Black Root of Sugar Beets as Influenced by Various Cropping Sequences and Their Associated Mycofloras

R. E. Deemis and H. C. Young[1]

Summary

Black root of sugar beets at Hoytville, Ohio, during 1954-1955 was almost exclusively caused by *Aphanomyces cochlioides* Drechs. Soil was cropped to sugar beets during 1953-1954 to increase black root incidence. In May, 1955, sugar beets planted in soil from any portion of the plot became approximately 100 percent black rooted. Alfalfa, corn, oat, and sugar beets were cropped on this soil during 1955 and the soils assayed monthly for black root incidence and mycofloras. Cropping to corn and oat resulted in decreased black root. Corn was the most effective, decreasing disease incidence to 10 percent after three months and maintaining that level, or lower, through November. Oat decreased disease to 25 percent during the first four months, after which the oat was "plowed-down"; immediately black root increased to 76 percent and continued to increase through November. Alfalfa did not significantly decrease the amount of black root below the control sugar beet plots in which 95 to 100 percent of sugar beets black rooted at each monthly sampling from May through November.

There were several quantitative differences in the fungi isolated from the variously cropped plots. Increases and decreases in different fungi are correlated to black root incidence. It is probable that the effects of mycofloras on *A. cochlioides* are combination effects of the various fungi. Differences in associated mycofloras were primarily due to frequency of occurrence of fungi rather than to the presence or absence of specific fungi in any particular cropping sequence. Corn soil contained more of the following fungi than did alfalfa or sugar beet soils: *Penicillium funiculosum* Thom, *P. janthinellum* Biourge, *P. purpurogenium* Stoll, *P. rugulosumh* series Thom, *Trichoderma viride* Pers. ex Fr., *Absidia Butleri* Lendner, and other fungi of less striking differences. Several fungi occurred less frequently in corn soil than in alfalfa or sugar beet soils: *Vermicularia* sp. Fr., *Gliocladium catenulatum* Gilman et Abbott, *Sporotrichum* sp. Link, and other fungi of less striking differences. Oat soil differed from alfalfa or sugar beet soils primarily in the predominance of *T. viride* and *Aspergillus fumigatus* Fres. Alfalfa soils differed from corn, oat, or sugar beet soils in containing higher numbers of *Myrothecium verrucaria* (Alb. et Schw.) Ditm. ex Fr., *Ascochyta* sp. Lib., and *Fusarium merismoides* Corda; and in containing lower numbers of *A. fumigatus, Cephalosporium acremonium* Corda, *P. oxalicum* Thom, and *F. oxysporum* Schlect. Sugar beet soils differed primarily from the other soils in higher numbers of *Acrostalagmus*, spp. Corda and *Fusarium roseum* (g) Link. Possible explanations of the occurrence and effects of fungi associated with different cropping sequences were discussed.

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[1] Research Assistant and Professor of Plant Pathology, respectively, Ohio Agriculture Experiment Station, Wooster, Ohio.

[2] Numbers in parentheses refer to literature cited.

Introduction

The possibility of controlling soil-borne pathogens by introducing specific antagonistic fungi to infested soils has been extensively investigated during the past four decedes. Antagonism has been readily demonstrated in diverse artificial cultures but attempts to reproduce such results in the field have been wholly unsatisfactory. A wide variety of diseases have been controlled by special crop sequences and by various organic and inorganic amendments. Empirically, such disease control has often been ascribed to effects on natural soil mycofloras relative to various pathogens (5, 11, 17, 18). Cropping sequences have received attention as methods of establishing desirable mycofloras. Total numbers of fungi have been correlated with special cropping effects on disease incidence. It is, however, difficult to understand the significance of total mycofloras in disease control. It seems much more logical to assume that control of specific pathogens should be associated with the increase or decline of specific members or groups of members of the mycoflora.

Coons and Kotila (2) reported that sugar beet black root was controlled if corn immediately preceded sugar beets in cropping sequences. Contrariwise, sweet clover or alfalfa immediately preceding sugar beets actually increased black root incidence. Several types of clover and alfalfa were later proven to favor the same fungi which incited black root (1). Soybeans and small grains were found to decrease black root. Clovers and alfalfa could safely precede sugar beets if they were "plowed-down" early enough to allow "adequate disintegration of residues." Coons suggested that these controls were effected by competition and succession of non-pathogenic fungi.

That the basic control mechanisms of specific cropping sequences were ascribable to associated mycofloras was indicated by Kommendahl and Brock (10). They analysed soil which had been monocropped to corn, or oat, or wheat for 38 years for numbers of fungi. In this stabilized situation *Aspergillus fumigatus* Fres. was the most abundant fungus isolated from oat soil. Isolates of this fungus controlled seedling blight of corn incited by *Gibberella zeae* (Schw.) Petch. Gibberella seedling blight was also controlled when corn was planted in oat soil. In artificial culture, wheat was protected from seedling blights incited by *Fusarium* spp. Link ex Fr. by the following fungi in the order listed: *A. clavatus* Desm., *Penicillium* sp. Link ex Fr., *Trichoderma lignorum* (Tode) Harz., *P. lilacinum* Thom, *Nigrospora* sp. Zimm., *Penicillium* sp., and *A. fumigatus*. Herr (8) more thoroughly investigated the mycofloras associated with corn, oat, and wheat in these same continuously cropped plots. Four kinds of fungi predominated: *A. fumigatus, P. funiculosum* Thom, *Trichoderma* spp. Pers. ex Fr., and *Fusarium* spp. Striking differences in the frequency of these fungi were apparent. *A. fumigatus* was the predominant fungus from oat plots; *P. funiculosum* was the predominant fungus from corn plots; while *Trichoderma* spp., *Fusarium* spp., and *A. fumigatus* occurred with equal frequency in the wheat plots. *Trichoderma* spp. were never isolated from the oat plots.

The purpose of this study was to establish whether different characteristic mycofloras developed during one season's cropping to alfalfa, corn, oat, and sugar beets following two seasons of sugar beets and to correlate such differences, if they occurred, with the effects of these crops on black root incidence.

Materials and Methods

To insure adequate increase of black root, an 86 foot by 172 foot plot of Brookston Clay was planted to sugar beets at Hoytville, Ohio, in 1953. This same plot was planted to sugar beets in 1954 and assayed weekly, from May 6 through September 9, for soil mycoflora and black root incidence. At the end of this period, approximately 100 percent of the sugar beets seeded in soil from any portion of the plot became black rooted. This plot was divided into twelve 44 foot by 14 foot plots in the spring of 1955 and planted to alfalfa, corn, oat, and sugar beets. The soil mycoflora and black root incidence in each plot were assayed monthly using the following methods. Aseptic techniques were used throughout.

Each plot was randomly sampled by taking eight 1 inch by 8 inch cores perpendicular to and including the surface. These eight cores were thoroughly mixed by grinding and agitation. The amount of moisture in each sample was determined using a 100° C. oven.

Black root was assayed by planting segmented sugar beet seeds in a portion of each pooled sample and counting the diseased seedlings after three weeks. Diseased seedlings were cultured for black root pathogens. To determine the number of seedlings diseased with *Aphanomyces cochlioides* Drechs., seedlings were washed thoroughly, blotted of excess water, placed in sterile distilled water, and later examined.

Soil fungi were isolated by a soil dilution plate technique (14). Samples of the pooled soil were suspended in a 1 percent CMC[3] solution with a mechanical stirrer and diluted 1:10,000. One ml aliquots of this final dilution were added to each of 16 petri dishes. Ten ml of a medium developed at the Ohio Agricultural Experiment Station (see Table 1), maintained at 52° C, were placed in each plate, the cultures mixed, and then incubated

Table 1.—Culture Medium Developed at the Ohio Agricultural Experiment Station for the Isolation of Saprophytic Soil Fungi.

Agar	20.0 g
Yeast Extract	2.0 g
Sodium Nitrate	1.0 g
Potassium Phosphate (monobasic)	1.0 g
Magnesium Sulfate	0.5 g
Glucose	5.0 g
Oxgall	1.0 g
Sodium Propionate	1.0 g
Streptomycin	50.0 mg
Chloromycetin	50.0 mg
Distilled Water	1,000.0 ml

Autoclave at 11 pounds pressure for 15 minutes

at 24° C. for 5 days. After incubation, fungal colonies were marked and each fungus identified as soon as possible. If a fungus could not be classified to a well recognized category, it was named to the best of the author's ability and constantly there assigned on reoccurrence. Subsequently, most of these colonies were correctly identified. Several "keys" to the fungi were used in this research (3, 4, 6, 7, 13, 16).

The kinds and total numbers of fungi were tabulated for each replication of each crop and recorded as the numbers per gram of oven dried soil to facilitate comparisons.

[3] CMC - 120 High Viscosity Carboxy-methyl cellulose, Hercules Powder Company, Wilmington 99, Delaware.

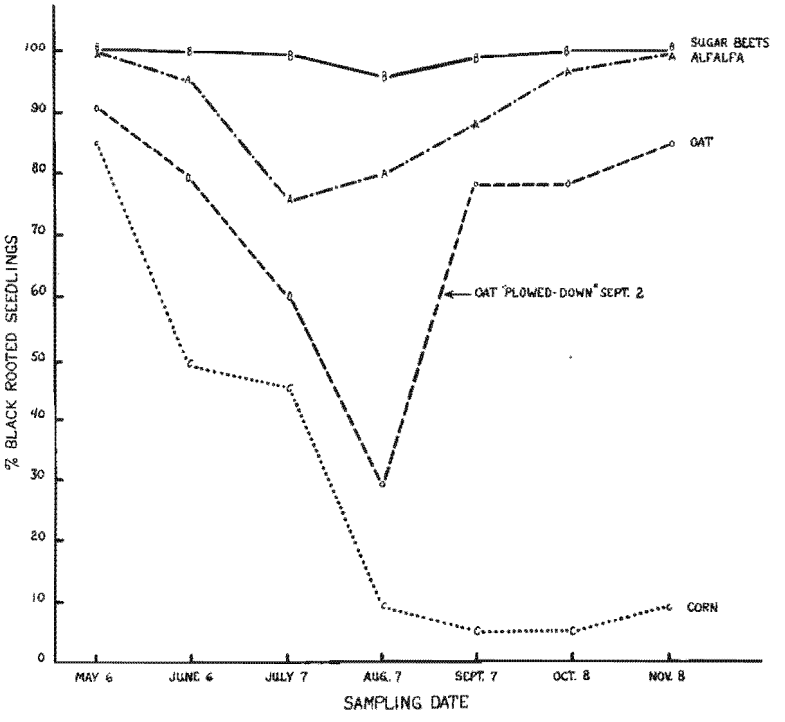

Figure 1.—Incidence of black root pathogens in sugar beet soil (2 years) cropped to alfalfa, corn, oat, and sugar beets at Hoytville, Ohio, during 1955.

Results

Pathogens causing black root.

Black root incidence is a limiting factor in sugar beet production in Ohio. The disease has most often been attributed to *A. cochlioides*, *Rhizoctonia solani* Kuhn, and *Pythium* spp. Pringsheim. Investigation of the cause of black root during 1954 and 1955 established that the disease was almost exclusively incited by *A. cochlioides*. Of 2,548 diseased seedlings examined, *A. cochlioides* was cultured from 2,487, *Fusarium* spp. from 34, *R. solani* from 12, and various other organisms from 15. A bacterium tentatively identified as *Erwinia carotovora* (L. R. Jones) Holland was usually associated with *A. cochlioides*. This bacterium incites a soft rot of sugar beet tissues when introduced but does not appear to be a primary invader.

Effects of cropping sequences on black root incidence.

Striking effects on black root incidence resulted from cropping soil thoroughly infested with black root pathogens to alfalfa, corn, oat, and sugar beets (see Figure 1). Sugar beets maintained a 95 to 100 percent inoculum potential in the soil throughout the season. Alfalfa slightly decreased black root during May and June, but thereafter steadily increased black root through November. Oat steadily decreased black root to 25 percent through the August 7 sampling. "Plowing-down" of oat September 2 resulted in an increase in black root to 76 percent at the September 7

sampling date. Black root increased through the succeeding sampling dates. Corn was the most effective crop, decreasing black root to 10 percent after three months and maintaining that level, or lower, through the remainder of the season.

Effects of cropping sequences on soil mycofloras.

In May of 1955, when the soil was seeded to alfalfa, corn, oat, and sugar beets, the mycoflora was little different from that established for the plot during 1954. There were also little differences between the mycofloras of the plots seeded to alfalfa, corn, oat, and sugar beets. Throughout the growing season of 1955, the differences between mycofloras could, therefore, be attributed to the different crops or associated agronomic practices. Generally, the same kinds of fungi were isolated from all the plots in 1955. The principal differences between treatments were in the frequencies of the different types of fungi.

One season's cropping of sugar beet soil to alfalfa, corn, oat, and sugar beets had little effect on the total numbers of fungi isolated (see Table 2). There were no important differences in numbers of fungi associated with the different crops; except in case of alfalfa and oat at the October 8 sampling and oat at the September 7 sampling. The low number of fungi associated with alfalfa at the October 8 sampling can be attributed to no known factor. In the case of oat, the extremely high number of fungi at the September 7 sampling immediately followed "plowing-down" of the crop. The entire difference was due to an epidemic of two species of Fusarium: *F. lateritium* Nees v. *minus* Wr. and *F. avenaceum* (Fr.) Sacc. The sharp decrease in total numbers at the next sampling was primarily due to a complete absence of these two Fusaria. The variability in total numbers (Table 2) is probably due to differential availability of moisture.

Table 2.—Total Fungi[1] in Sugar Beet Soil (2 yrs.) Cropped to Alfalfa, Corn, Oat and Sugar Beets at Hoytville, Ohio, During 1955 (Each Number is the Average of 3 Replications).

Crop	Sampling Date							Av. No. of Fungi During Season
	May 6	June 6	July 7	Aug. 7	Sept. 7	Oct. 8	Nov. 8	
Sugar Beets	128.8	81.8	55.5	80.3	106.4	100.4	114.0	95.3
Alfalfa	108.4	71.2	34.4	88.4	89.0	50.6	133.3	82.2
Corn	134.3	75.3	57.5	101.0	89.6	111.2	137.3	100.9
Oat	96.6	69.8	34.6	81.6	207.6[2]	42.6	77.7	87.2

[1] Thousands of Fungi per gram of Oven-Dried Soil.

[2] Of this total, 119.4 are Fusarium avenaceum and Fusarium lateritium var. minus.

During 1955, 145 different species of fungi belonging to 85 different genera were isolated from the plots. It is noteworthy that 60 to 80 percent of the fungi isolated at any one sampling were *Aspergillus* spp., *Gliocladium* spp., *Fusarium* spp., *Penicillium* spp., and *T. viride*.

Fungi isolated from oat plots are not directly comparable with those from alfalfa, corn, and sugar beet plots after the August 7 sampling. *A. fumigatus* and *T. viride* were more abundant in oat soil than in alfalfa or sugar beet soils. This difference may partially account for decreased black root in oat soils up to "plowing-down." More importance should probably

be placed on the early predominance of *F. equiseti* (Corda) Sacc. and *F. episphaeria* (Tode) Snyder et Hansen in oat soil. These two Fusaria are important components of grassland soils. They are excellent colonizers of organic materials and possibly competed more successfully for nutrients than did *A. cochlioides*.

Corn most effectively reduced black root incidence, whereas alfalfa and sugar beets did not. Correlations between corn soil vs. alfalfa and sugar beet soils are possible. Various fungi occurred in different numbers in corn soil than in alfalfa and sugar beet soils. Several fungi were strikingly higher in corn soil. *Penicillium* spp. were most distinctive in this respect with *P. funiculosum*, *P. janthinellum* Biourge, *P. purpurogenum* Stoll, and *P. rugulosum* series Thom being outstanding. *Absidia Butleri* Lendner, *Robillardia* sp. Sacc., *Geotrichum candidum* Link, *Spicaria divaricata* (Thom) Gilman et Abbott, and *T. viride* were also more abundant. Those fungi which occurred less in corn soil were *Vermicularia* sp. Fr., *Phoma* sp. Desm., *Gliocladium catenulatum* Gilman et Abbott, *Hyalopus ater* Corda, *P. nigricans* Bain., *Sporotrichum* sp. Link, *Pullularia pullulans* (deBary) Berkhout, *Alternaria tenuis* Nees, *Fusarium* spp., and *F. solani* (Martius) Appel et Wollenw.

The organisms favored by cropping to alfalfa were *Myrothecium verrucaria* (Alb. et Schw.) Ditm. ex Fr., *Ascochyta imperfecta* Peck, and *F. merismoides* Corda. *M. verrucaria* is noted as an important cellulytic fungus of

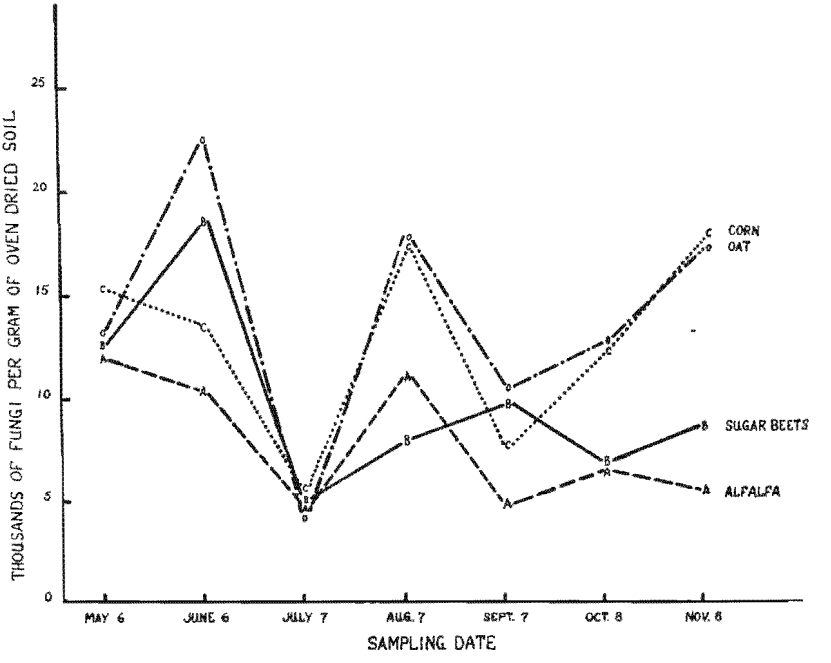

Figure 2.—Occurrence of *aspergillus fumigatus* and *trichoderma viride* in sugar beet soil (2 years) cropped to alfalfa, corn, oat, and sugar beets at Hoytville, Ohio, during 1955.

cosmopolitan distribution. The presence of debris containing high amounts of cellulose in the alfalfa plots late in the season may account for its increase. Blackstem of alfalfa was common in the plots late in the season accounting for the increased *A. imperfecta*. Those fungi decreased by alfalfa were *A. fumigatus*, *Cephalosporium acremonium* Corda, *P. oxalicum* Thom, and *F. oxysporum* Schlecht. *Fusarium conglutinans* Wollenw. occurred predominantly in sugar beet soil. No attempt has been made to prove the pathogenicity of this fungus; but a variety is known to be the causal agent of sugar beet yellows. *Acrostalagmus* spp. Corda and *F. roseum* Lk. (g) were also higher in sugar beet soils. *R. solani* and *Pythium* spp. were isolated infrequently due to the method used. These two organisms were, however, consistently obtained from soil samples when special isolation procedures were used.

Occurrences of the four fungi best correlatable with decrease of black root at various samplings are illustrated in Figures 2, 3, 4, and 5. *Penicillium* spp. continually increased through the season in corn soils (see Figure 3). The two species most responsible for this increase late in the season were *P. funiculosum* (see Figure 4) and *P. janthinellum* (see Figure 5). *P. purpurogenum* and *P. rugulosum* series were important fungi early in the season. The occurrence of *A. fumigatus* and *T. viride* is illustrated in Figure 2. In general, there was a greater frequency of the latter two organisms in corn and oat soils than in alfalfa and sugar beet soils. The seasonal variations of

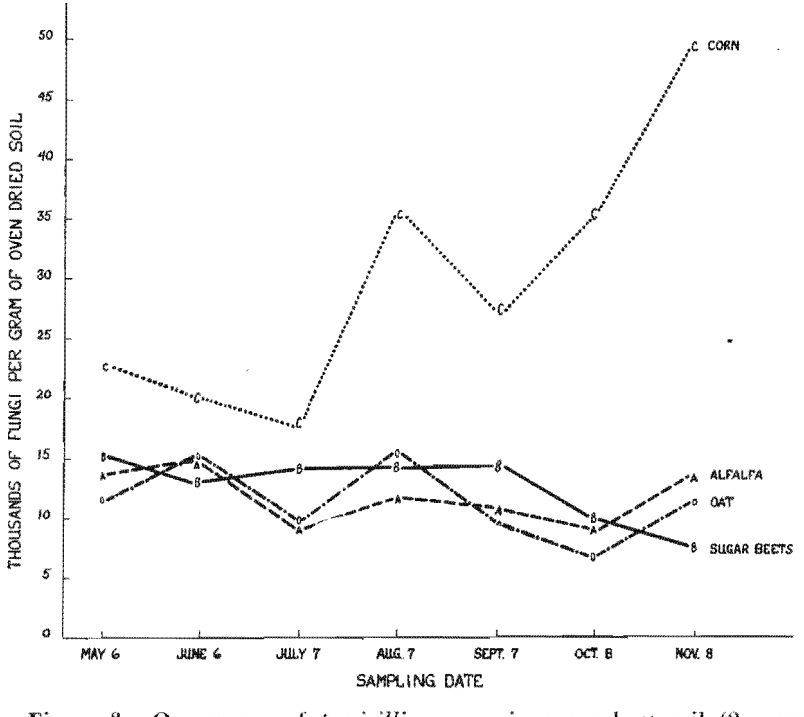

Figure 3.—Occurrence of *penicillium* spp. in sugar beet soil (2 years) cropped to alfalfa, corn, oat, and sugar beets at Hoytville, Ohio, during 1955.

these four organisms do not obscure the definite relationships. Even with fluctuations, the fungi remain in the same relative order in the variously cropped soils. Such seasonal fluctuations were common with most of the genera identified.

Discussion

There has been considerable question by various investigators concerning the establishment of characteristic different mycofloras by various crops. Herr (8) proved that mycofloras were different when corn, oat, and wheat were continuously cropped for 39 years. There is little doubt, from the present investigation, that characteristic different mycofloras may develop in one season. In fact, the mycofloras become different early in the development of the crops. Cropping influences are stronger than impinging environments since similar relationships in frequency of specific organisms were maintained through the season. There was little difference in mycofloras during the latter samplings in 1954 and that at planting time in 1955, but is this the normal? A strikingly different situation was the share increase of black root when oat was "plowed-down" September 2. The desirable effect on black root was immediately negated. Certainly the mycoflora was critically disturbed. *F. avenaceum* and *F. lateritium* v. *minus* were probably introduced from above ground portions of oat plants. *Penicillium* spp. decreased as did several other genera.

The majority of saprophytic soil fungi in Ohio apparently belong in 6-10 genera with a large number of minority inhabitants and invaders. The

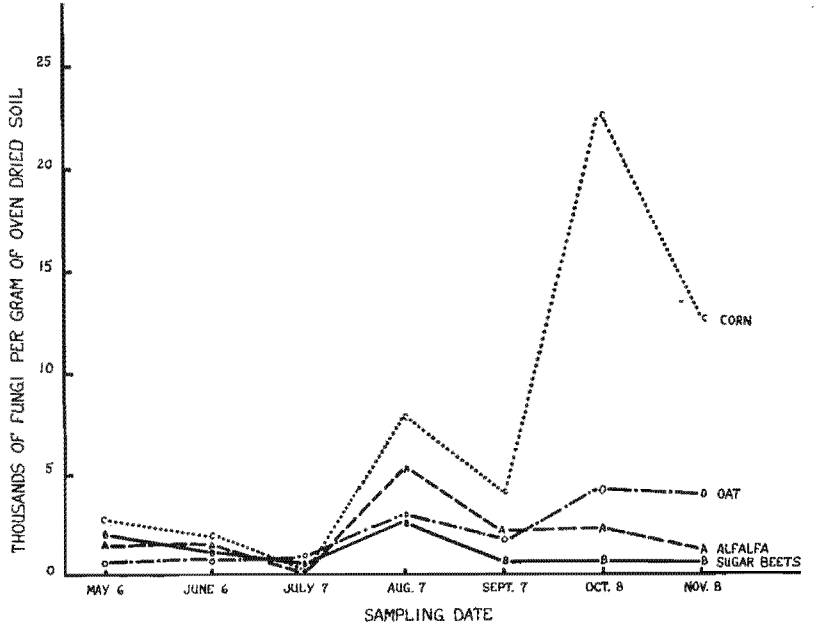

Figure 4.—Occurrence of *penicillium funiculosum* in sugar beet soil (2 years) cropped to alfalfa, corn, oat, and sugar beets at Hoytville, Ohio, during 1955.

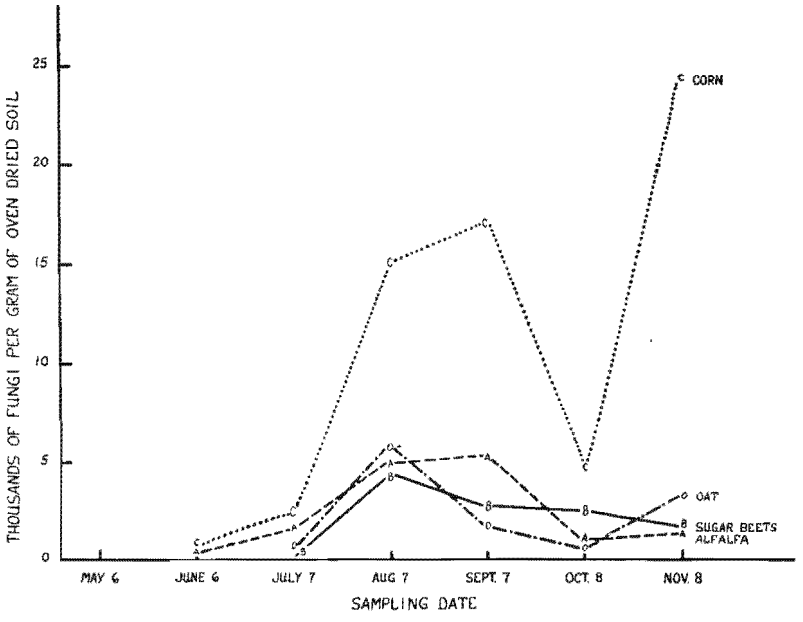

Figure 5.—Occurrence of *penicillium janthinellum* in sugar beet soil (2 years) cropped to alfalfa, corn, oat, and sugar beets at Hoytville, Ohio, during 1955.

primary concept to be recognized is that while genera may vary in occurrence, this variability is due to differences in numbers of individuals of species and varieties. Such species and variety differences are of primary importance in mycoecology.

The slight early decrease of *A. cochlioides* disease potential in alfalfa soil followed by an increase to 100 percent is quite interesting since *A. cochlioides* has never been proven pathogenic to alfalfa. It is possible that some organisms or groups of organisms are antagonistic to an organism or group of organisms antagonistic to the pathogens. *P. funiculosum*, *P. janthinellum*, *P. purpurogenum*, and *P. rugulosum* series may be considered antagonistic to *A. cochlioides*. These species occur infrequently in alfalfa and sugar beet soils and have, in general, an intermediate occurrence in oat soils. A *Vermicularia* sp., *G. catenulatum*, and a *Sporotrichum* sp. occur in precisely the opposite pattern. It is possible that the latter organisms are antagonistic to the *Penicillium* spp. Therefore, *Penicillium* spp. do not increase to a level where they are antagonistic to *A. cochlioides*.

Correlations, such as those described in the present investigation, may indicate "biological control" by fungi such as *Penicillium* spp., *Aspergillus* spp., *T. viride*, *Fusarium* spp., or others. The soil fungi are in a state of equilibrium with their environments. Various cropping sequences and soil amendments favor development of different members of the soil population.

Different fungi increase at the expense of other fungi. The principal mechanisms are probably antibiosis and differential competition for available nutrients. Differential cropping is an effective means of varying mycofloras. Results reported here are not yet conclusive; however, an understanding of the mode of black-root control by crop sequences is indicated.

Members of the soil population other than fungi are undoubtedly important in "biological control." Actinomycetes, bacteria, nematodes, and protozoa are also probably differentially influenced by cropping sequences. Any change in the soil population may affect pathogens differentially. This program, at present, concerns only fungi. The belief is that the more completely the soil population is understood, the more intelligently and, therefore, more effectively can root rot problems be solved.

Several factors are important in establishment of characteristic mycofloras. Differences depend on competition, antagonism, antibiosis, or parasitism among the component fungi. The degree and type of differences may be conditioned by: (a) Agronomic practices peculiar to each crop; (b) quantity and quality of crop debris returned to the soil during the growth of and after the death of individual crops; (c) different growth habits of plants such as transpiration rates, type of root system, quantity and type of materials removed from the soil by plants, and root diffusates; (d) pH; (e) temperature; (f) moisture; and (g) physical qualities of the soil.

Inasmuch as the soil mycofloras are probably associated with multitudes of microhabitats near various substrates, it is quite possible that antibiotics could be an important factor in the distribution of many types of fungi. Certainly many strains of fungi are capable of producing antifungal antibiotics. Diffusates which accelerate the growth of fungi may also be important. Such conditions probably occur in the soil and the quantity of effective antibiotic or acceleratory substances could be relatively small if concentrated at limited loci. Pramer and Starkey (12) have indicated that the quantities of antibiotics necessary for action in the soil are much higher than in in vitro research. Furthermore, antibiotics are often unstable in some soils. Such research has not been sufficiently investigated on a micro-scale.

Strains of several fungi isolated in this investigation are capable of producing antifungal antibiotics (see Table 3). Three of the antibiotics are effective against Phycomycetes (9). The authors are of the opinion that mechanisms involving antibiotics are partially responsible for the occurrence of different mycofloras and may be effective in controlling soil borne pathogens.

The soil-dilution plate technique used in this investigation has been proven quite satisfactory for the type of study described. The method does favor isolation of sport producing fungi; but this is not disadvantageous since the drawback is recognized. Close agreement between members of similar samples emphasizes the reliability of the method. Accuracy of the procedure continges upon proper sampling and thorough mixing. Too much care cannot be exercised in identifications. The fungi listed in this research were all identified by one investigator and constancy has been emphasized throughout.

Table 3.--Some Antibiotics from Soil Fungi of Possible Importance in These Investigations.

Antibiotic	Organism	Biological Activity
Expansine	Aspergillus clavatus, A. terreus	Many Phytopathogens—Pythium spp., Ceratostomella ulmi, etc.
Gliotoxin	Gliocladium catenulatum (= G. fimbriatum) Trichoderma viride (= all yellow and green T's) Aspergillus fumigatus	Inhibits Claviceps purpurea, Phytophthora erythroseptica, Sclerotinia sclerotiorum, and Stereum purpureum
Aspergillic Acid	Aspergillus flavus	Inhibits Phytophthora erythroseptica, Pythium ultimum, etc.
Glutinosin	Myrothecium verrucaria	Inhibits Phoma betae, Mucor mucedo, and other fungi
Viridin	Trichoderma viride	Inhibits Botrytis allii, Cephalosporium sp., Fusarium coeruleum, F. culmorum, Stachybotrys atra, Penicillium spp., Trichothecium roseum, and other fungi
Trichothecin	Trichothecium roseum	Inhibits some Fungi
Uncharacterized	Penicillium citreo-viride, P. funiculosum, P. nigricans, P. oxalicum, and Scopulariopsis sp.	Inhibits some Fungi

Significant levels have not been completely established for these data. Investigators at the OAES (15) have evidence that differences of 60 to 100 percent are significant for total numbers of fungi. We are using this level at present to judge significance between genera. Recent results indicate that lesser differences may be significant for genera.

A type of mycoflora associated with a soil which has a low incidence of black root has been described. The authors believe that assays such as these could be used to predict black root free soil. More supplementary data are necessary for several different soils before such analyses are practicable. These data are also preliminary to an understanding of the basic control mechanisms operative in desirable cropping sequences.

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