SOIL MYCOFLORA ASSOCIATED WITH CONTINUOUS CROPPING OF CORN, OATS, AND WHEAT[1]

LEONARD J. HERR

Department of Botany and Plant Pathology
Ohio Agriculture Experiment Station, Wooster, Ohio

Root rots and seedling blights are among the most destructive and most ineffectively controlled of plant diseases. According to Garrett (1944), the oldest and most effective method of controlling root rots of field crops is crop rotation. In regard to seedling blights and root rots of corn and wheat, it has been reported that the incidence of these diseases was reduced when these crops were preceded in the rotation by a crop of oats (Broadfoot, 1934; Sanford, 1946; Tyner, 1948; Odland *et al.*, 1950; and Kommedahl and Brock, 1954). Efforts are now being made to discover the underlying causes of variations in disease severity which accompany variations in crop sequence.

Among the proposed explanations of the effect of crop sequence on the incidence of root rots, one which has received a great deal of attention from plant pathologists is the relation of soil microflora to these diseases (Sanford, 1946; Weindling, 1946; Clark, 1949; Weindling *et al.*, 1950; Lochhead, 1952; Waksman, 1952; Simmonds, 1953; and Kommedahl and Brock, 1954).

In 1952 a continuing series of investigations into this problem was initiated at The Ohio Agricultural Experiment Station. Kommedahl and Brock (1954) assayed soil from continuously cropped corn, oats and wheat for numbers of fungi and found that the fungus counts from the oat plot were consistently higher than those from the corn or wheat plots. In agreement with the workers previously cited they found that, in greenhouse tests, less seedling blight of corn and wheat occurred when those crops were planted in soil taken from the oat plot than when they were planted in soil taken from the corn or wheat plots.

The study reported here was made during the growing season of 1953. The same continuously cropped plots used by Kommedahl and Brock were assayed for both numbers and kinds of fungi. The principal objectives of this study were: 1) to determine whether the fungi isolated from the different plots would differ in kind, and 2) to determine whether the total numbers of fungi isolated from the different plots would again have the same relation to each other (oats highest) as in 1952.

METHODS

The soil samples assayed in this investigation were obtained from three plots located on the agronomy rotation range of The Ohio Agricultural Experiment Station, Wooster, Ohio. These three plots had been continuously cropped since 1915 to either corn, oats, or wheat. The soil was a silty loam. Each of these plots received equal amounts of 0–14–7 fertilizer each year. No nitrogen had been added to these plots since 1936, when they were manured. After the harvest of the wheat, volunteer black medick (*Medicago lupulina L.*) grew in this plot and may have supplied some nitrogen. After harvest of the oats, a nearly pure stand of ragweed covered the plot. Little plant growth other than corn was present on the corn plot. The soil samples collected from these plots were assayed for numbers and kinds of fungi by a standardized soil dilution-plate technique.

[1]Work completed while employed as Research Assistant at The Ohio Agricultural Experiment Station, submitted in partial fulfillment of the requirements for Master of Science Degree at The Ohio State University in 1953. O.A.E.S. Paper No. 25–57 and Paper No. 603, Department of Botany and Plant Pathology, The Ohio State University.

The plots were assayed for numbers and kinds of fungi on four dates; June 30, July 7, August 18, and September 11, 1953.

1. *Collection of samples.* The soil samples were collected in sterilized screw-cap vials at three different depths (surface, 2 in., and 4 in.) from each of the plots. A vertical face was cut in the soil; the vials were held at right angles to the face at the proper depths and were filled by screwing the vial into the soil. The surface sample included soil to a depth of approximately ¼ of an inch. One sample was collected at each depth.

2. *Dilution technique.* An accurately weighed aliquot of approximately 0.100 g. of each soil sample was added aseptically to 10 ml. of sterile distilled water contained in a 125 ml. Erlenmeyer flask. The flasks were placed on a platform shaker (60 oscillations per minute) for 15 minutes to break up the soil particles. They were then shaken manually for approximately 20 seconds and 1 ml. of the suspension was immediately withdrawn with a sterile pipette and transferred to 50 ml. of sterile distilled water contained in a 250 ml. Erlenmeyer flask. The dilution was 1:5000. The resulting suspension was shaken thoroughly and 1 ml. aliquots were transferred onto the surface of each of 5 agar plates for each individual soil sample. Warcup's (1950) medium (Czapeks-Dox plus 0.5 percent yeast extract), acidified with lactic acid to pH 4.0, was used for the assays.

3. *Determination of the numbers and kinds of fungi.* The fungus colonies were counted following a 3-day incubation at 26°C. The colonies were consecutively numbered on the bottom of each of the petri plates. The mean number of colonies per set of 5 plates was then used to calculate the number of fungi per gram of oven-dry soil. Portions of all soil samples were dried for 24 hours at 110°C and the dry weight equivalent of the soil used in the dilution was computed. This dry weight equivalent was then used to calculate the number of fungi per gram of oven-dry soil.

Seven to 10 days after preparing the plates, the fungi reached a stage of growth at which they could be identified. All colonies not having a corresponding number on the bottom of the plates were assumed to be secondary. Some slow-growing fungi may have been excluded because of this assumption. The presence of secondary colonies obviously derived from sporulating original colonies made counts beyond the 3-day incubation period highly unreliable.

The fungus colonies were identified on the original dilution plates. When "spreaders," *Trichoderma spp.* or *Rhizopus spp.*, were present, it was still possible to identify most of the other fungus colonies in the plate, provided the identification was undertaken early.

The fungi were identified to genus except in the case of Aspergilli and Penicillia, which were identified to the groups as outlined by Thom and Raper (1945) in their "Manual of the Aspergilli" or to the series as outlined by Raper and Thom (1949) in their "Manual of the Penicillia." In the remainder of this paper the groups of the Aspergilli and the series of the Penicillia shall be referred to by the name of the specific fungus which characterizes the group or series. Two other extremely useful keys were "A Manual of Soil Fungi" by Gilman (1945) and "The Genera of Fungi" by Clements and Shear (1931).

EXPERIMENTAL RESULTS

1. *Distribution and frequency of the kinds of fungi isolated.* From the three plots, corn, oats, and wheat, four kinds of fungi predominated in the isolations. These were *Aspergillus fumigatus*, *Penicillium funiculosum*, *Trichoderma spp.*, and *Fusarium spp.* A collective total of 1,967 colonies of fungi was isolated during this study; 1,344 of the total colonies (approximately 70 percent) consisted of the four fungi previously mentioned. The distribution and frequency of isolation of these four fungi according to crop (averages of all sampling depths and sampling dates) are graphically presented in figure 1.

Striking differences in distribution and frequency of isolation of these four fungi were associated with the particular crops. From the oat plot soil samples *Aspergillus fumigatus* was the predominant fungus isolated and accounted for 64 percent of the total fungi isolated from oat soil. From the corn plot soil samples *Penicillium funiculosum* was the predominant fungus isolated and accounted for 57 percent of the total fungi isolated from corn soil. No one fungus predominated in isolations from the wheat plot soil; instead *Aspergillus fumigatus*, *Trichoderma spp.*, and *Fusarium spp.* were isolated with approximately equal frequency. *A. fumigatus* accounted for 13 percent, *Trichoderma spp.* for 21 percent, and *Fusarium spp.* for 16 percent of the total fungi isolated from the wheat soil. Collectively, from wheat soil these three fungi accounted for 50 percent of the total fungi.

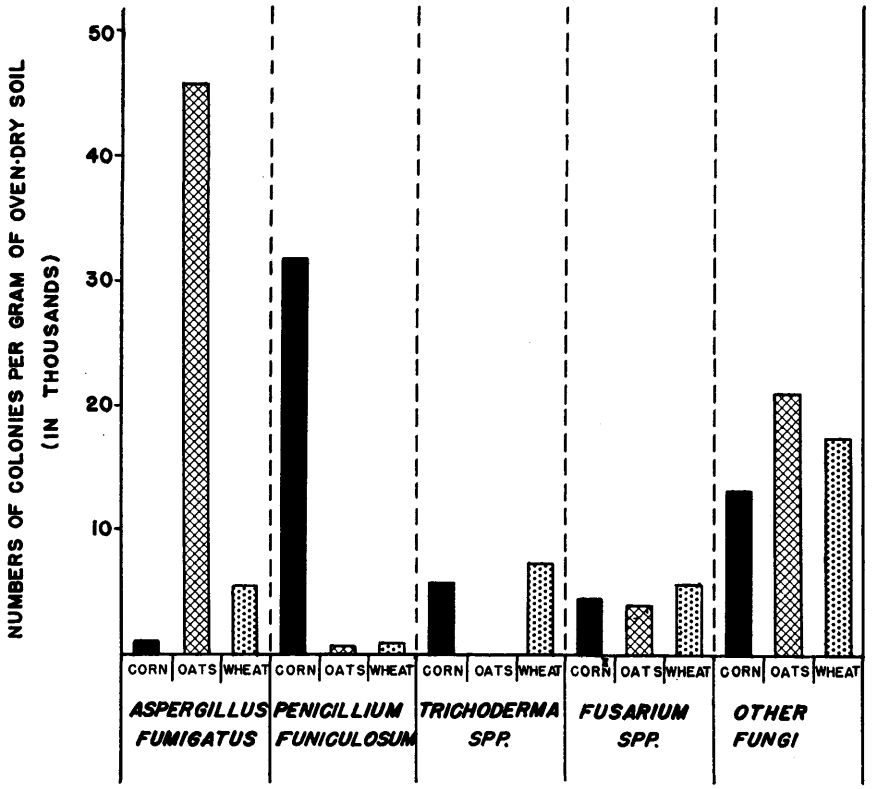

FIGURE 1. Average frequency of isolation (average of all sampling depths and sampling dates) of the four predominate fungi, as well as all other fungi combined, from each of the three plots, corn, oats, and wheat.

With the exception of *Trichoderma spp.*, which were never isolated from the oat plot samples, these four fungi were, at least occasionally, isolated from every plot. Thus, the distinctiveness of the mycoflora associated with each of the three crops was primarily due to the relative frequency of isolation of these fungi and not to their complete absence. The differences in distribution and frequency of isolation of these fungi existed at all sampling depths and on all sampling dates (fig. 2) although fluctuations associated with sampling depths and dates occurred. The experimental design was such that these fluctuations which were apparently

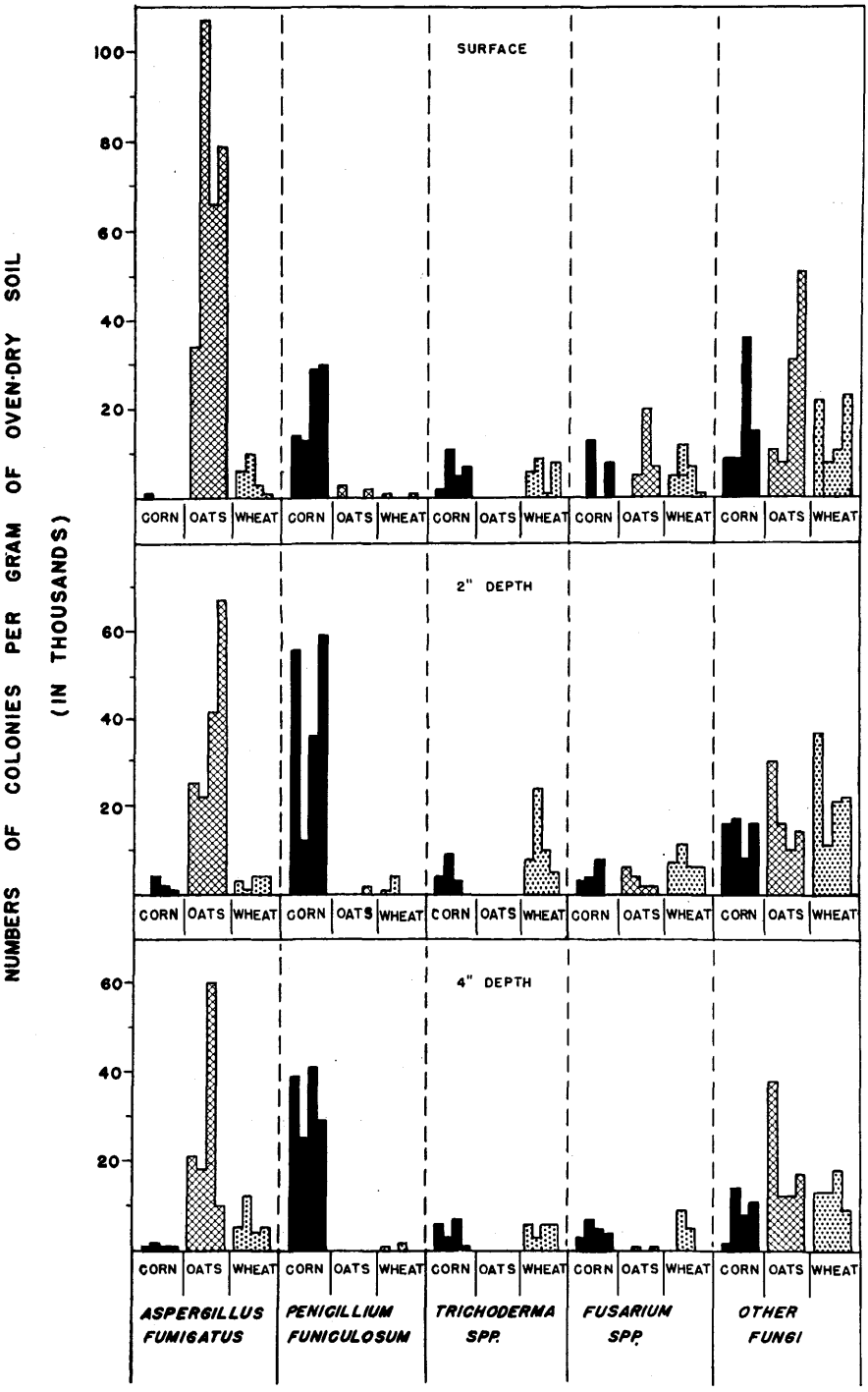

FIGURE 2. Distribution and frequency of the predominate fungi, and all other fungi combined, isolated from the corn, oats, and wheat plots for each sampling date and each sampling depth. Each of the four bars drawn for each crop and a given fungus refers to a sampling date, the bar on the left representing the first sampling date, the next bar the second sampling date, etc. The sampling dates were June 30, July 7, August 18, and September 11.

TABLE 1

List of the kinds of fungi isolated and the total numbers of each isolated from the corn, oats, or wheat plots. Fungi listed in order of frequency of isolation

Fungi	Corn	Oats	Wheat
Aspergillus fumigatus	13	551	58
Penicillium funiculosum	383	7	10
Fusarium spp.	55	48	69
Trichoderma spp.	58		92
Cladosporium spp.	14	22	8
Oospora spp.	1	28	4
Alternaria spp.	4	23	2
Penicillium nigricans	9	5	13
Tilachlidium spp.	1	22	
Monilia spp.	13	2	6
Penicillium oxalicum	14	2	5
Penicillium lilacinum	3	3	14
Acrostalagmus spp.	6		10
Hyalopus spp.	1	2	13
Rhizopus spp.	1		15
Phoma spp.	6	9	
Spicaria spp.	7	2	6
Rhizoctonia spp.	6	3	5
Sacchromyces spp.	4	9	1
Monotospora spp.			10
Penicillium janthinellum	9		1
Gliocladium spp.		1	7
Monosporium spp.	1	2	5
Scopulariopsis spp.		8	
Sporotrichum spp.	2	3	3
Chaetomium spp.		4	3
Papularia spp.			6
Hormiscium spp.		3	2
Mucor spp.	3	1	1
Spondylocladium spp.		5	
Penicillium purpurogenum			4
Cylindrocarpon spp.	1	1	1
Penicillium herquei			3
Penicillium implicatum		3	
Verticillium spp.	1		2
Aspergillus clavatus			2
Cylindrophora spp.	2		
Penicillium canescens	2		
Penicillium citrinum	2		
Penicillium pallidum	1	1	
Penicillium turbatum	2		
Ustilago spp.	2		
Acremonium spp.			1
Aspergillus niger	1		
Aspergillus ustus			1
Aspergillus versicolor			1
Botrytis spp.		1	
Botrytrichum spp.	1		
Cephalosporium spp.	1		
Cepahlothecium spp.		1	
Ciliciopodium spp.			1
Cunninghamella spp.		1	
Mycogone spp.			1
Papulospora spp.	1		
Pestalotia spp.	1		
Pullularia spp.	1		
Stachylidium spp.		1	
Stemphylium spp.	1		
Trichotheeium spp.			1
Unidentified colonies	35	84	53
Totals	669	858	440

due to sampling depths and dates could not validly be evaluated. It was found that the total kinds of fungi (genera, groups, or series) isolated decreased with increased sampling depth; thus, 43 genera, groups, or series were found at the surface, 38 at the 2-inch depth, and 35 at the 4-inch depth.

In table 1 a complete list of the kinds of fungi isolated and the numbers of colonies of each fungus isolated from each of the three plots during this study is given. A total of 59 genera, groups, or series was isolated from all the plots. Thirty-eight genera, groups, or series were isolated from corn soil; 31 from oat soil; and 37 from wheat soil. The unidentified category accounted for approximately 9 percent of the total fungi isolated.

2. *Total fungus colony counts.* As previously mentioned, Kommedahl and Brock (1954) assayed these same continuously cultivated plots during the growing season of 1952 and found that the total fungus colony counts from the oat plot were consistently significantly higher than those from the corn or wheat plots.

TABLE 2

Total colony counts (per gram of over-dried soil) of fungi isolated from corn, oats, or wheat plots at surface, 2-inch and 4-inch depths on the different sampling dates

Sampling Date	Depth	Colonies per gram oven-dried soil		
		Corn	Oats	Wheat
June 30	Surface	26,000	48,000	42,000
	2-inch	85,000	64,000	59,000
	4-inch	57,000	63,000	26,000
July 7	Surface	49,000	122,000	47,000
	2-inch	52,000	45,000	60,000
	4-inch	57,000	34,000	46,000
August 18	Surface	72,000	119,000	25,000
	2-inch	63,000	57,000	47,000
	4-inch	66,000	78,000	42,000
September 11	Surface	62,000	142,000	36,000
	2-inch	84,000	92,000	42,000
	4-inch	50,000	27,000	26,000

All their samples were taken at a depth of 2 inches and the same dilution and plating procedure was used as that employed in this work. The total colony count data for this present study are given in table 2. The total colony counts from the oat plot were consistently higher than those from the corn or wheat plots at the surface sampling depth, but were not consistently higher at the 2 and 4-inch sampling depths. It should be noted that the assays of Kommedahl and Brock were made earlier in the growing season than were the assays in this study. This may account for the conflicting data obtained at the 2-inch sampling depth. It seems reasonable that the relative numbers of the kinds of fungi comprising total counts are of more importance in regard to the incidence of root rots than are the total numbers themselves.

DISCUSSION

In the past, several workers have suggested or reported that there are differences in microflora from the same type of soil cropped to different plants. According to a review by Katznelson *et al.* (1948) the Russian worker Zukovskoya reported that potatoes, flax, and clover growing in the same soil enhanced their own specific microflora. Timonin (1941) found differences in the relative numbers of certain

fungi in "artificial rhizosphere" studies with two varieties of flax. Bisby *et al.* (1933) reported that there are definite differences in the mycoflora from the same type of soil cropped to different plants. Katznelson *et al.* (1948) stated ". . . there are suggestions that different plant species or even varieties may favor development of a specific rhizosphere microflora, but much more work is required before this can be regarded as established."

In the study reported in this paper it was found that the mycoflora of each of three plots, continuously cultivated to either corn, oats, or wheat for 38 years, were characterized by differences in the relative frequency of isolation of four major fungi. It is readily admitted that these continuously monocropped plots represent an unusual and unique cropping situation. They also represent the simplist possible cropping sequence, a desirable condition for preliminary investigations of such a complex problem as soil mycoflora studies. Viewed in the light of the continuing research on soil mycoflora by other members of the Department of Botany and Plant Pathology of The Ohio Agricultural Experiment Station, the choice of the simple continuous plots was indeed fortunate at that stage of the research program. The mycoflora of the continuously cropped plots had reached a degree of stability which made the simple techniques used satisfactory; whereas, new techniques (particularily sampling techniques) and methods had to be developed for the study of more complex cropping sequences. (These new techniques as such are not yet published. References to them were made by Williams and Schmitthenner, 1956; Deems, 1956; and Deems and Young, 1956). Furthermore, it should not be inferred that mycoflora under more complex cropping sequences necessarily bear any relation to those found in this study on the monocropped, nitrogen-deficient continuous plots.

Since the completion of this study in 1953, Deems (1956) and Deems and Young (1956) reported that corn, alfalfa, oats, and sugar beets each developed a characteristic mycoflora during one cropping season (the immediate preceding cropping history of the field was two years of sugar beets). The mycoflora were characterized by the relative frequency of isolation of certain fungi from the variously cropped plots rather than the presence or absence of these fungi. The differences in mycoflora were correlated with increase or decrease in the incidence of black root of sugar beets. As well may be expected, considering the contrasting cropping histories, the work of Deems differed in several respects from that reported in this paper. *Trichoderma spp.* were never isolated from oat soil under continuous cropping; whereas Deems found *Trichoderma viride* to be one of the dominant fungi under oats. The dominant fungi from the monocropped oats and corn plots sampled in this study constituted a greater percentage of the total fungi isolated from each of these plots than did the dominant fungi under given crops in the work of Deems.

Of more interest than the differences between this work and that of Deems are the similarities. *Penicillium funiculosum*, the predominant fungus from monocropped corn soil, was also found to be a characteristic dominant fungus under corn by Deems. *Aspergillus fumigatus*, the predominant fungus under monocropped oats, was also found in relatively large numbers under oats by Deems. In regard to these similarities, Menon (1956) studied the effects of temperature, moisture, and cropping on soil mycoflora and reported that the primary factor influencing soil mycoflora was the crop.

In the opinion of the author, which is also shared by Deems (1956), the kinds of fungi and the relative frequency of their isolation under different crops are of more importance in relation to incidence of root rots than are the total numbers of fungi isolated under the various crops. Since the major fungi (those isolated in the largest numbers) generally are found under all the crops, it is essential that quantitative methods be used, such as the soil dilution plate method, instead of qualitative methods since the principal differences in soil mycoflora are quantitative not qualitative with respect to kinds of fungi.

SUMMARY

Soil from three plots cropped continuously since 1915 to either corn, oats, or wheat was assayed by the dilution-plate technique for numbers and kinds of fungi. The fungi were identified to genus, except for the Penicillia and Aspergilli which were identified to series and groups respectively.

From the three plots four kinds of fungi predominated in the isolations; *Aspergillus fumigatus*, *Penicillium funiculosum*, *Trichoderma spp.*, and *Fusarium spp.* Marked differences in the distribution and frequency of isolation of these four fungi were found to characterize the mycoflora under each of the three crops. *Aspergillus fumigatus* was the predominant fungus isolated from the oat plot; *Penicillium funiculosum* was the predominant fungus isolated from the corn plot; whereas, no one fungus predominated in the isolations from the wheat plot; instead, *Aspergillus fumigatus*, *Trichoderma spp.*, and *Fusarium spp.* were approximately equal in frequency of isolation.

These differences in mycoflora under the crops consisted primarily of differences in the relative frequency of isolation of the major fungi rather than to their complete absence (with the exception of *Trichoderma spp.* which were never isolated from the oat plot).

In contrast to the findings of Kommedahl and Brock (1954), the numbers of fungi isolated from the plots at the 2-inch depth were not found to be consistently higher from the oat plot.

ACKNOWLEDGMENT

The writer wishes to express his sincere gratitude to Dr. Thor Kommedahl for his invaluable help in regard to the identification of the fungi and for other assistance.

LITERATURE CITED

Bisby, G. R., N. James, and **M. I. Timonin.** 1933. Fungi isolated from Manitoba soil by the plate method. Can. Jour. Res. 8: 253–275.

Broadfoot, W. C. 1934. Studies on foot and root rot of wheat. III. Effect of crop rotation and cultural practice on the development of foot rot of wheat. Can. Jour. Res. 10: 115–124.

Clark, F. E. 1949. Soil microorganisms and plant roots. Adv. in Agron. 1: 241–288.

Clements, F. E. and **C. L. Shear.** 1931. The Genera of Fungi. 496 pp. H. W. Wilson Co., New York.

Deems, R. E. 1956. Black root of sugar beets as influenced by various cropping sequences and their associated mycofloras. 217 pp. Ph.D. dissertation. The Ohio State University, Columbus, Ohio.

——— and **H. C. Young.** 1956. Black root of sugar beets as influenced by various cropping sequences and their associated mycofloras. Jour. Am. Soc. of Sugar Beet Technologists. 9: 32–43.

Garrett, S. D. 1944. Root Disease Fungi. 177 pp. Chronic Botanica Co., Waltham, Mass.

Gilman, J. C. 1945. A Manual of Soil Fungi. 392 pp. Collegiate Press, Inc., Ames, Iowa.

Katznelson, H., A. G. Lochhead, and **M. I. Timonin.** 1948. Soil microorganisms and the rhizosphere. Bot. Rev. 14: 543–587.

Kommedahl, T. and **T. D. Brock.** 1954. Studies of the relationship of soil mycoflora to disease incidence. Phytopathology 44: 57–61.

Lochhead, A. G. 1952. Soil microbiology. Ann. Rev. Microbiol. 6: 185–206.

Menon, S. K. 1956. Some factors influencing soil mycofloras. 72 pp. Ph.D. dissertation, The Ohio State University, Columbus, Ohio.

Odland, T. E., R. S. Bell, and **J. B. Smith.** 1950. The influence of crop plants on those which follow. V. Rhode Island Exp. Sta. Bull. 309.

Raper, K. B. and **C. Thom.** 1949. Manual of the Penicillia. 875 pp. Williams and Wilkins Co., Baltimore, Md.

Sanford, G. B. 1946. Soil-borne diseases in relation to the microflora associated with various crops and soil amendments. Soil Sci. 61: 9–21.

Simmonds, P. M. 1953. Root rots of cereals II. Bot. Rev. 19: 131–146.

Thom, C. and **K. B. Raper.** 1945. Manual of the Aspergilli. 373 pp. Williams and Wilkins Co., Baltimore, Md.

Timonin, M. I. 1941. The interaction of higher plants and soil microorganisms III. Effect of by-products of plant growth on activity of fungi and actinomycetes. Soil Sci. 52: 395–408.

Tyner, L. E. 1948. Effect of crop debris, plant roots, and crop sequence on the microbial flora of the soil in relation to root rot in cereal crops. Can. Jour. Res. (C) 26: 86–93.

Waksman, S. A. 1952. Soil Microbiology. 356 pp. John Wiley and Sons, Inc., New York.

Warcup, J. H. 1950. The soil-plate method for isolation of fungi from soil. Nature 166: 117–118.

Weindling, R. 1946. Microbial antagonism and disease control. Soil Sci. 61: 23–30.

——— **H. Katznelson,** and **Helen P. Beale.** 1950. Antibiosis in relation to plant diseases. Ann. Rev. of Microbiol. 4: 247–260.

Williams, L. E. and **A. F. Schmitthenner.** 1956. Genera of fungi in Ohio soils. 7 pp. Ohio Agric. Exp. Sta. Res. Circ. 39.