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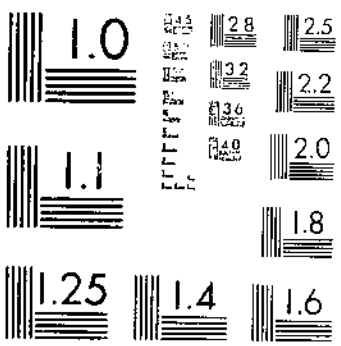

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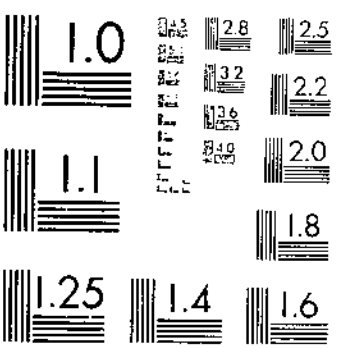

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Experiments Toward the Control of the Take-All Disease of Wheat and the Phymatotrichum Root Rot of Cotton[1]

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INTRODUCTION

Root diseases cause heavy losses of many important crops. So serious are these losses over a half century that Texas alone has at times estimated the annual reduction in value of the cotton crop at many millions. Although the reduction in the value of the wheat crop by take-all cannot be estimated, it has been great enough to stimulate research in many lands and from many angles. These are only two of a long list of parasites that operate below ground. In the last few years, recognition that these root-rot organisms are a part of an exceedingly complex soil population has led to studies of their relation to the normal soil flora, as another line of attack that offers hope of practical results.

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[2] These investigations were conducted under an allotment from the Special Research Fund authorized by Title I of the Bankhead-Jones Act of June 29, 1935. The author acknowledges his indebtedness to Roland B. Mitchell for assistance with studies on the phymatotrichum root rot of cotton; to Charles R. Stumbo for nitrate-nitrogen and available phosphorus determinations; to H. V. Jordan for supervision of and assistance with analysis of data on elimination of viable and killed sclerotia; and to Dalton R. Hooton for cooperation in work at Greenville, Tex. Deep appreciation is expressed to Charles Thom, principal mycologist, and to many others, for valuable suggestions during the course of this work.

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Microbiologists have known for many years that micro-organisms may produce antagonistic substances in culture. Certain of these toxic or inhibitory substances have recently been isolated and described, and they are believed to offer promise in the control of various diseases of man, lower animals, and plants. In soil, however, some of the parasitic fungi capable of destructive attacks upon plant roots are distributed through considerable depths. *Ophiobolus graminis* Sacc., the fungus causing take-all disease of wheat, and *Phymatotrichum omnivorum* (Shear) Dug., commonly known as the cotton root-rot fungus, are two soil-borne parasites in this category. Where the mass of infested soil amounts to several million pounds per acre, the preparation and application of antibiotic or germicidal substances appears impractical. Also it has not thus far been found possible to establish a microbial antagonist in soil by simple inoculation procedures.

The possibility that organic manures can be used to provide soil conditions under which antibiotic effects are obtained through stimulation of components of the saprophytic microflora of the soil is well recognized. King (*17*)[3] and King and Loomis (*19*) have made a practical application of this line of attack in their use of deep placement of manure for field control of the phymatotrichum rot of cotton in the irrigated Southwest. Much earlier, and considerably before the soil-sanitation value of organic manure was assumed to be due largely to the stimulation of saprophytic members of the soil microflora, Pammel (*23*) had recognized the *Phymatotrichum*-infested fields responded favorably to applications of stable manure. Similarly, even before the fungus nature of the take-all disease was indicated, the liberal use of animal manure was observed to give field control (*21*, *33*). The favorable response of take-all infested soils to applications of organic manures is now generally recognized.

Investigations in this laboratory have been directed toward definition of the factors in manurial treatments contributing to soil-sanitation effects and of the conditions that must be established in soil in order to obtain control or elimination of undesirable members of the soil-microbial population. In earlier work, already reported in part (*2*, *3*, *4*, *22*, *30*), some working hypotheses were developed that in turn have determined the course of the current studies.

Organic amendments, although producing striking changes in the soil microflora, do not alter appreciably the microfloras associated with the roots of crop plants (*2*, *4*). This observation suggested that any antagonistic effects that might be occasioned by manuring must be accomplished primarily in the soil during the comparatively unsheltered phase of the parasite's existence. Once the parasite is actually attached to living host tissue, factors of host resistance or factors of virulence of the causal organism are probably more important than microbic activities in determining the amount of host damage, or at least more important than microbic antibiosis, which may be ascribed directly to manurial treatments. This reasoning was believed confirmed when it was shown that for wheat-cropped *Ophiobolus*-infested soil, successful take-all control could be obtained, even though significant differences in microbial numbers were not occasioned, when simply by inorganic fertilization procedures the fertility content of the experimental soil was maintained at levels suitable for good plant

[3] Italic numbers in parentheses refer to Literature Cited, p. 26.

growth (*30*). In view of these results, the statement (*16*) that organic manures are effective in controlling take-all because of their action on the parasite and not because of their benefits to the host plant may not explain accurately the mechanism of control in organic-amended, wheat-cropped soils. That *Ophiobolus*-infested fields so frequently respond favorably to phosphatic fertilization alone suggests in itself that the fertility contributions of organic manures to take-all infested soils should be carefully considered.

Working with another root-rotting parasite, Vanterpool (*38*) has shown the importance of nutritional factors in disease resistance of wheat to pythium seedling blight. It remains unknown whether organic or inorganic amendments that do check take-all damage in wheat-cropped soil actually reduce *Ophiobolus* infestation in soil to the extent that the incidence of the disease is reduced or eliminated when that soil is again cropped to wheat.

The possibility has been raised that in the absence of the host plant, unbalanced soil-nutrient conditions, such as nitrogen deficiency, may actually be desirable for soil sanitation. Garrett (*11*, *12*) has reported that it is characteristic of organic manures which control take-all that they contain insufficient or just sufficient nitrogen for their decomposition. After showing that the viability of *Ophiobolus graminis* in infested stubble buried in soil was reduced most markedly by the application of low-nitrogen amendments, he suggested that an inadequate nitrogen supply was possibly desirable from the standpoint of soil sanitation, in that it encouraged competition among soil micro-organisms for nitrogen and in this manner led to the elimination of the parasite. In this work, it must be remembered that Garrett was dealing with the parasite in the absence of the living host.

Mitchell, Hooton, and Clark (*22*), working on the phymatotrichum rot of cotton, obtained considerable support of the hypothesis that a soil-borne parasite can be most effectively attacked by a rotting process when the soil is free from living-host tissue. When known numbers of sclerotia of *Phymatotrichum omnivorum*, were buried in experimental soils, greater numbers of sclerotia were eliminated from soil amended with organic material than from soil receiving no such amendment. This elimination of sclerotia was obtained with such materials as stable manure, which possesses a relatively narrow carbon-nitrogen ratio. No information was presented as to whether, in further exploitation of Garrett's work, materials with wide carbon-nitrogen ratios would have been even more effective. Amended soils were shown to contain considerably higher populations of saprophytic micro-organisms, and it was assumed, although not demonstrated with certainty, that the increased sclerotial elimination observed was due to microbial interactions or antibioses. More exact definition of the conditions most favorable for the destruction of root parasites during the soil-borne phase of their existence remains an important objective in soil sanitation. Sclerotia of *P. omnivorum* offer suitable material for studying factors affecting the persistence or viability of a root parasite in soil. Such sclerotia can be produced in unlimited number in laboratory-soil culture, and these bodies may be added to soil variously amended or maintained, subject to subsequent recovery and viability tests. As sclerotia are known to persist in field soil for many years and to offer continuing centers of infestation for subsequent crops, the desirability

of defining conditions that affect the persistence or viability of sclerotia in soil is apparent.

There also remains the question of whether conditions on root surfaces may be so changed by plant treatment that the parasite is eliminated more quickly. Many workers have observed that *P. omnivorum* can persist on cotton roots that it has parasitized for varying periods, but that it gradually disappears from or dies with the roots to which it is attached. Rea (*25*) considered food exhaustion a possible explanation for this phenomenon of fungus disappearance. Ezekiel (*8*) noted that decreased survival percentages of *P. omnivorum* can be obtained following plant injury, and that, coincidentally, there is a reduction in alcohol-soluble solids contained in the roots. These results suggest also that food exhaustion or changes in root composition are limiting factors for survival of the parasite. Noting that when *Phymatotrichum*-infected roots were placed in an environment of intensified microbial activity the parasite was quickly eliminated, Mitchell, Hooton, and Clark (*22*) emphasized the influence of associated microbial activities on fungus survival. As cotton roots injured during the late summer or early fall showed pronounced increases in the micropopulations associated with root surfaces, a partial explanation appeared possible for the observation of Adams et al. (*1*) that early-fall plowing of cotton stalks reduced the incidence of root rot in the cotton crop of the following year.

It is becoming apparent that no single explanation is adequate to account for all of the phenomena attendant with the appearance or control of root-rot diseases. The attractiveness of the idea that the micropopulation of the soil is an asset that can be directed toward the decomposition of an intensely destructive parasite can be realized only through thorough knowledge of many component factors within a major problem. The recognition in recent years of the importance of soil-borne and host-borne stages of root-infecting fungi and of the influence of physical and micro-organic factors upon the persistence or virulence of parasitic fungi within the soil environment have been worthy contributions. Continued advances have been made in knowledge of the ecology and physiology of root-rotting parasites and in their attendant morphological and pathogenic phenomena. It is the purpose of this bulletin to consider some of the specific contributions of manurial and tillage treatments to soil sanitation and to define certain interrelationships between saprophytic micro-organisms of the soil and the parasitic fungi concerned in the ophiobolus rot of wheat and the phymatotrichum rot of cotton.

NUTRITIONAL FACTORS IN THE CONTROL OF TAKE-ALL DISEASE OF WHEAT BY DECOMPOSING ORGANIC MATTER

It was repeatedly observed, during the course of 4 years of study, that liberal fertilization of *Ophiobolus*-infested soil with chicken manure or chopped alfalfa provided take-all control, as reported by Fellows (*9*), whereas materials of wide carbon-nitrogen ratios, such as wheat straw, failed to provide control. Following the demonstration of successful take-all control entirely by means of inorganic fertilizations (*30*), it was considered desirable to compare the fertility contributions to soil of certain organic materials differing in their effec-

tiveness for take-all control. Accordingly, the following organic materials were added to an *Ophiobolus*-infested silt loam at the rate of 1 part by volume to 5 parts of soil: chicken manure, chopped green alfalfa, barley and oat kernels mixed in equal parts and boiled, horse manure, and crude potato flour. These materials, when applied at such a heavy rate (*9*), have been tested for take-all control. The order of their effectiveness has been judged approximately as that in which they are listed; chicken manure provides excellent control, and potato flour little or no control.

Treated soil lots, together with an unamended check, were potted in duplicate in half-gallon glazed earthenware jars and then maintained under greenhouse conditions for a period of 15 weeks at optimum moisture content. At weekly intervals for 7 weeks, and after 15 weeks, soil cores were removed for determinations of nitrate-nitrogen and available-phosphorus content. Total numbers of micro-organisms were determined after intervals of 1, 4, and 10 weeks. The laboratory techniques employed have been summarized elsewhere (*3, 30*). Available-phosphorus content of soil samples was determined according to the method of Truog (*37*), and nitrate nitrogen by the phenoldisulfonic acid method as modified by Harper (*13*). As Kansas soils are generally rich in potassium (*36*) and as it had already been shown that *Ophiobolus* control could be achieved in such soil by adequate phosphoric and nitrogenous fertilization, determination of soil potassium content was not undertaken.

A summary of the laboratory data obtained is presented in table 1.

TABLE 1. *Nitrate nitrogen, available phosphorus, and microbial numbers in soil following application of organic manures*

[Manures are arranged in descending order of their effectiveness for take-all control]

NITRATE NITROGEN

Organic material added	Content after differing periods of incubation following application of soil amendment							
	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks	After 6 weeks	After 7 weeks	After 15 weeks
	P. p. m.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
Chicken manure	0.0	0.0	25.4	28.7	58.9	77.8	114.7	670.8
Chopped alfalfa	0	0	13.3	63.3	108.5	132.5	163.5	625.0
Barley and oats	0	0	2.9	4.4	32.9	74.0	166.5	405.1
Horse manure	0	0	0	4.4	14.1	14.9	29.4	81.0
Potato flour	0	0	0	0	0	0	0	0
None (control soil)	8.5	7.2	10.0	13.3	13.6	15.0	13.4	46.0

AVAILABLE PHOSPHORUS

Chicken manure	115.3			123.9			129.3	
Chopped alfalfa	42.5			36.7			38.3	
Barley and oats	37.2			52.4			69.7	
Horse manure	69.2			78.0			73.7	
Potato flour	43.0			23.7			16.2	
None (control soil)	23.2			24.1			21.4	

BACTERIA

	Number [1]	*Number* [1]	*Number* [1]	*Number* [1]	*Number* [1]	*Number* [1]	*Number* [1]	*Number* [1]
Chicken manure	3,215			1,543			[2] 636	
Chopped alfalfa	2,063			2,267			[2] 147	
Barley and oats	5,950			4,800			[2] 218	
Horse manure	2,467			903			[2] 170	
Potato flour	10,000			7,155			[2] 5,300	
None (soil only)	110			91			[2] 58	

[1] Millions per gram of air-dry soil.

[2] At 10 weeks.

From these data, it is apparent that those materials noted as most valuable for take-all control are the ones that contribute most heavily to the nitrate-nitrogen and available-phosphorus supply in soil. On the other hand, the greatest increase in microbial number was occasioned by potato flour, which is not effective in the control of *Ophiobolus*.

In an effort to determine why organic matter is most effective in depressing phytopathogenic fungi during the period of its most active decomposition, comparison was made of the microbial and soil-fertility contributions of fresh and partially composted manures when added to wheat-cropped soil. Fresh chicken manure and chopped alfalfa tops were dried and finely ground, and portions thereof were stored dry for 3 months and then added to naturally infested soil at the rate of 6.6 percent by weight. Meanwhile, duplicate portions of these same two materials were composted in large glass dishes for 3 months in the absence of soil at 26° C., and with sufficient water being added as necessary to keep each material in a thick, pasty condition. These compost residues were then dried, ground, and added to naturally infested soil at a rate equivalent to 6.6 percent by weight of dried fresh material. All experimental soil lots, together with untreated check lots, were then cropped to wheat. Unglazed 6-inch clay pots, each containing 1,400 gm. of soil, were employed. Two wheat plants were allowed to grow in each pot. Pots were maintained in the greenhouse for 190 days, with water being added as necessary to maintain the soil at approximately 60 percent of its moisture-holding capacity.

During the first 6 weeks of crop growth, both the fresh-manured and compost-manured soil lots supported vigorous and apparently healthy plants. But by 2 months after planting, compost-manured, as well as untreated, soil showed plants with take-all symptoms. A comparison of plant growth in soil receiving fresh chicken manure and in compost-manured soil is shown in figure 1.

Soil analyses from samples removed 30 days and 70 days after planting of wheat showed available-phosphorus levels adequate in both series of amended soils. At the first sampling, the soils treated with fresh materials showed considerably higher microbial populations than did the soils amended with composted materials, but at 70 days, the total microbial number in both series was similar. At this time all amended soils showed microbial populations several times greater than those of the check soils (table 2). However, at about the time of onset of distinct take-all damage, differing influences of composted and fresh organic material became manifest in nitrate-nitrogen accumulations. At 70 days, nitrate nitrogen was virtually absent from all soil lots except those receiving fresh organic materials. Take-all damage, apparently, followed depletion of nitrate nitrogen, regardless of whether soil-microbial populations were at high or low levels.

After 190 days of growth, wheat plants were removed and the root systems washed clean of soil. Observations on the extent of disease at this time, together with average weight of plant material per pot, are also given in table 2.

These results, together with the data (table 1) obtained from study of uncropped soil, suggested that organic manures when applied to wheat-cropped soil are valuable for take-all control to the extent that

they provide for good plant nutrition, regardless of their effect on or their stimulation of the saprophytic microflora of the soil. Before evidence is presented that control obtained in the presence of the host plant does not necessarily mean that the parasite is eliminated from the soil, attention is called briefly to the following observation.

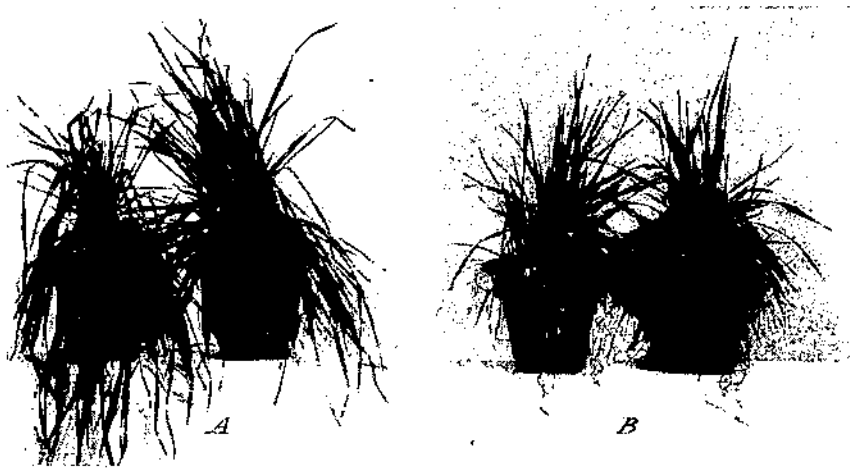

FIGURE 1.—Wheat plants grown in soil naturally infested with *Ophiobolus graminis*: *A*, Fresh chicken manure added at seeding time; *B*, composted chicken manure added at seeding time.

TABLE 2.—*Total microbial number and nitrate-nitrogen content in fresh-manured and compost-manured soil lots after 30 days and 70 days of crop growth, and severity of take-all disease at plant maturity*

Organic material added	After 30 days		After 70 days		At plant maturity		
	Bacteria	Nitrate nitrogen	Bacteria	Nitrate nitrogen	Top growth (dry weight)	Root growth (dry weight)	Severity of take-all disease
	Number[1]	*P. p. m.*	*Number*[1]	*P. p. m.*	*Grams*	*Grams*	
Fresh chicken manure	2,346	27.1	598	165.5	15.30	8.80	None.
Fresh alfalfa tops	3,875	60.6	1,320	215.8	22.30	4.00	Slight.
None (check soil A)	115	10.3	123	1.6	2.05	.35	Very severe.
Composted manure	606	181.7	780	1.8	23.07	2.68	Moderately severe.
Composted alfalfa	961	18.6	981	.5	20.05	1.80	Severe.
None (check soil B)	66	5.9	93	.5	3.55	.90	Very severe.

[1] Millions per gram of air-dry soil

Nine kilograms each of two *Ophiobolus*-infested soils were potted in each of two large containers. These were brought to optimum moisture, and one wheat plant was allowed to grow in each container. Three-kilogram portions of these same soil lots were potted in each of four containers, and in each of these three wheat plants were allowed to grow. Wheat established in all containers had previously been vernalized 30 days at 2° C. After transfer to soil, such wheat was allowed to grow 110 days after vernalization. Sufficient illumination was employed to provide a daily plant illumination period in the greenhouse of 16 hours. At no time was any differential soil treat-

ment employed between the large and small-container series. In the two series, markedly superior wheat developed in the larger pots; upon removal, such plants showed considerably less take-all injury than did those plants grown under more crowded conditions. Representative root systems obtained from the two series are shown in figure 2.

FIGURE 2.—Root systems of wheat grown in differing volumes of naturally infested soil: *A*, Root system of wheat plant grown in 1 kg. of soil; *B*, root system of wheat plant grown in 9 kg. of soil.

PERSISTENCE OF OPHIOBOLUS GRAMINIS IN WHEAT-CROPPED, FERTILIZED SOIL, AND ITS ELIMINATION FROM UNCROPPED SOIL

Ophiobolus-infested soil lots in which wheat had been grown successfully in the greenhouse, following either (a) adequate inorganic phosphatic and nitrogenous fertilization or (b) manurial treatment with effective organic materials, such as chicken manure or chopped green alfalfa, were employed, without further amendment, in subsequent

experiments in order to determine whether *O. graminis* had disappeared from such soil lots.

Immediately upon removal of the first wheat plants grown in any given soil lot, the soil was air-dried, and then maintained in that condition until it was again potted. In repotting, unglazed 3-inch pots were employed, and wheat was seeded at the time the soil in these containers was brought to optimum moisture content. Eight to ten seedlings were allowed to grow in each pot of soil. Such extreme overcrowding of seedlings in a small volume of soil provided almost ideal conditions for disease development in the event that any *O. graminis* was still present. Severity of take-all disease was rated according to observed damage on roots, crowns, and culms, and for plants allowed to go to maturity, by the extent of tillering and heading and by the oven-dried weights of root and top material.

The following soil lots were repotted in the manner described and tested for the persistence of *O. graminis:* (a) Silt loam fertilized during the time of the first cropping with 1,000 p.p.m. superphosphate and with 976 p.p.m. nitrogen, applied as potassium nitrate and ammonium nitrate; (b) silt loam, fertilized during the first cropping with 200 p.p.m. superphosphate and 240 p.p.m. nitrogen added as ammonium nitrate; (c) soil fertilized immediately prior to first cropping with 6.6 percent by weight of dried fresh chicken manure; (d) as above, but using dried chopped alfalfa tops; and (e) check or untreated soil lots in which the first wheat plants grown had shown severe take-all damage.

All wheat plants grown in these soils following this repotting were found severely damaged by take-all disease. *O. graminis* was not eliminated from soil in which wheat had been grown successfully following adequate soil fertilization.

The disappearance of take-all infestation from uncropped soil kept moist in the greenhouse has been noted by Fellows[4]. An elimination of *O. graminis* from artificially infested wheat stubble buried in soil amended with organic material possessing a wide carbon-nitrogen ratio has been reported by Garrett (*11*). The following experiment was conducted so that the elimination of *O. graminis* from naturally infested soil maintained under differing soil fertility and microbiological conditions and in the absence of the host plant might be considered.

One half of a naturally infested clay loam was divided into 15 portions of 1,400 gm. each. These portions potted in glazed jars and treated initially, as shown in table 3, were maintained for 3 months at optimum moisture content and under temperature conditions favoring optimal bacterial activity. The remaining half of the soil lot was stored air-dry during this period, and then aliquot portions were given equivalent treatments and brought to optimum moisture content. Wheat was seeded in this series 4 days later, at the same time that the lots maintained moist for 3 months were seeded to wheat. Three plants were allowed to grow in each jar.

Soil samples collected 4 days and 90 days after application of amendments but prior to seeding of wheat showed that differences both in

[4] Personal communication in the files of the Division of Soil and Fertilizer Investigations.

microbial numbers and in available phosphorus and nitrate-nitrogen content were established by the treatments employed. The data accumulated suggested that with certain treatments (Nos. 8 and 13, and possibly also 11, table 3) adequate nitrate nitrogen was present both at the beginning and at the end of the fallow period. Subsequent soil analyses during the wheat growing period showed differences between treatments both in microbial numbers and in soil-nutrient conditions for several months following seeding of wheat. After 94 days, however, by reason of plant growth of wheat and small volume of soil per plant, all soils were devoid or nearly devoid of nitrate nitrogen, and thereafter all wheat showed the stunting and yellowing of top growth characteristic of nitrogen starvation.

TABLE 3.—*Initial soil treatments employed, and microbial number, nitrate nitrogen, and available phosphorus in soil 4 days and 90 days after application of amendments but prior to seeding of wheat*

Soil No.	Initial treatments			After 4 days			After 90 days		
	Wheat straw	Superphosphate	Potassium nitrate	Bacteria	Nitrate nitrogen	Available phosphorus	Bacteria	Nitrate nitrogen	Available phosphorus
	P. p. m.	*P. p. m.*	*P. p. m.*	*Number*[1]	*P. p. m.*	*P. p. m.*	*Number*[1]	*P. p. m.*	*P. p. m.*
1				103.8	13.7	23.1	40.9	76.4	17.7
2	5,000			272.5	0	17.5	121.8	74.5	21.8
3	5,000	50		210.8	0	33.6	81.4	76.4	38.5
4	5,000	100		297.8	0	41.3	106.5	58.2	30.1
5	5,000	300		397.8	0	105.2	110.3	85.1	66.2
6	5,000	50	50	508.0	0	27.0	101.2	102.3	32.2
7	5,000	100	100	360.0	0	33.9	75.6	107.0	35.7
8	5,000	300	300	248.0	43.2	83.3	73.2	91.0	75.7
9	5,000		50	335.0	0	17.9	79.4	76.4	18.3
10	5,000		100	360.0	0	15.9	81.8	131.0	19.4
11	5,000		300	396.5	25.9	22.5	91.7	76.2	24.2
12	10,000			260.5	0	20.0	130.3	122.8	18.3
13		100	100	(2)	21.3	36.7	73.2	153.8	21.0
14		300	300	151.4	62.5	116.2	63.2	102.5	48.5
15				154.4	10.3	18.4	51.6	(2)	21.7

[1] Millions per gram of air-dry soil.
[2] Lost.

Plants were removed from soil Nos. 1, 4, 7, and 10 after 98 days of growth, from Nos. 3, 5, 8, 9, 12, 14, and 15 after 138 days, and from Nos. 2, 6, 11, and 13 after 167 days. All plants removed from soil that had been maintained moist for 3 months were uniformly free from disease, whereas plants from soil lots correspondingly fertilized but not maintained moist for 3 months prior to seeding were severely damaged by *O. graminis*. Representative root systems from two soil treatments in each series are shown in figure 3.

Following removal of plants from soils Nos. 3, 5, 8, 9, 12, 14, and 15 in the series that had been maintained moist for 3 months prior to seeding, sufficient soil was recovered from each container to fill two unglazed 3-inch clay pots. These were again cropped to wheat, and 8 to 10 wheat plants were allowed to grow in each pot. Seedlings removed 53 days later were found entirely free of take-all lesions, whereas seedlings removed from soil lots not maintained moist for any

considerable period while devoid of susceptible roots were severely damaged by *O. graminis*.

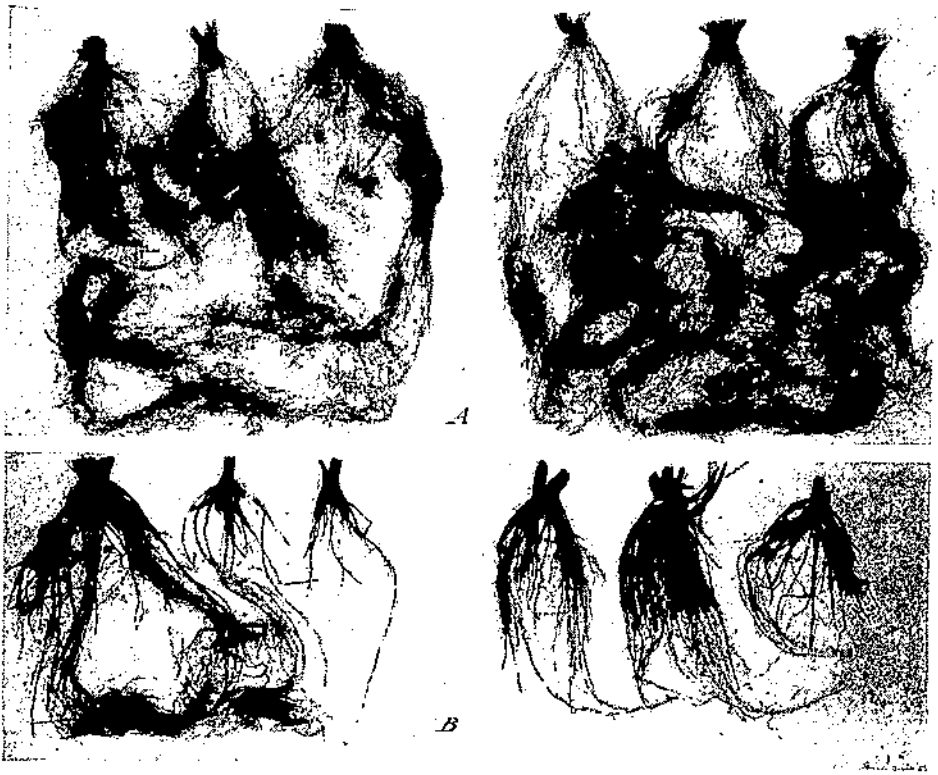

FIGURE 3.—Root systems of wheat grown in soil naturally infested with *Ophiobolus graminis*: *A*, Soil maintained moist for 3 months prior to seeding with wheat; *B*, soil maintained moist for only 4 days prior to seeding with wheat.

FACTORS AFFECTING THE PERSISTENCE OR VIABILITY OF PHYMATOTRICHUM OMNIVORUM SCLEROTIA IN SOIL

A more complete study of the factors affecting the destruction of a parasitic fungus in soil devoid of susceptible roots was undertaken with sclerotia of *Phymatotrichum omnivorum*. Especial attention was given to the increased destruction of such sclerotia in organic-amended soil. The ability of *Phymatotrichum* sclerotia to persist in soil under natural conditions is well established. Rogers (*34, p. 204*) found at least two-thirds of the number of sclerotia buried for 38 months at depths of 1, 2, and 3 feet viable upon recovery. Both King and Eaton (*18*) and Taubenhaus and Ezekiel (*32*) have noted the effects of differing moisture contents on persistence of sclerotia in

laboratory soil. That viability of sclerotia is destroyed in field soil receiving heavy manurial treatment was suggested by King.[5] Sclerotia found in soil from untreated or check field plots were viable almost without exception; sclerotia obtained from manured plots were seldom viable. Subsequently, Mitchell, Hooton, and Clark (22), working with known numbers of sclerotia added to experimental soils, demonstrated convincingly that greater numbers of sclerotia are eliminated from organic-amended than from unamended soil.

The sclerotia employed in these investigations were produced in laboratory soil culture (7). Soil cultures employed for sclerotia production consisted of (a) 100 gm. aliquots of Hunt clay sieved 20-mesh and placed in 300-milliliter Erlenmeyer flasks; (b) 5 gm. of either milo seed or navy beans added to the surface of the soil; and (c) sufficient water to adjust the soil moisture to 65 percent of its moisture-holding capacity. After soil flasks had been cotton-stoppered and autoclaved sterile, each was inoculated with an agar disk from a stock culture of *Phymatotrichum omnivorum*. Soil cultures were incubated at 28° C. Although sclerotia formation was usually apparent by 10 or 15 days, flasks were routinely incubated for approximately 45 days in order that sclerotia could become well-matured. The final sclerotia yields from several flasks were combined to provide gross lots from which sclerotia could be selected for burial in soil.

All the soil employed was taken from the top 2 to 4 inches of fields in clean cultivation. Types of organic material used as soil amendments, rates of application, and conditions of incubation are given for each separate experiment. Immediately upon addition of sclerotia to an experimental soil the moisture content was adjusted to the desired level, so that no desiccation of sclerotia was permitted.

After incubation, sclerotia-loaded soils were washed on a 40-mesh screen, and the number of sclerotia surviving incubation was determined. Viability of recovered sclerotia was determined by incubation on moistened filter paper in Petri-dish containers.

Microbial and Other Factors in the Elimination of Sclerotia from Hunt Clay Amended with Corral Manure

The initial experiment was designed to consider the elimination of viable and killed sclerotia under sterile and nonsterile conditions of incubation in soil receiving corral manure at differing rates of application or with differing supplemental inorganic fertilizations. Corral manure was added to Hunt clay at rates of 1.0, 3.0, and 5.0 percent by weight (dry basis). Aliquots of all manured soil lots as well as soil receiving no manure were also employed with supplemental inorganic fertilizations as follows: (a) ammonium nitrate, applied at a rate of 0.125 percent; (b) dipotassium phosphate, applied at a rate of 0.135 percent; and (c) ammonium nitrate and dipotassium phosphate in combination, each applied at the same rate as when used singly.

From a lot of laboratory-grown sclerotia, shown to be 100 percent viable, 25,600 sclerotia of generally uniform size and appearance were selected. Half of this number were killed by pasteurization

[5] Personal communication in the files of the Division of Soil and Fertilizer Investigations.

at 85° C. for 10 minutes. From both the viable and killed lots, 6,400 sclerotia were selected for surface sterilization in bichloride of mercury solution (1:1,000), following which they were washed thoroughly in sterile water, and thereafter maintained in an uncontaminated condition. It was found that uncontaminated sclerotia could readily be obtained by this method and that no loss of sclerotial viability was occasioned.

Thirty-two Petri dishes, each containing 70 gm. of soil, were prepared for each of 16 experimental soil lots. One-half of the number of containers prepared for each soil lot were autoclaved sterile, and these were kept uncontaminated thereafter. Such containers received either uncontaminated viable or uncontaminated killed sclerotia. The remaining 256 containers were established with unsterilized soil, and these received sclerotia that had not been given surface sterilization. Fifty sclerotia were added to each container. Moisture was added to 57 percent of moisture-holding capacity. All containers were incubated at 28° C. Incubation periods of 10, 20, 30, and 40 days were employed prior to washing for sclerotia recovery.

An outline of the soil amendments and incubation conditions employed is given in table 4, together with the number of sclerotia

TABLE 4.—*Elimination of viable and killed sclerotia from variously amended soils under nonsterile and sterile conditions of incubation*

EXPERIMENTAL DATA

Treatment	Replicates	Sclerotia eliminated after various incubation periods (days) under nonsterile conditions								Sclerotia eliminated after various incubation periods (days) under sterile conditions							
		Viable sclerotia				Killed sclerotia				Viable sclerotia				Killed sclerotia			
		10	20	30	40	10	20	30	40	10	20	30	40	10	20	30	40
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Unamended Hunt clay	a	3	8	15	5	42	45	46	47	7	4	9	3	7	7	4	11
	b	5	7	17	9	38	50	45	46	6	7	3	6	2	2	4	3
Hunt clay plus N	a	7	1	8	9	45	44	46	44	7	10	7	8	4	3	7	5
	b	7	8	9	23	40	43	34	49	6	0	3	6	3	8	4	9
Hunt clay plus P, K	a	9	15	13	10	46	48	49	46	3	3	6	4	4	4	3	7
	b	7	10	12	9	42	42	45	49	7	4	7	4	6	0	7	1
Hunt clay plus N, P, K	a	8	11	18	16	42	43	42	46	9	8	7	6	0	8	9	10
	b	14	15	10	21	40	39	46	44	2	13	2	1	5	8	4	2
Hunt clay plus 1 percent manure	a	5	9	18	20	38	44	47	46	4	9	6	3	3	10	9	1
	b	6	7	12	24	31	45	43	48	3	3	0	4	7	10	11	4
Hunt clay plus 1 percent manure, plus N	a	4	10	16	26	40	47	48	43	4	10	3	9	4	7	4	6
	b	6	13	13	21	47	43	46	44	4	2	7	6	9	6	12	2
Hunt clay plus 1 percent manure, plus P, K	a	6	13	11	18	33	45	49	47	0	2	8	9	1	4	9	10
	b	7	9	18	23	39	48	41	50	1	0	3	3	9	10	0	10
Hunt clay plus 1 percent manure, plus N, P, K	a	5	15	17	29	39	44	46	50	0	11	0	6	2	1	5	11
	b	8	9	23	31	39	46	50	49	4	3	6	7	5	9	7	8
Hunt clay plus 3 percent manure	a	3	18	22	23	33	47	47	48	4	5	10	7	8	11	8	0
	b	4	21	21	26	31	43	44	48	1	0	5	8	4	2	6	9
Hunt clay plus 3 percent manure, plus N	a	16	20	23	31	39	46	46	47	3	7	3	3	5	9	7	6
	b	11	19	26	29	38	45	49	50	4	3	9	4	5	7	7	3
Hunt clay plus 3 percent manure, plus P, K	a	7	14	20	26	32	45	48	46	10	10	5	2	9	4	8	7
	b	4	19	18	26	32	45	45	49	1	5	5	10	8	10	3	8
Hunt clay plus 3 percent manure, plus N, P, K	a	17	20	29	30	32	43	45	45	0	9	7	5	7	11	4	4
	b	13	24	33	33	29	46	45	49	7	3	12	5	3	0	4	9
Hunt clay plus 5 percent manure	a	7	33	26	31	34	49	47	43	5	13	1	3	7	6	10	7
	b	9	17	39	36	36	45	48	48	4	8	9	9	1	7	3	8
Hunt clay plus 5 percent manure, plus N	a	10	18	33	39	31	44	46	46	9	9	3	7	5	8	9	2
	b	9	26	38	35	42	50	50	45	10	5	8	8	7	3	7	0
Hunt clay plus 5 percent manure, plus P, K	a	11	18	29	24	30	46	44	49	3	6	12	9	3	4	11	8
	b	5	23	26	39	41	47	49	47	4	2	6	4	5	4	3	4
Hunt clay plus 5 percent manure, plus N, P, K	a	21	24	33	40	34	49	50	48	1	3	2	0	8	6	11	9
	b	16	31	41	46	36	49	50	46	9	3	15	9	5	7	4	5

TABLE 4.—*Elimination of viable and killed sclerotia from variously amended soils under nonsterile and sterile conditions of incubation*—Continued

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square	*F* value			Recapitulation of percentages of sclerotia destroyed
			Found	Required		
				0.5	0.1	
Total	511					
Incubation conditions:						
Nonsterile vs. sterile	1	81,003.13	6,700.01**	3.87	6.72	61.7 vs. 11.4
Viable vs. killed in nonsterile	1	44,047.52	3,643.30**	3.87	6.72	35.5 vs. 87.9
Duration in viable nonsterile:						
10 days vs. 20 days	1	870.25	71.98**	3.87	6.72	16.9 vs. 31.6
30 days vs. 40 days	1	228.77	18.92**	3.87	6.72	42.9 vs. 56.8
10 to 20 days vs. 30 to 40 days	1	4,038.76	334.00**	3.87	6.72	24.3 vs. 46.7
Duration in killed nonsterile:						
10 days vs. 20 days	1	1,118.89	92.13**	3.87	6.72	74.4 vs. 91.0
30 days vs. 40 days	1	12.25	1.01	3.87	6.72	92.3 vs. 94.0
10 to 20 days vs. 30 to 40 days	1	855.95	70.80**	3.87	6.72	82.8 vs. 90.6
Viable vs. killed in sterile	1	5.06	----------	3.87	6.72	11.1 vs. 11.7
Duration in viable sterile:						
10 days vs. 20 days	1	19.14	1.58	3.87	6.72	9.4 vs. 11.6
30 days vs. 40 days	1	3.52	----------	3.87	6.72	12.2 vs. 11.3
10 to 20 days vs. 30 to 40 days	1	11.28	----------	3.87	6.72	11.5 vs. 11.7
Duration in killed sterile:						
10 days vs. 20 days	1	9.77	----------	3.87	6.72	10.3 vs. 11.9
30 days vs. 40 days	1	3.52	----------	3.87	6.72	12.8 vs. 11.8
10 to 20 days vs. 30 to 40 days	1	11.28	----------	3.87	6.72	11.1 vs. 12.3
Fertilizers (inorganic):						
Nitrogen	1	168.82	13.96**	3.87	6.72	37.7 vs. 35.9
Phosphorus and potassium	1	29.07	2.40	3.87	6.72	37.5 vs. 36.1
Nitrogen × phosphorus and potassium	1	22.78	1.88	3.87	6.72	37.0 vs. 36.6
Fertilizers (manure):						
0 percent vs. 5 percent	1	1,126.44	93.17**	3.87	6.72	32.8 vs. 41.2
1 percent vs. 3 percent	1	93.55	7.76**	3.87	6.72	35.0 vs. 37.4
0 to 5 percent vs. 1 to 3 percent	1	20.32	1.68	3.87	6.72	37.0 vs. 38.1
Inorganic fertilizers × manure	9	7.03	----------	1.90	2.46	----------
Incubation conditions × fertilizers (inorganic)	45	23.39	1.93**	1.42	1.64	----------
Incubation conditions × manure	45	131.44	10.87**	1.22	1.32	----------
Incubation conditions × fertilizers × manure	135	4.65	----------	----------	----------	----------
Error	256	12.09	----------	----------	----------	----------

**Highly significant.

per container, out of the initial 50 added, that could not be recovered. Data of table 4 have been subjected to statistical analysis in order to determine significant differences due to amendment or incubation conditions employed. A summary of an analysis for variance is given. In the last vertical column of this section of the table there is also included a recapitulation of the percentages of sclerotia destroyed under differing experimental conditions.

From these data, it is apparent that the activity of the saprophytic microflora is essential for the destruction of *P. omnivorum* sclerotia in artificially infested, organic-amended soil. Uncontaminated viable sclerotia survived in sterile, organic-amended soil equally as well as in sterile, unamended soil. In short, not the organic material itself but the accompanying microbial activity was detrimental to sclerotia.

ELIMINATION OF SCLEROTIA FROM SOIL BY WIDELY DIFFERING TYPES OF ORGANIC MATERIALS

The slightly greater effectiveness of manure with added nitrogen over manure without added nitrogen as an amendment for the elimination of sclerotia from soil suggested that materials of narrow

carbon-nitrogen ratios might prove more valuable for rotting out sclerotia than would organic materials of extremely wide ratios. Although earlier work had shown that several different materials (namely, manure, chopped sorghum fodder, chopped cotton roots, cottonseed meal or hulls, and ground alfalfa hay) could be used to obtain destruction of sclerotia in soil (*22*), the possibility remained that more extensive comparisons might reveal a class or type of material of superior value for soil-sanitation purposes. To this end, an experiment was established in which differing rates of application of each of a series of organic materials were compared in Hunt clay, Wilson sandy loam, and washed sand.

The following materials were employed as soil amendments: Starch, cellulose, ground wheat straw, chopped sorghum fodder, chopped cotton roots, ground alfalfa hay, barnyard manure, and commercial peptone. These materials were added to soil or to washed sand at rates of 0.5, 1.0, 3.0, and 5.0 percent. Temperatures of incubation, moisture contents, shape of container, and amount of soil per dish were the same as in the initial experiment (pp. 12 and 13). Each of the 108 containers established were given 30 viable sclerotia, and all washings for sclerotia recoveries were made after 31 days.

No significant differences in relative effectiveness of the several organic materials for destruction of sclerotia were obtained. Destruction of sclerotia appeared comparable in Hunt clay and Wilson sandy loam, but slightly poorer elimination was observed in washed sand. In agreement with the results reported in table 4, increasing rates of application of organic materials were again found to be increasingly destructive to sclerotia (table 5).

TABLE 5. *Average percentage elimination of viable sclerotia from Hunt clay, Wilson clay, Wilson sandy loam, and washed sand, following amendment with organic materials at differing rates of application*

Rate of application of organic materials	Elimination of viable sclerotia from—		
	Hunt clay	Wilson sandy loam	Washed sand
	Percent	*Percent*	*Percent*
None (unamended check)	18.4	16.7	8.3
0.5 percent	25.9	28.0	17.2
1.0 percent	35.7	37.1	26.3
3.0 percent	43.1	39.5	38.5
5.0 percent	55.3	52.5	43.8

In subsequent work in which pure cellulose, wheat straw, crimson clover tops, hairy vetch tops, sorghum fodder, stable manure, and commercial peptone were employed as organic amendments in Hunt clay, such widely differing materials as cellulose and peptone were again noted to provide for comparable destruction of sclerotia. After incubation at 30° C. for 22 days and with each amendment applied at a 3.0 percent rate, the following percentages of viable sclerotia were eliminated:

Treatment:	*Percent eliminated*
Unamended soil	22.6
Cellulose added	81.4
Wheat straw added	71.4

Treatment—Continued.	Percent eliminated
Clover tops added	82.6
Vetch tops added	82.0
Chopped sorghum	35.1
Manure added	32.0
Peptone added	85.4

In this comparison the lots of chopped-sorghum fodder and stable manure employed provided only slightly greater destruction of sclerotia than that provided by the check soil. Other lots of chopped-sorghum fodder and of manure have been found highly effective in the course of previous work (*22*). The reason for discrepancies between separate experiments in which different lots of organic material have been employed is not immediately apparent, but work reported in subsequent paragraphs suggests that conditions of incubation can markedly affect the results to be obtained with a single lot of organic material. Some results recorded (see table 8) show that under more favorable conditions of incubation, the amount of stable manure and sorghum fodder employed above can markedly reduce the sclerotial infestation of soil.

Influence of Temperature of Incubation on Elimination of Sclerotia

In an experiment designed to study the effect of temperature of incubation on the elimination of sclerotia from soil, 100-gram aliquots of Hunt clay were employed in narrow-mouth flint-glass bottles of 8-ounce capacity. Half of the containers prepared were fertilized with finely ground dry alfalfa tops applied at a rate of 2 percent. Each container then received 50 sclerotia, from a lot shown to be 100 percent viable, and soil water contents were adjusted to 60 percent of moisture-holding capacity. Two containers each of both unamended and amended soil were incubated at each of the following temperatures: 2°, 12°, 28°, and 35° C.

After 30 days all containers were examined for numbers of sclerotia still remaining, and viability of sclerotia recovered was tested in moist-chamber incubations. Percentages of viable sclerotia recovered from soil lots incubated at differing temperatures are shown in table 6.

The marked differences in numbers of sclerotia destroyed in organic-amended soil lots incubated at differing temperatures again make it appear that not the organic matter itself, but the soil conditions established by an active soil microflora are responsible for the increased destruction of sclerotia.

Table 6.—*Percentages of sclerotia remaining viable after 30 days of exposure in soil lots incubated at differing temperatures*

Soil amendment	Viable sclerotia remaining after 30 days of incubation at various temperatures			
	2° C.	12° C.	28° C.	35° C.
	Percent	*Percent*	*Percent*	*Percent*
None (check)	66	73	66	66
Ground alfalfa, 2 percent	88	70	28	9

Influence of Moisture on the Elimination of Sclerotia From Soil

To determine the influence of moisture content of soil upon destruction of sclerotia, 100-gm. aliquots of Hunt clay, amended with commercial molasses applied at a rate of 3.0 percent, were established in flint-glass bottles of 8-ounce capacity. Laboratory-grown sclerotia, shown to be 100 percent viable, were added to each of 36 containers at the rate of 50 sclerotia per container. In one-third the total number of containers, soil-water content was adjusted to 35 percent of moisture-holding capacity; in another one-third, to 58 percent; and in the remainder, to 80 percent. All incubations were at 28° C. and of 55 days' duration. All sclerotia recovered were tested for viability.

For the soil-moisture contents employed, greater destruction of sclerotia was obtained at the higher soil-moisture conditions. Observations are summarized briefly as follows: 59.5 percent of the sclerotia added to amended soil were destroyed in soil aliquots incubated at 35 percent of moisture-holding capacity; 66.2 percent of the number added were destroyed in soil at 58 percent moisture; and 76.7 percent, in soil at 80 percent of moisture-holding capacity. In unamended Hunt clay, 37.0, 38.0, and 33.0 percent of the sclerotia added were eliminated by incubation at soil-moisture contents of 35, 58, and 80 percent, respectively.

Influence of Soil Reaction

All preceding studies here reported on the persistence of *Phymatotrichum omnivorum* in soil were made on neutral or slightly acid soils collected on the United States Department of Agriculture Cotton Field Station (Greenville, Tex.) grounds. For the most part, Hunt clay was employed. Comparison of sclerotia destruction in Hunt clay and in Wilson sandy loam revealed that very similar results could be obtained in these two soils (table 5). These soils, although differing in character, did not differ appreciably in soil reaction. To determine whether soil reaction influences the extent of sclerotia decomposition in organic-amended soil, three of the Greenville-station soils with distinctly different pH values were selected.

Soil A was Hunt clay from the surface area of land that had been cropped continuously to cotton for many years. This particular area had been treated with sulfur 11 years previously, and for the soil lot collected a pH value of 4.5 was determined by a soil paste method (*6*), with readings taken on a glass electrode. Soil B was typical Hunt clay, as routinely employed, with a pH value of 6.5. Soil C was Houston clay, eroded phase; the lot collected was a yellowish-brown highly calcareous plastic clay, with a pH value of 8.0. To obtain differing pH values in aliquots of a single soil lot, either 0.1 N hydrochloric acid or lime was added to soil B. Results of sclerotia-decomposition tests, in which ground alfalfa tops at the rate of 3.0 percent were added as the organic amendment and with standardized conditions of incubation, are summarized in table 7.

TABLE 7.—*Influence of soil reaction on the destruction of Phymatotrichum omnivorum sclerotia in soil amended with ground alfalfa tops at the rate of 2.0 percent*

Soil lot	pH value	Acid or lime added to 100 gm. of soil	Buried sclerotia rendered nonviable
			Percent
A	4.5	None	65.0
B	6.5	do	75.0
C	8.0	do	94.0
B (plus acid)	3.0	8 ml. N/10 HCl	88.0
Do	4.1	4 ml. N/10 HCl	58.0
Do	4.6	2 ml. N/10 HCl	53.0
Do	5.3	1 ml. N/10 HCl	56.0
B (plus lime)	7.9	Not measured	96.0

During the course of incubation of this series of soil lots in loosely covered dishes, it was observed that those soil lots with pH values within the range of 4.0 to 5.5 showed excessive overgrowth of filamentous fungi, whereas the surfaces of the two alkaline soils were macroscopically free of filamentous fungi. The greatest destruction of sclerotia was observed in alkaline soil.

INFLUENCE OF AERATION ON ELIMINATION OF SCLEROTIA FROM SOIL

Comparison was made between two differing conditions of aeration that could be obtained by use of differently shaped containers. To provide for incubation of soil in shallow layers in which superior aeration could be expected, Petri dishes 15 mm. deep and 100 mm. in diameter were employed; for incubation under less favorable conditions of aeration, pyrex tubes 200 mm. deep and 25 mm. in diameter were used.

In a preliminary comparison, four replicates each of Hunt clay aliquots, either unamended or amended with ground alfalfa tops at the rate of 3.0 percent, were started both in shallow and in deep containers. To each of 16 containers 50 viable sclerotia were added, and the soil-moisture content was adjusted to 55 percent of the moisture-holding capacity. Washings for sclerotia recovery were made after 30 days of incubation at 28° C.

In unamended soil in shallow containers 22.0 percent of the sclerotia added were rendered nonviable, and in deep containers, 37.5 percent. In alfalfa-amended soil in shallow containers, 52.0 percent of the number added were destroyed, and in deep containers 100 percent.

Confirmation of the superiority of deep containers as an aid in the destruction of sclerotia was then attempted in soil amended with differing types of organic material. Aliquots of Hunt clay amended with organic material (commercial peptone, stable manure, chopped-sorghum fodder, vetch or clover tops, wheat straw, and cellulose) at a rate of 3.0 percent were started in 200×25 mm. glass tubes. With the exception of the use of a differently shaped container, the incubation conditions were identical with those employed above during the comparison of these same organic materials in Petri-dish containers.

The extent to which *Phymatotrichum omnivorum* sclerotia, after

burial in fertilized soil maintained 22 days in deep tubes, were found nonviable, together with the increase in loss of viability over that noted in shallow containers, is shown in table 8.

TABLE 8.—*Influence of aeration on the destruction of Phymatotrichum omnivorum sclerotia in soil receiving organic amendments*

Soil treatment	Sclerotia rendered nonviable by incubation in deep containers	Increase in loss of viability over loss in shallow containers	Soil treatment	Sclerotia rendered nonviable by incubation in deep containers	Increase in loss of viability over loss in shallow containers
	Percent	*Percent*		*Percent*	*Percent*
Commercial peptone	92.0	6.6	Clover tops	91.0	11.4
Stable manure	92.6	60.6	Wheat straw	66.6	4.8
Sorghum fodder	85.4	50.3	Cellulose	63.1	18.0
Vetch tops	94.6	12.6			

CHANGES IN THE FUNGUS FLORA OF COTTON ROOTS FOLLOWING PARASITIC AND MECHANICAL INJURIES

Although earlier studies (*4*) had shown that application of organic amendments to soil did not alter appreciably root-surface microfloras, injury of cotton roots during the late summer or early fall was found to increase greatly the number of micro-organisms associated with root surfaces. Such increases in root-surface micropopulations following plant injury were proportionately greater than those in the soil micropopulation following application of organic amendments. The possibility that *Phymatotrichum omnivorum* might be attacked during the host-borne stage of its existence was thus suggested.

A field area in cotton was selected in which a fairly uniform distribution of *Phymatotrichum omnivorum* rot was apparent. On August 18, 1941, the following plant injuries were inflicted: (a) Girdling immediately above the crown portion; (b) clipping immediately above the crown portion; and (c) clipping approximately 2 inches below the crown. Plots subjected to these treatments, together with check, or untreated plots, were laid out in randomized block arrangement, with each treatment replicated five times. Individual plots were 25 feet by 30 feet in size.

After 10, 18, and 57 days cotton roots were collected from plots given each treatment. In sampling, 24 plants were taken at random in each treatment replicate. From each root system excavated, that section of the taproot, together with stubs of laterals, occurring within the interval of 3 inches to 6 inches below the crown was removed and placed immediately into a Petri dish containing several thicknesses of moistened filter paper. Following incubation in these moist chambers for 5 to 6 days at 28° C., root segments were examined for fungus mycelium growing over the surface. Identifications of fungi present were made either by direct microscopic examination of growth on root segments or from subcultures prepared therefrom. Fungi observed, with but few exceptions, could be grouped into the following: *Aspergillus* species, *Penicillium* species, *Trichoderma* species, Dematiaceae, Mucorales, and sterile mycelium types.

There were no significant differences in the numbers of live and apparently nonparasitized roots recovered from the plots given different treatments 10 days and 18 days following the infliction of the injuries. On each of these dates of sampling, however, the number of root segments from healthy plants showing saprophytic fungi developing in moist-chamber incubation was significantly greater for the clipped-below-crown treatment than for the other treatments. Although there were no differences between treatments in total numbers of diseased or decaying roots recovered in the 10-day and in the 18-day sampling, the former sampling revealed a trend toward reduction, and the latter, a highly significant reduction, in the number of diseased roots in the clipped-below-crown treatment from which *Phymatotrichum omnivorum* mycelium could be recovered. At the end of 57 days, all treated plots when compared with the check showed marked reduction in the number of diseased roots from which *P. omnivorum* could be cultured.

Accompanying the increased difficulty of recovery of *Phymatotrichum omnivorum*, an influence of plant injury upon types of saprophytic fungi developing in moist-chamber incubations was also apparent. All qualitative observations on fungi may be treated summarily. From all the root samples collected, a total of 1,898 observations on fungi were made. When these observations are grouped according to treatment, regardless of disease or of types of fungi encountered, 432 observations were obtained from root segments from untreated plants; 471, from girdled-above-crown treatment; 462, from clipped-above-crown treatment; and 533, from clipped-below-crown treatment. If condition or treatment of plants is disregarded, and subgroups of fungi only are considered, sterile mycelium types were noted 80 times; Dematiaceae, 148 times; *Penicillium* species, 325 times; *Trichoderma* species, 328 times; *Aspergillus* species, 442 times; and Mucorales, 573 times. The distribution of these subgroups of fungi according to treatment and to condition of roots (diseased or nondiseased) is shown graphically in figure 4.

DISCUSSION

Analyses of uncropped soil given organic fertilization at rates known to prevent take-all damage under greenhouse conditions revealed that the relative capacities of such amendments to promote take-all control were paralleled by their relative capacities to enrich the nitrate-nitrogen and available-phosphorus content of soil. The order in which such amendments favored nitrate-nitrogen accumulation was also similar to that which could be expected from consideration of carbon-nitrogen ratios of the amendments employed. As take-all of wheat can be controlled simply by maintaining both the nitrate-nitrogen and available-phosphorus content of naturally infested soil at suitable levels, it seems reasonable that the fertility values of organic manures aid in obtaining the reductions in take-all incidence that can be observed following their application. For the organic manures employed, no correlation was evident between microbial numbers and soil-sanitation benefits. It would appear therefore that in the presence of both the host plant and the parasite, the important function of saprophytic micro-organisms is one of making certain plant nutrients available from added organic materials rather than one of direct microbial antagonism.

Differences in the severity of take-all damage in soil lots receiving either fresh or partially composted manures furnish further support to this view. Large microbial populations were maintained in the compost-manured soil even until the onset of take-all damage—in fact, at this time microbial populations were as great in the compost-manured as in the fresh-manured soil. The essential difference ap-

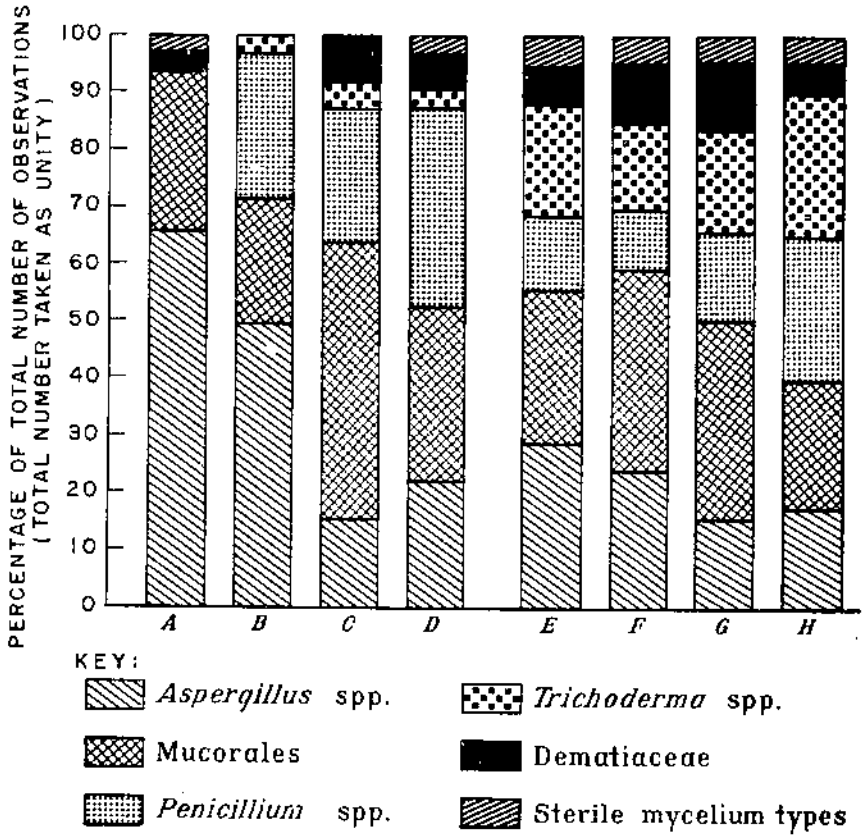

FIGURE 4.—Relative frequency of occurrence of certain saprophytic fungi on non-parasitized and on *Phymatotrichum*-parasitized cotton roots following differing methods of mechanical injury. *A–D*, Nonparasitized series; *E–H*, parasitized series. *A* and *E*, Untreated roots; *B* and *F*, roots girdled above the crown; *C* and *G*, roots clipped above the crown; *D* and *H*, roots clipped below the crown.

peared rather to be the virtual absence of nitrate nitrogen in the compost-manured soil in contrast to an abundant supply in the fresh-manured soil.

Thom and Morrow (*35*) have considered that organic manures are most effective in depressing pathogenic fungi during the period of active decomposition. It is possible that upon reaching the stage of soil humus, organic manures become inert for the control of certain diseases because they no longer release an excess of plant-nutrient materials. The demonstration of appreciable reduction in take-all injury merely by providing a larger soil volume per plant also

emphasizes the importance of providing the growing crop with favorable plant-nutrient conditions. Reductions in the incidence of take-all disease in fields with thin stands, "border effect" in the lack of disease along the borders of drill misses, and the occurrence of "escape" plants in take-all patches may thus be explained.

Recropping to wheat under conditions favorable for take-all development has shown that *Ophiobolus graminis* is not eliminated from soil in which a preceding crop has been grown successfully because good plant-nutrient conditions were provided. However, when naturally infested soil was maintained for 3 months at moisture and temperature favorable for microbial activity, but devoid of susceptible roots, *Ophiobolus graminis* was completely eliminated. Elimination of the parasite was considered accomplished because such soil could again be cropped to wheat, with extreme overcrowding of seedlings, without evidence of take-all disease. It would appear therefore that take-all control may be obtained either through augmentation of host resistance by proper nutrition or by subjecting the parasite to a soil-borne phase of existence favorable for microbic activity but with the soil devoid of susceptible roots.

With proper nutrition of the host plant, *Ophiobolus graminis* does not cause appreciable damage to root systems, even in cases in which it is not eliminated from soil. On the other hand, if the parasite is forced to grow or to compete with saprophytic microorganisms in the absence of susceptible roots for a sufficiently long period, it apparently loses its viability altogether, or becomes unable to invade wheat plants regardless of adequate or inadequate host nutrition.

Recognition of both host-nutritional and soil-antibiotic phenomena as important in determining the incidence of take-all disease contributes toward a better understanding of the extensive literature concerning *Ophiobolus graminis*. Certain major trends within this literature may be summarized briefly. Early reports on take-all disease came from European and Australian sources, and, broadly, characterized field conditions which permitted take-all development and suggested field control measures. The association of take-all disease with nonfertile, poorly drained, alkaline, or otherwise unfavorable soils was frequently emphasized. Remarks that take-all disease is nothing more than starvation of the plant, or poverty of the soil, are illustrative (*24, 43*). High winter moisture, early rank growth, and late spring frosts were also recognized by various workers as predisposing wheat plants to take all disease (*29*). Although the opposing view was not uncommon, many workers apparently believed that *Ophiobolus graminis* was widely distributed in wheat farming areas and appeared as a crop enemy only under unfavorable crop-growing conditions. Many recent investigations confirm and extend the evidence that favorable crop conditions should be provided if serious take-all damage is to be avoided (*12, 14, 38*).

Earlier recommendations for take-all control included the use of organic and inorganic fertilizers, drainage, late sowing, crop rotation, and such tillage operations as rolling and early plowing (*26, 27, 31*). But evidence against any treatment being generally effective was also accumulated. There appeared no adequate interpretation of many of the conflicting results that could be obtained. Part of the con-

fusion was undoubtedly due to the failure at times to distinguish between different root diseases of wheat (*5*) and to incorrect identification of *Ophiobolus graminis* and take-all disease.

Recognition of take-all disease as a crop menace in America (*10, 15, 20*) occurred just prior to the earlier papers on microbial antagonism as an important factor in soil sanitation. Following the development of the hypothesis that an active soil micropopulation is inimical to root-rotting parasites, much of the work of the last two decades has attempted to explain relationships of soil-environmental and soil-fertility factors to take-all disease from the point of view of the influence of such factors on soil-microbial populations. Several reviews of this later phase of the take-all problem are available (*12, 39*). But just as earlier there developed conflicting evidence concerning the soil-fertility hypothesis, likewise some recent evidence makes it questionable whether successful take-all control is entirely a question of soil-microbial phenomena. Undoubtedly, final interpretation of much of the more recent, as well of the earlier, literature on take-all disease must take into consideration not only host-plant nutrition and microbial antibioses but also the direct effects of physical factors on *Ophiobolus graminis*. In the present evaluation of antibiosis in relation to take-all disease, it would appear best to consider antagonistic phenomena of prime importance only in the absence of the host plant.

Some further information concerning the persistence of a parasitic fungus in soil devoid of susceptible roots was obtained during the course of studies on *Phymatotrichum omnivorum* sclerotia. Activity of the saprophytic microflora of the soil appeared essential for obtaining marked destruction of sclerotia in organic-amended soils. Uncontaminated viable sclerotia survived in sterile, organic-amended soil equally as well as in sterile, unamended soil. The importance of microbial activity was also shown when sclerotia were incubated in nonsterile soil at differing temperatures. Incubation temperatures favorable for microbial activity provided the greatest destruction of sclerotia.

In comparisons in which organic materials with greatly dissimilar carbon nitrogen ratios were added to sclerotia-loaded soils, destruction of *Phymatotrichum omnivorum* sclerotia was obtained both with highly nitrogenous and with nitrogen-deficient substances. Such physical incubation factors as aeration, moisture, and temperature appeared of greater importance than did type of organic material added. Garrett (*11*), working with *Ophiobolus graminis* in artificially infected stubble, noted that soil amendments deficient in nitrogen gave greater destruction of that fungus than did highly nitrogenous materials. He has suggested that the soil saprophytes attack *Ophiobolus graminis* as a source of nitrogen whenever other supplies of nitrogen are not available, and in this manner effect destruction of the parasite. The destruction of *Phymatotrichum omnivorum* sclerotia in soil lots amended with highly nitrogenous materials, such as peptone, suggests that competition for nitrogen is not the most important factor in the destruction or elimination of the cotton root-rot fungus in manured soil.

The susceptibility of *Phymatotrichum omnivorum* sclerotia in organic-amended soil characterized by an active saprophytic microflora emphasizes the importance of farming procedures that terminate the sheltered, host-borne stage of the parasite's existence as early as practicable. As cutting and girdling of cotton plants influence the bacterial number associated with root surfaces, it is possible that antibiosis rather than exhaustion of food supply functions as the important factor limiting survival of the parasite. In the current work, changes in the fungus flora of cotton roots have been apparent following parasitic and mechanical injuries. Approximately two-thirds of all root segments recovered from nonparasitized and uninjured cotton plants failed to show saprophytic fungi developing when incubated under moist-chamber conditions. This was also true of the nonparasitized plants that had been injured above the crown by girdling or clipping. Clipping below the crown, however, opened up root systems for invasion by saprophytic fungi; only one-fifth of the root segments obtained from nonparasitized plants injured in this way failed to show saprophytic fungi following moist-chamber incubation. It is possible that the increased aeration provided by cutting below the crown aided in obtaining a better colonization of fungi in that section of the cotton-root system examined. Cutting below the crown, which encouraged saprophytic fungi, also hastened the disappearance of *Phymatotrichum omnivorum* from diseased root systems.

SUMMARY

Investigations were undertaken to determine the factors in manurial treatments that contribute toward soil sanitation and to define the conditions that must be established in order to obtain control or elimination of undesirable members of the soil microbial population. Experimental work was limited to the take-all (*Ophiobolus graminis*) disease of wheat and the *Phymatotrichum omnivorum* root rot of cotton.

Fertility and soil-sanitation contributions of different organic manures to take-all infested soils, as revealed by nitrate-nitrogen and available-phosphorus content, microbial number, and incidence of take-all disease on wheat, were studied in greenhouse experiments. Organic materials giving excellent take-all control, such as chicken manure and alfalfa tops, markedly increased nitrate-nitrogen and available-phosphorus content in soil, when applied at rates adequate for take-all control. When partially composted and fresh or noncomposted manures were compared, only the latter appeared effective for take-all control. Nitrate nitrogen disappeared from the compost-manured soils about the time of onset of take-all damage, although microbial numbers in the two series remained comparable.

When naturally infested soil in which wheat had been grown successfully following adequate fertilization was recropped to wheat under conditions favorable for *Ophiobolus graminis*, the failure of fertilization to eliminate the parasite from soil was revealed. On the other hand, *O. graminis* was eliminated from naturally infested soil maintained for 3 months under moisture and temperature conditions favorable for microbial activity but completely devoid of susceptible roots.

The activity of the saprophytic microflora of the soil appeared essential for the destruction of *Phymatotrichum omnivorum* sclerotia in artificially infested, organic-amended soil. Uncontaminated, viable sclerotia survived in sterile, organic-amended soil as well as they survived in sterile, unamended soil. Widely differing types of organic material (ranging from pure cellulose to commercial peptone) were employed successfully for the destruction of sclerotia in soil. When suitable incubation conditions were employed, destruction of sclerotia was obtained with either low-nitrogen or high-nitrogen types of amendments.

Increasing rates of application (0.5, 1.0, 3.0, 5.0 percent) of organic material rendered nonviable increasingly greater percentages of the number of viable sclerotia initially present. Incubation temperatures favoring general microbial activity were more destructive to sclerotia than low-temperature incubations. From organic-amended soil lots maintained at 2°, 12°, 28°, and 35° C., 12.0, 30.0, 72.0, and 91.0 percent, respectively, of the number of viable sclerotia initially present were eliminated. Attention was also given to the importance of favorable soil moisture and to the possible importance of aeration and soil reaction.

In an effort to determine whether the host-borne stage of *Phymatotrichum omnivorum* was terminated by plant injury, the incidence of common saprophytic fungi on uninjured and on mechanically injured cotton roots of healthy and diseased (i. e., phymatotrichum root rot) cotton plants and the persistence of *P. omnivorum* on roots that it had parasitized were studied.

Cutting of nondiseased roots below the crown hastened colonization by saprophytic fungi; injuries inflicted above the crown portion of the stem provided no increased colonization of roots in comparison with untreated plants. Recovery of *Phymatotrichum omnivorum* was more difficult from parasitized roots that had been subjected to plant injury, and especially difficult from roots cut below the crown.

From plants parasitized by *Phymatotrichum omnivorum* but not mechanically injured, *Penicillium* and *Trichoderma* spp., Dematiaceae, and sterile mycelium types were encountered with greater, and *Aspergillus* spp. and Mucorales with less relative frequency on root segments given moist-chamber incubation. Following infliction of mechanical injuries either above or below the crown, a similar shift in the fungus flora of nonparasitized roots and an accentuated shift in the flora of parasitized roots was evident.

In a brief evaluation of the role of antibiosis in the control of root-rotting parasites, it is recognized that antibiotic effects are not necessarily the only contribution of organic amendments to soil sanitation. For take-all disease, antibiosis appeared of prime importance only in the absence of the host plant. For wheat-cropped soil, the fertility contributions of organic manures appeared responsible for observed reductions in take-all disease following manurial treatment. Evidence is considered sufficient that a root-rotting parasite can be completely eliminated from soil devoid of susceptible roots. Activity of the saprophytic microflora of soil appeared responsible for the marked destruction of *Phymatotrichum omnivorum* sclerotia in organic-amended soils. Competition for nitrogen did not appear to be

the factor limiting survival of this parasite in manured soil. The development of agronomic procedures exploiting microbial antagonisms is believed to offer a practical line of attack against root-rotting parasites.

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