10 cc. of chloropicrin; the other remained non-disinfected. Manure in all disinfected flats was added before disinfection with the chloropicrin. All disinfected flats were placed in a large metal box for 72 hours after they received the chloropicrin and then were placed on the greenhouse bench for aeration. In the non-disinfected soil flats, the effects of the different treatments under conditions of natural competition of the soil microflora were tested. In the disinfected flats, competition of the soil microorganisms was eliminated.

Two weeks later, all flats, except No. 1 in both sets, were inoculated with eleven-day-old cultures of Aphanomyces cochlioides grown in potato dextrose agar in Petri dishes. Six Petri dishes of inoculum per flat were used, three of culture No. 720, obtained from Dr. H. W. Bockstahler, University of Minnesota, University Farm, St. Paul, Minnesota, and three of culture No. 721, obtained from Dr. L. D. Leach, University of California, Davis, California. Flat No. 17 in both sets of soil was also inoculated with spores and mycelium from eleven-day-old cultures of *Trichoderma lignorum*, using six Petri dishes per flat. Three rows of segmented sugar beet seeds were then planted in each flat the same day at a rate of 40 seeds per row. This seed was treated two days previously with New Improved Ceresan on the basis of 8 oz. per 100 lbs. of seed. The beets emerged in about 4 days and reading began 6 days later. Beets were grown for approximately one month and readings of healthy and diseased seedlings were taken at frequent intervals during this period. The last reading of healthy and diseased beets was taken at harvest time. Weights of tops and roots were also taken and the average weight per beet calculated.

RESULTS

THE EFFECT OF PRECEDING CROPS ON AMOUNT OF DISEASE IN NON-DISINFECTED SOIL

Alfalfa, potatoes, sugar beets, beans, oats, and corn were grown in naturally infested soil in greenhouse flats. After a growing period of approximately two months, the crops were harvested in such a way as to approximate field conditions. Each flat was then inoculated with the mycelium of a mixture of 2 cultures of *Aphanomyces cochlioides*, and sugar beet seeds were planted. The beets were harvested 30 days after emergence and final readings as to healthy and diseased beets were taken.

The amounts of disease during the month after emergence in the soils in which the different crops were grown are compared in figure 1. Beets following alfalfa showed the smallest amount of disease, 66%; followed in order by corn, 79%; the inoculated and non-inoculated checks, 91%; potatoes, 95%; beets, beans, and oats, all 100%. Although there was less disease following alfalfa than corn, the beets were attacked more severely in the alfalfa flat. All other flats showed a severe manifestation of the disease. No attempt was made to determine exactly the pre-emergent killing off of seedlings. However, the number of seedlings which emerged in the check, alfalfa, and beet flats, was about 75% below that emerging in the other flats. The number of seedlings in the remaining flats was about equal.

The results shown here are somewhat at variance with those of some other investigators. Coons et al. (15) found that legumes favor the fungi causing black root, whereas alfalfa, in this experiment, shows the least percentage of disease. Afanasiev et al. (8) found under greenhouse conditions in non-inocculated soil that corn was conducive to the least amount of disease and the leguminous crops, beans and alfalfa, to a greater amount.

Under the conditions of this experiment alfalfa and corn are the only two crops tested which gave any decrease in the amount of seedling disease. Beets following potatoes, beans, beets and oats showed more disease than either the non-inoculated or inoculated check. In addition, the appearance of the beets was no better than the checks. Beets were graded as green, light green, or yellow. Those in the checks and following potatoes and corn showed a light green color; all others were yellow.

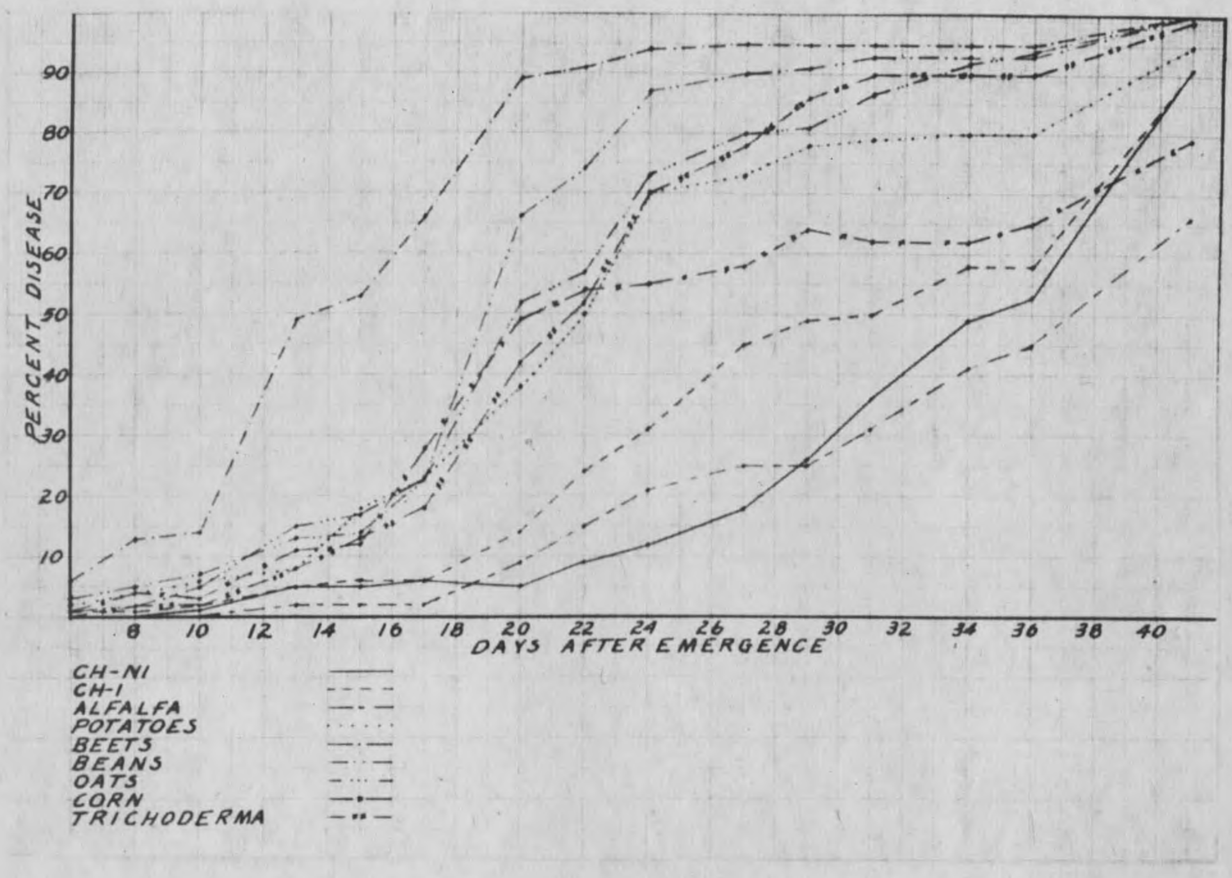

Figure 1. Percentage of Seedling Disease after Various Preceding Crops in Non-disinfected Soil.

THE EFFECT OF PRECEDING CROPS ON AMOUNT OF DISEASE IN DISINFECTED SOIL

The same crops were grown as in the previous experiment. However, previous to planting to beets, the soil was disinfected with chloropicrin and after the disappearance of the fumes from the soil, it was inoculated as before and beets planted. Results on the first test were negative, probably due to residual effect of the chloropicrin, so the experiment was repeated on the same flats.

The effects of the different crops on amount of seedling disease are presented in figure 2. As would be expected, the non-inoculated check showed no disease. The beets grown in the inoculated check showed the lowest amount of seedling disease (32%) while beets planted after beans showed the greatest amount (95%). In between, in order of increasing disease, were beets grown after alfalfa (39%), potatoes (55%), beets (59%, corn (85%), and oats (93%). All beets following these crops had severe symptoms of the disease; symptoms of the check were medium. All had a light green color except those following beets, which were yellow.

The beets of the inoculated check showed some effects from residual toxicity of the chloropicrin. It is possible that this was a factor in the low amount of disease in the check. However, Melhus et al. (10) observed in Iowa that tip rot of sugar beets attributed to <u>Aphanomyces cochlioides</u> did not appear in a field in 1939 whereas the same field in 1932 had been severely attacked. An adjacent plot planted to beets at intervals during the 7 years carried a great amount of it. It appears possible that after disinfection of the soil the population of the organism, even after inoculation, was not great enough to cause severe damage. The presence of the crop residues in the other flats quite conceivably might furnish sufficient organic matter for the organism to increase to a point where its attack on beets would cause considerable disease.

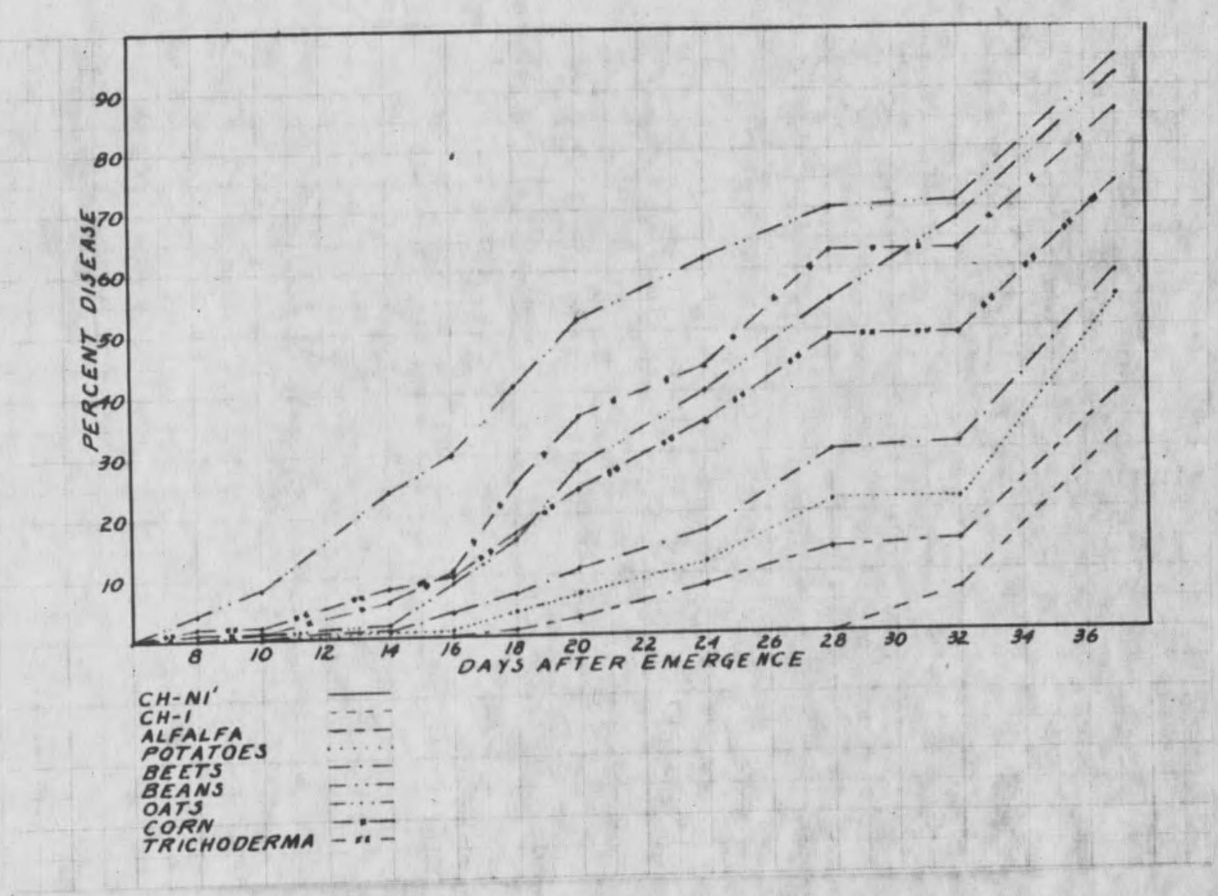

Figure 2. Percentage of Seedling Disease after Various Preceding Crops in Disinfected Soil.

1 There were no diseased seedlings in the non-inoculated check.

THE EFFECT OF SOIL AMENDMENTS ON AMOUNT OF DISEASE IN NON-DISINFECTED SOIL

An experiment was conducted to determine the effects of various soil amendments on the amount of seedling disease. In addition to inoculated and non-inoculated checks, flats were treated with sodium nitrate, treble superphosphate, potassium chloride, manure, and combinations of these. Each flat was inoculated with a mixture of two cultures of *Aphanomyces cochlioides*.

The results of this experiment are presented in figure 3. The order of disease was NPK (5%), NP (16%), NPKM (18%), N (30%), P (59%), K (70%), PM (79%), M (84%), non-inoculated check (91%), inoculated check (91%). Beets grown in manured flats showed considerably more disease than those grown in flats containing the same amendments without manure. Beets in the flat containing NPK had but 5% disease as compared with 18% for those in the NPKM flat. Beets in the PM flat had 79% diseased seedlings compared with 59% for those in soil treated with P alone. The flat treated with manure alone had the greatest amount of disease of any of the treated flats. Afanasiev and Morris (6) found in the field that manured rotations sometimes had a large amount of seedling disease but that the vigor of the beets resulted in a high percentage of recovery. In this test, however, the symptoms were very severe and it is doubtful if there would have been any recovery. The composition of manure is quite variable and its effect depends to a large extent on its nutritional and biological properties.

The beets in both checks displayed severe disease symptoms as did those grown in soil containing K and M. The N, P and PM treatments produced beets with symptoms of moderate severity, the NP and NPKM treatments produced beets with light symptoms, and the symptoms of beets in the NPK flat were very slight.

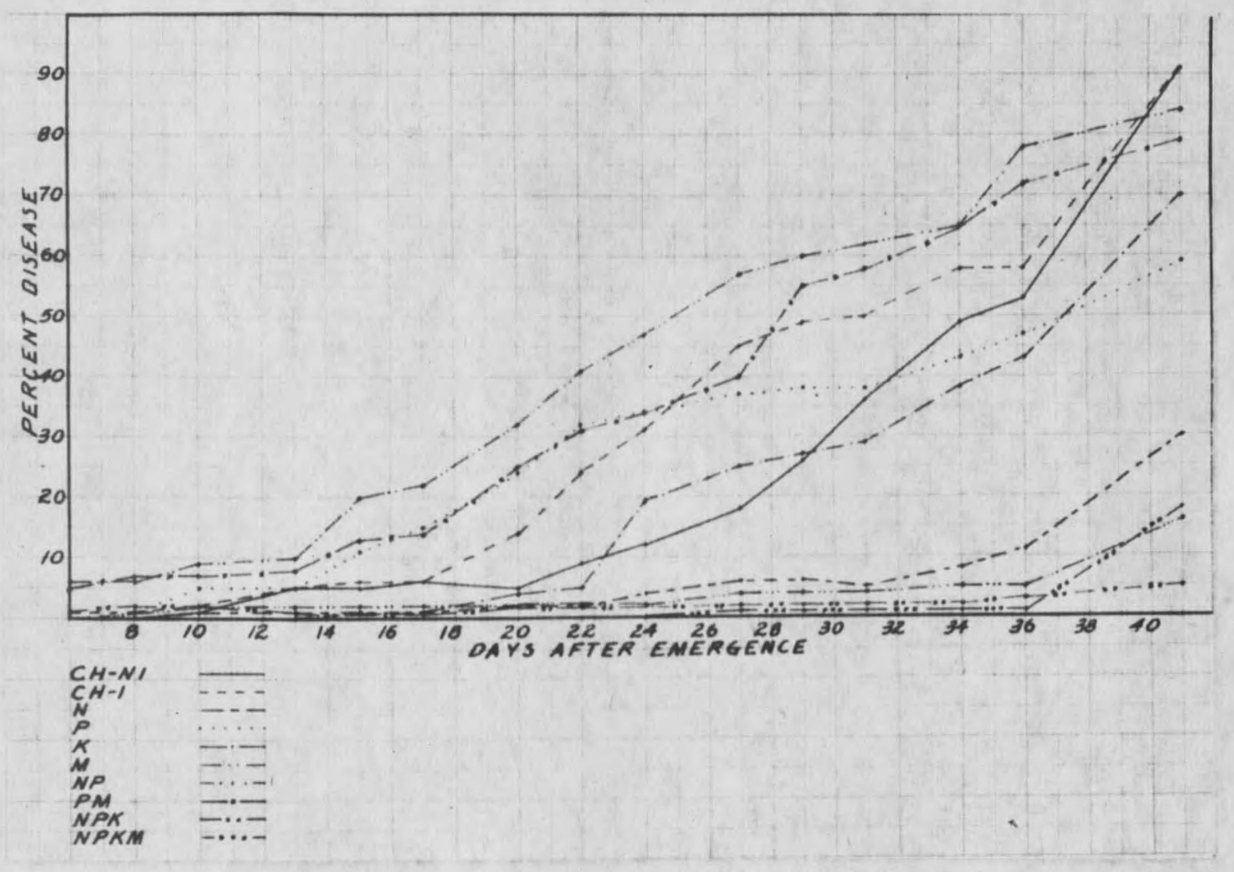

Figure 3. Percentage of Seedling Disease with Different Levels of Fertilization in Non-disinfected Soil.

THE EFFECT OF SOIL AMENDMENTS ON AMOUNT OF SEEDLING DISEASE IN DISINFECTED SOIL

The same treatments were applied to the soil, after disinfection with chloropicrin, as in the previous experiment.

The results are shown in figure 4. Beet seedlings in the non-inoculated check showed no disease at harvest, while those in the inoculated check showed 32%. The greatest amount of disease occurred to beets grown in soil fertilized with P alone (62%). The least amount of disease (8%) occurred to beets in soil fertilized with NPKM. In between were beets grown in soils with the following treatments: NPK (9%), M (11%), PM (15%), K (19%), NP (21%), N (23%), and the inoculated check (32%). These results indicate that a well balanced fertilizer is conducive to freedom from seedling disease, at least in soils where Aphanomyces cochlioides is predominantly the pathogen concerned. However, manure alone was effective in controlling the disease in this test.

Quite striking differences were noted in the expression of disease symptoms in the various treatments. In general, those treatments which resulted in a low incidence of disease also resulted in very mild symptoms. The NPKM, NPK, M, and PM treatments resulted in the lowest amount of disease of beet seedlings and the expression of a slight amount of disease. The K, NP, and N treatments resulted in symptoms of moderate severity as did the inoculated check. Beets grown in soil treated with phosphate showed severe symptoms.

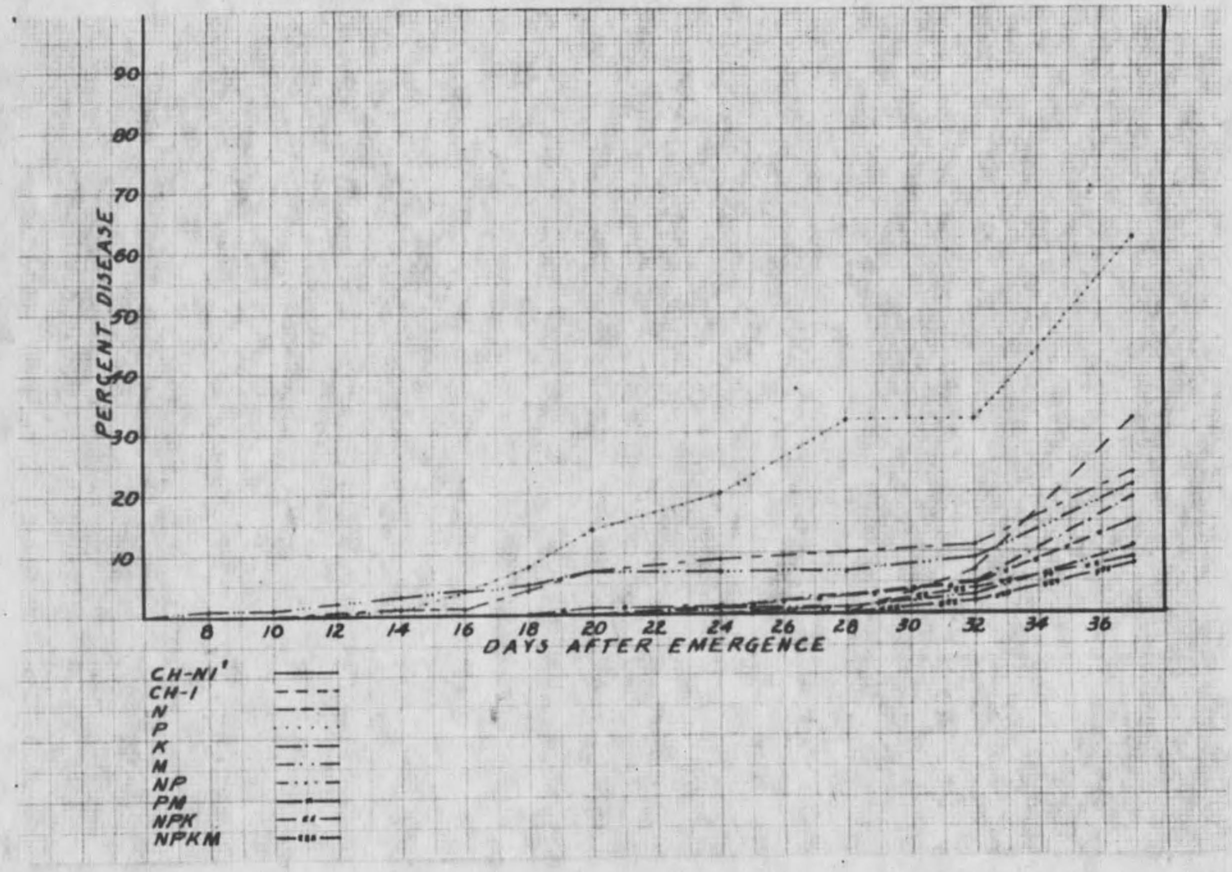

Figure 4. Percentage of Seedling Disease with Different Levels of Fertilization in Disinfected Soil.

1 There were no diseased seedlings in the non-inoculated check.

EFFECT OF *TRICHODERMA LIGNORUM* ON AMOUNT OF DISEASE IN DISINFECTED AND NON-DISINFECTED SOILS

Two flats, one of which was disinfected with chloropicrin and the other not, were inoculated with *Trichoderma lignorum* in addition to *Aphanomyces cochlioides*. Each flat received six Petri dishes of the inoculum which consisted of mycelium and spores. Various investigators (9, 16, 21, 26, 30) have shown that *T. lignorum* exerts an antagonistic effect on some pathogenic organisms.

In this test, *Trichoderma lignorum* exerted no beneficial effect in control of the disease. Results are shown in figures 1 and 2. The non-disinfected flat had 99% diseased seedlings as compared to 91% for both inoculated and non-inoculated checks. Disinfected soil inoculated with *T. lignorum* showed 74% diseased seedlings, the check 32%. Slagg and Fellows (26) found that *Rhizopus nigricans* produced substances inhibitory at first to *Ophiobolus graminis*; later they appeared to stimulate it. Although this test is too restricted to allow any conclusive results, it indicates that possibly rather than exerting an antagonistic effect on *Aphanomyces cochlioides*, *T. lignorum* may in some way aid the pathogen.

THE EFFECT OF SOIL TREATMENTS AND PRECEDING CROPS ON GROWTH OF SUGAR BEET SEEDLINGS IN NON-DISINFECTED SOIL

At harvest time, the average height of all beets, healthy and diseased, was determined for each flat. In addition, the fresh weight in grams of roots and leaves of both healthy and diseased beets was taken and then the average calculated for each flat. The results are tabulated in table I.

Height of Sugar Beets. There was practically no difference in height of seedlings grown in soil treated with NP, NPK, and NPKM. These showed the greatest increase in height of all treatments used. Beets grown in soil treated with NPK averaged 20 cm. in height; NPKM, 19 cm.; NP, 18cm. The beets in the checks were small, averaging but 6 cm. in height. The height of all other beets ranged between 4 cm. and 9 cm. The addition of nitrogen resulted in a slightly greater growth of beets than was shown by the checks. Similar results were obtained in soils where beets were grown after potatoes, beans and alfalfa. Growth was somewhat below normal in soils treated with M and K and *Trichoderma lignorum* and in soils in which beets, oats and corn were grown as preceding crops. The only treatments which showed significant beneficial effects on growth of beets were those of NP, NPK, and NPKM.

Weight of Tops. In all cases, the average weight of tops was greater in healthy than in diseased seedlings. The absorptive capacity of diseased roots was apparently decreased to such an extent that normal growth was not possible. In both healthy and diseased seedlings, the greatest weight of tops was obtained in soil with the complete fertilizer.

Any treatment which added nitrogen to the soil resulted in greater weight of tops in healthy seedlings except the PM treatment. The growing of potatoes as a preceding crop also resulted in greater weight of tops than the checks. There were no healthy seedlings in the bean, beet and oat soils. The weight of healthy seedlings grown after corn (.21 g.) was considerably less than that of the checks. Seedlings grown in soil treated with *Trichoderma lignorum*, PM, P, and K had lighter tops than the checks.

The weight of the tops of diseased seedlings generally followed the trend of the healthy seedlings. The tops of the beets of the non-inoculated check were about twice as heavy as those of the inoculated check, suggesting that the severity of disease was greater in the inoculated check. The NPKM treatment resulted in the greatest weight of tops. As in healthy seedlings, the corn as a preceding crop resulted in the lightest weight of tops. All treatments furnishing nitrogen, except the PM treatment, resulted in greater weights than that of the inoculated check. The PM treatment resulted in beets with an average weight approximately equal to that of the inoculated check. No benefit was derived from corn, beets or oats, nor *Trichoderma lignorum*, P and PM.

Weight of Roots. The greatest average weight of healthy roots was in beet seedlings grown after potatoes (.27 g.). Beets grown after corn had root weights which averaged but .05 g., as was also the case of beets grown in *Trichoderma* inoculated soil. Considering the inoculated check (.09 g.) as representative of a soil low in plant nutrients and with an amount of inoculum equal to that of the treatments, the corn, Trichoderma, N, and K treatments gave no benefits in regard to root size. P, NPKM, M, NP, NPK, alfalfa, PM, and potatoes gave increasingly greater root weights in that order.

The average weights of roots of diseased beet seedlings were more than those of the healthy in NP and NPKM treated soils. The degree of disease was light and the averages for both were calculated on small numbers of diseased beets which may account for the seeming discrepancy. Diseased seedlings grown in the Trichoderma and N treated flats equalled the weights of the healthy seedlings in these flats. Roots of diseased seedlings grown in the soils of other treatments were lighter than the roots of healthy seedlings. Beet roots grown in soils treated with NP were the heaviest (.35 g.) and those grown in NPK treated soil were the lightest (.02 g.). It would be expected that the average root weight of the beets in the NPK treated soil would be considerably greater, but this figure was obtained on the basis of one diseased beet.

Beets grown in soils with the following treatments had roots of decreasing weights in the order given: NPKM, potatoes, alfalfa, P, M, beans, K, N, oats, Trichoderma, inoculated check, non-inoculated check, beets, PM, and corn.

Table I. The Effect of Soil Treatments and Preceding Crops on Growth of Sugar Beet Seedlings in Non-disinfected Soil.

Treatment	Average Weight in Grams				Average Height in cm.	Color of Leaves
	Tops		Roots			
	Healthy	Diseased	Healthy	Diseased		
1. Check NI[1]	.77	.73	.12	.04	6	light green
2. Check I[2]	.61	.37	.09	.04	6	light green
3. N	1.09	.79	.07	.07	8	light green
4. P	.47	.36	.12	.09	6	light green
5. K	.57	.43	.09	.07	4	yellow
6. M	1.38	.54	.17	.08	4	light green
7. NP	2.99	1.41	.18	.35	18	green
8. PM	.44	.35	.25	.04	6	light green
9. NPK	3.18	1.0	.19	.02	20	green
10. NPKM	3.54	2.75	.15	.20	19	green
11. Alfalfa	1.23	.6	.19	.09	7	yellow
12. Potatoes	.91	.6	.27	.10	9	light green
13. Beets	---	.17	---	.04	4	yellow
14. Beans	---	.46	---	.07	7	yellow
15. Oats	---	.21	---	.06	4	yellow
16. Corn	.21	.10	.05	.03	4	light green
17. T[3]	.4	.28	.05	.05	5	light green

1 Non-inoculated check
2 Inoculated check
3 _Trichoderma lignorum_

THE EFFECT OF SOIL TREATMENTS AND PRECEDING CROPS ON GROWTH OF SUGAR BEET SEEDLINGS IN DISINFECTED SOIL

The results were obtained in the same manner as described for non-disinfected soil. Results are shown in table II.

Height of Sugar Beets. The greatest height of beet seedlings was obtained in the N, NPK, and NPKM flats in which they averaged 20 cm. The NP treatment was nearly as effective, the seedlings averaging 18 cm. Those in the non-inoculated check averaged 9 cm.; those in the inoculated check, 7 cm., probably due to a greater incidence of disease. P, K, and PM treatments resulted in beets of 11 cm. average height. The height of those in the manured soil averaged 10 cm. None of the preceding crop treatments produced seedlings of much greater height than the inoculated check. Soils in which potatoes were grown as a preceding crop produced beets of 11 cm. average height; alfalfa and beet soils, 10 cm.; oat and corn soils, 9 cm.; bean soil, 8 cm. *Trichoderma lignorum* treated soil produced beets 8 cm. in height.

Weight of Tops. Treatments involving direct application of nitrogen resulted in the greatest weight of tops in healthy beet seedlings. Tops of beets grown after beans, potatoes and alfalfa were slightly less than half as heavy as those grown in soils where nitrogen was applied. Tops of beets grown in the non-inoculated check were about twice as heavy as those grown in the inoculated check, which had the lightest tops of all seedlings. *Trichoderma* inoculated soil produced beet tops slightly heavier than those of the inoculated check. Manure and oats had some effect in increasing weight of tops but not so much as corn, P, beets, PM, and K, all of which produced tops about twice as heavy as the check.

Diseased seedlings followed the general pattern of healthy seedlings in regard to weight of tops. Direct nitrogen applications resulted in the greatest average weights, although NP was considerably lighter than the others. The beets in the inoculated check had the lightest tops.

Weight of Roots. Beets grown in the inoculated check had the lightest roots (.04 g.) of all healthy seedlings. Beet seedlings grown after beets and beans and in soils treated with NP and NPKM produced the heaviest roots (.19 g.). Roots of seedlings grown in the P and NPK treated soils were almost as heavy (.18 g.) as those above. These were followed in order of decreasing weight by roots of seedlings grown in the flats which received the following treatments: corn, potatoes, N, K, PM, alfalfa, M, non-inoculated check, oats and *Trichoderma*.

All diseased seedlings had roots lighter than those of the healthy with the same treatment. The average weight of beet roots grown in the non-inoculated check was lowest (.02 g.), while roots grown in the NPK treatment had the greatest average weight (.12 g.). The P treatment was slightly less effective, followed by beets, potatoes, alfalfa, N, PM, M, *Trichoderma*, NPKM, corn, NP, oats, beans, and K in order of decreasing weight of roots.

Table II. The Effect of Soil Treatments and Preceding Crops on Growth of Sugar Beet Seedlings in Disinfected Soil.

Treatment	Average Weight in Grams				Average Height in cm.	Color of Leaves
	Tops		Roots			
	Healthy	Diseased	Healthy	Diseased		
1. Check NI[1]	.81	--	.12	--	9	light green
2. Check I[2]	.44	.22	.04	.02	7	light green
3. N	2.9	1.54	.16	.09	20	green
4. P	.84	.75	.18	.1	11	light green
5. K	.96	.32	.16	.04	11	light green
6. M	.64	.27	.12	.07	10	light green
7. NP	2.8	.91	.19	.05	18	green
8. PM	.92	.56	.15	.08	11	light green
9. NPK	2.7	1.54	.18	.12	20	green
10. NPKM	2.7	1.44	.19	.06	20	green
11. Alfalfa	1.2	.75	.13	.09	10	light green
12. Potatoes	1.1	.86	.16	.09	11	light green
13. Beets	.85	.74	.19	.09	10	yellow
14. Beans	1.1	.36	.19	.04	8	light green
15. Oats	.71	.45	.11	.04	9	light green
16. Corn	.83	.50	.17	.06	9	light green
17. T[3]	.57	.42	.11	.07	8	yellow

1 Non-inoculated check
2 Inoculated check
3 *Trichoderma lignorum*

DISCUSSION

Summaries of the results are presented in figures 5 and 6. The results indicate that some decrease in the amount of disease of sugar beet seedlings might be expected where either alfalfa or corn is grown as a preceding crop. In non-disinfected soil to which a quantity of *Aphanomyces cochlioides* inoculum had been added, beets grown after alfalfa showed 66% seedling disease and beets after corn, 79%, compared to 91% for the checks. Although beets grown after corn had a higher disease rate than those after alfalfa, they were not attacked so severely. It is probable that the greater amount of nitrogen available to beets grown after alfalfa and their resulting increased tenderness made them subject to greater injury by the pathogen when once attacked. Oats, beans, potatoes and beets as preceding crops resulted in greater amounts of seedling disease than was present in the checks.

The soil microflora is of an extremely complex nature. Predominant development of parasitic or saprophytic groups of microorganisms in the soil is largely dependent upon the predisposing conditions which favor growth of one or another group of microorganisms. It is conceivable that one crop residue, because of its composition and properties, may support greater numbers of one group of organisms than another crop residue. If so, the above mentioned considerations may have some bearing on the findings of this study that corn and alfalfa as preceding crops seemed to give a certain amount of control of seedling disease. Under conditions of a large soil population of microorganisms, the intense competition might seriously reduce the pathogen concerned to a state where it is unable to cause any serious amount of disease, or at least less than would be possible under conditions of less intense competition.

Other factors operate in determining the qualitative and quantitative composition of the soil microflora, among them soil temperature and soil moisture. Variation in either of these factors probably results in variation in the soil microflora. Coons et al. (15) and Afanasiev and Morris (8) found that alfalfa as a preceding crop increased the amount of seedling disease of sugar beets--a finding which is in direct opposition to the findings of this study. The explanation of this contradiction may lie in the fact that differences in the types of soils, in their temperature and moisture, and in other factors, such as soil pH, materially alter the host-parasite relationship with resulting variation in experimental results. Also, if a balanced nutrition for the host is necessary for control of seedling disease, soils of different nutritional value would react differently to different soil amendments and crop residues as far as control of the disease is concerned.

In disinfected soil, no benefit was received by the beets from any of the six preceding crops tested. Beets grown in all flats of disinfected soil had greater amounts of the disease than did those grown in the check flats. A quite different situation prevails here than in non-disinfected soil. Presumably all, or nearly all, soil microorganisms were destroyed by the chloropicrin, eliminating the competitive factor of the soil population from the picture. It may be concluded, then, that the only variable factor in this test which would influence seedling disease was the type of crop grown, since an equal amount of inoculum was added to all flats. It is possible that some crop residues may enhance the saprophytic activity of the pathogen more than others. Under the conditions of this experiment, the alfalfa residue may not have been as favorable a medium for growth of the organism as were beans, which showed the greatest amount of disease. The check, which contained no organic matter except that which was already present in the soil, probably did not allow a sufficient amount of inoculum to be built up to cause a great amount of the disease, whereas it is possible that the pathogen was able to increase in the crop residues to an extent where the greater amount of inoculum was responsible for a greater amount of disease.

In both disinfected and non-disinfected soils, another factor which may have influenced the amount of disease was the nutrition of the host itself. The nutritional factor in each set of flats differed from that in others. The effect on the beet seedlings may have been that certain crops such as alfalfa and corn conferred a certain amount of resistance to the beet seedlings, possibly through products of their decomposition. All, or most, environmental factors which affect the beet seedlings also affect the soil microflora, so that it is very difficult to ascribe the effect of any crop residue to either its effect on the host alone or to its effect on the pathogen and the other soil microorganisms. It seems probable that a complex relationship exists between host and pathogen which may be materially altered by a change in any one of many environmental factors.

Quite definite results were obtained in non-disinfected flats treated with various soil amendments. The NPK treatment gave best control of seedling disease, while NP and NPKM were slightly less effective. Fairly good control of disease was accomplished with nitrogen, although not as effective as that achieved with NP and NPKM. While in all other flats with soil amendments, the beets had less disease than those in the controls, the application of these amendments alone cannot be recommended. The results show that the use of manure alone or with phosphorus resulted in a rather high incidence of disease. Also, when used with a complete fertilizer, NPK, the use of manure resulted in more disease than that present in the flats where complete fertilizer was used alone. Although manure improved the structure of the soil, this benefit may have been more than offset by a higher incidence of seedling disease. Apparently, complete fertilization resulted in beets of such vigor that the incidence of disease was low, and its degree of severity was relatively light. In addition to escaping from disease, beets treated with a complete fertilizer were benefited by greater growth, so that increased yields could be expected at the end of the growing season. It is probable that a disease rate of up to 20% would not greatly affect the final yield of these beets. Even if all diseased seedlings should die, the slight reduction in stand reduces competition of the sugar beets for available plant nutrients so that surviving beets make a greater growth during the growing season.

Beets grown in disinfected soil in general had a lower rate of seedling disease than did those that were grown in non-disinfected soil.

The growth of the fungus in disinfected soil may have been slower than in the non-disinfected soil because of possible residual toxicity of the chloropicrin, which would account for this fact. Also, it must be assumed that Aphanomyces cochlioides is not the only pathogen concerned in non-disinfected soil and the inroads of the other parasitic organisms might have increased the amount of disease.

In the test with disinfected soil, NPKM and NPK proved most effective in controlling disease. Manure alone was almost as effective. It is possible that other pathogens were favored by the manure in the non-disinfected soil, and that the manure contributed to the increase in disease, while in the disinfected soil, no other pathogens were present.

No evidence of antagonism between Trichoderma lignorum and Aphanomyces cochlioides could be found. In both non-disinfected and disinfected soil, T. lignorum inoculations resulted in a higher incidence of disease than in the checks.

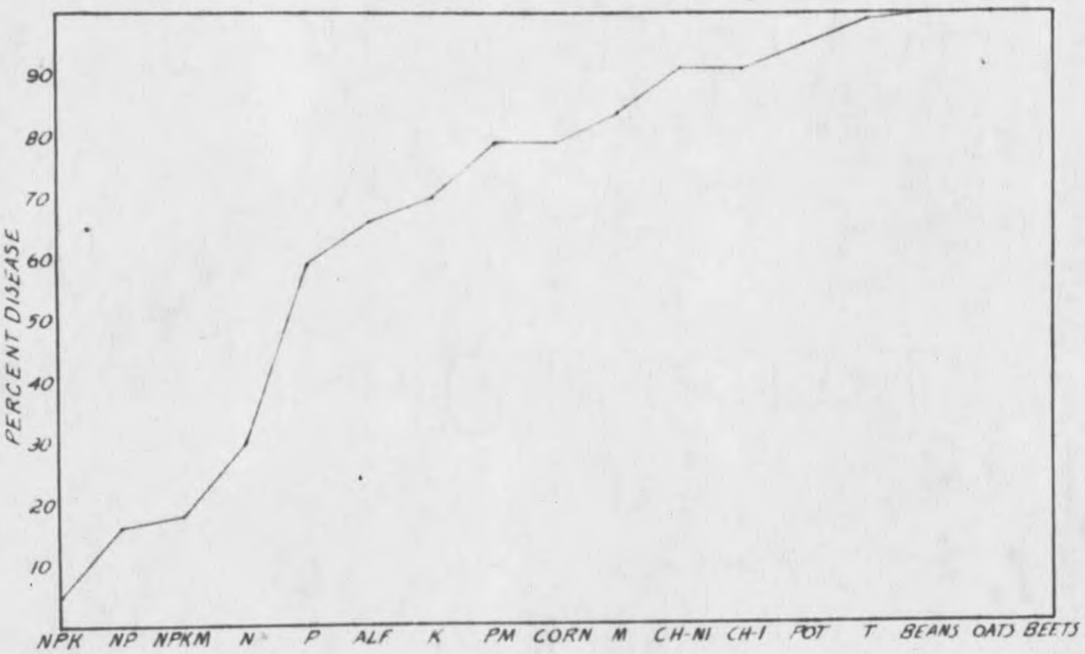

Figure 5. Percentage of Seedling Disease with Various Treatments in Non-disinfected soil.

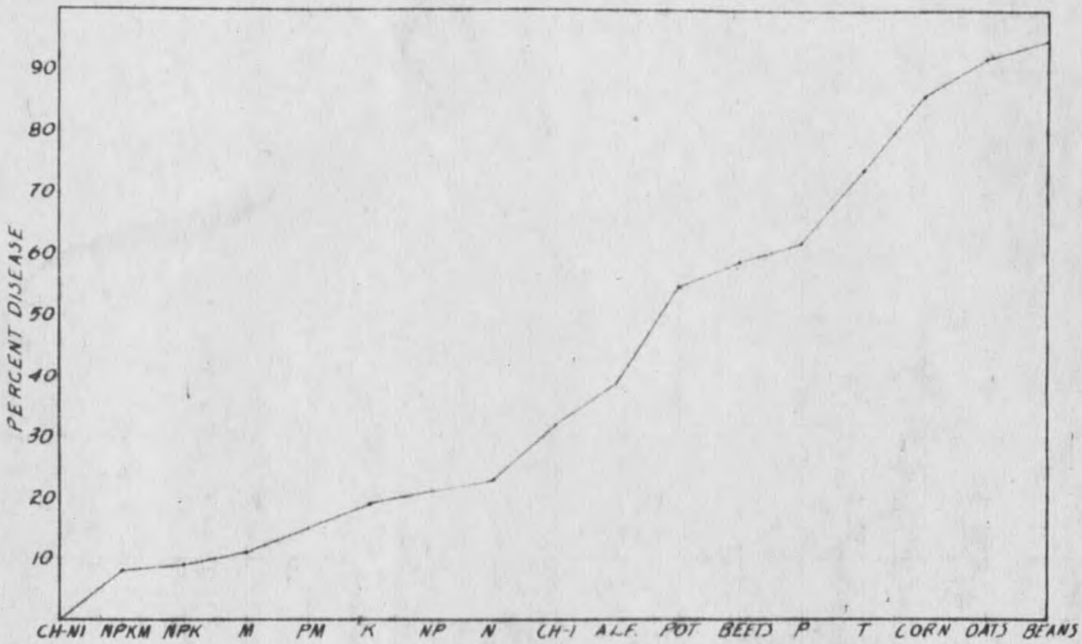

Figure 6. Percentage of Seedling Disease with Various Treatments in Disinfected Soil.

SUMMARY

1. The effects of various soil amendments and preceding crops on seedling disease of sugar beets were studied in non-disinfected and disinfected soil.

2. In non-disinfected soil alfalfa and corn were the only two preceding crops tested which provided any control of seedling disease. Although beets grown after corn had a greater amount of disease than those grown after alfalfa, the severity of the disease was less.

3. No preceding crop proved beneficial in reducing seedling disease of beets in disinfected soil.

4. Soil amendments most effective in controlling seedling disease in non-disinfected soil were NPK, NP, NPKM, and N, in the order written. While a certain amount of control was obtained with other amendments, their effectiveness was far below those named.

5. Although NPKM, NPK, and M proved to be the most effective in controlling seedling disease in disinfected soil, still all other treatments except P gave some control of disease.

6. The addition of a complete fertilizer gave far better control of seedling disease than any preceding crop tested.

7. *Trichoderma lignorum* exerted no antagonistic effect on *Aphanomyces cochlioides* under the conditions of this experiment.

8. In general, those treatments which resulted in a small amount of seedling disease produced only mild symptoms.

9. Potatoes and alfalfa generally produced beets of greater weight, both roots and tops, than other preceding crops.

10. Although results were somewhat variable, N, P, K, and M used singly as soil amendments produced smaller beets in roots and tops than NP, PM, NPK, and NPKM.

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