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BY OILSEED MEAL AMENDMENTS

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MICHAEL ABDI ZAKARIA

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John L. Lockwood
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REDUCTION IN *FUSARIUM* POPULATIONS IN SOIL BY OILSEED MEAL AMENDMENTS

By

Michael Abdi Zakaria

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ABSTRACT

REDUCTION IN FUSARIUM POPULATIONS IN SOIL BY OILSEED MEAL AMENDMENTS

By

Michael Abdi Zakaria

A sandy loam soil was artificially infested with Fusarium oxysporum and F. solani and incubated for eight weeks to obtain a constant chlamydospore concentration of about 10^5/g. The infested soil was amended with 1% (w/w) of ten different plant and animal residues and incubated in closed containers. Fusarium populations were estimated at intervals using standard dilution plate techniques and a medium selective for the genus.

Linseed, cottonseed, and soybean meals were effective in reducing Fusarium populations to 0.1% or less of the original after 4-5 weeks of incubation. Such reduction was also obtained using rates as low as 0.25% for soybean meal and 0.50% for linseed and cottonseed meals after six weeks of incubation. The oilseed meal amendments were also effective in reducing populations of five other Fusarium species tested. Compared to Fusarium, however, the total fungal, bacterial, and actinomycete populations were much less reduced.

Soil moisture levels influenced the effectiveness of linseed and cottonseed meal amendments, but not of soybean meal. Using linseed and cottonseed meals, Fusarium populations decreased most rapidly at 30-35% WHC; at 50% and 100% WHC, reduction was less rapid, and no reduction occurred at 15% WHC.

Fusarium populations decreased most rapidly and to a greater

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degree in oilseed meal-amended soils incubated in closed containers than in open containers. When infested soil samples were placed in planchets and incubated on oilseed meal-amended soils in closed containers, Fusarium populations in the soil samples were drastically reduced. These suggested that volatile substances were involved. The volatile substances were trapped in H_3BO_3 solution, indicating that ammonia and/or amines were possibly produced. With soybean meal amendment, these substances appeared to be solely responsible for reducing Fusarium populations, because the effectiveness of the amendment was completely nullified in the presence of H_3BO_3 or H_2O. The trapping solutions did not remove all of the toxic effect in the case of linseed and cottonseed meals.

The oilseed meal amendments were not effective in uncovered plots of Oshtemo-Boyer sandy loam or Conover loam soils in the field. In the laboratory, the oilseed meal amendments showed less activity in Conover loam than in the sandy loam soil, even when the soils were covered; the amendments were without any effect in Brookston loam soil. The pH of the sandy loam soil was increased following amendment with oilseed meals, whereas the pH of the two loam soils was decreased.

Fusarium chlamydospores applied to Nuclepore membranes were buried in oilseed meal-amended soils. At intervals, the membranes were removed from the soils and the chlamydospores were transferred onto agar discs followed by immediate microscopic observation. During the first three days of burial, most of the chlamydospores had germinated, the germ tubes had lysed, and new chlamydospores had been formed. However, prolonged exposure to the oilseed meal-amended soils resulted in complete loss of chlamydospore viablility, as verified by incubating

them on discs of a selective agar medium for 24 hours.

Reduction in Fusarium populations as affected by oilseed meal amendments was correlated with amount of Fusarium root rot of soybeans and peas. Linseed and cottonseed meal amendments, however, showed some phytotoxic residues even after the soils had been air-dried for one week. Soybean meal amendment, beside being the most effective in reducing Fusarium populations and root rot, did not show any phytotoxic residue after the period of air-drying.

To my wife and parents

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INTRODUCTION

Fusarium oxysporum Schlecht. and *F. solani* Mart. are often found associated with an extensive seed and seedling rot, and later a root rot on soybean grown in loamy sand soil in S.W. Michigan. The first evidence of the disease is poor emergence over restricted or extensive (several acres) areas in the field. Symptoms, including seed rot, curling and swelling of seedling hypocotyls, and necrosis are typical of the disease.

Fusarium disease of soybeans was first reported by Cromwell (31) in North Carolina in 1916. The disease, believed to be caused by *F. tracheiphilum* Smith, occurred during the first eight weeks of planting; symptoms included discoloration of root xylem tissue, followed by yellowing and stunting of the plants. The disease was favored by high temperature and sandy soils. Armstrong and Armstrong (6) found that certain races of *Fusarium* isolated from wilted cowpeas and cotton were pathogenic to soybeans and showed symptoms similar to those described by Cromwell. Based on the Snyder and Hansen system of classification, they suggested that the specific name, *F. oxysporum*, be used. They also found an isolate of *F. oxysporum* which caused a vascular wilt, especially on soybean plants that had passed the succulent stage.

In Iowa, a disease of soybean seedlings caused by *F. orthoceras* Appel & Wr. has been observed since 1953 (36). The disease caused

poor germination and late emergence of seedlings in the field. Growth of diseased seedlings were stunted and necrotic symptoms were found on succulent root tissues of the seedlings. The root systems of severely infected seedlings were sometimes completely destroyed. However, beyond the seedling stage, plants were rarely killed by the disease.

F. oxysporum was also found to be a potential threat to soybean crops in Minnesota (42). Soybeans in approximately 90% of the fields sampled in central and southern portions of the state were affected by root rot. Poor stand, stunted growth, and necrotic root symptoms were associated with the disease. F. oxysporum was readily isolated from diseased soybean roots and many of the isolates were highly pathogenic when tested.

The purpose of this study was to investigate possible control measures for Fusarium diseases in Michigan using organic amendments. It was also of interest to investigate various parameters which might influence the effectiveness of the amendments, and to elucidate the mechanism of action of effective amendments.

LITERATURE REVIEW

The genus Fusarium

Biology in Soil. Species of the genus Fusarium are among the most widely distributed in soil and on organic substrates (15) and also among the most frequently isolated by plant pathologists, either as pathogens or as saprophytes (154). As with many soil fungi members of this genus are abundantly endowed with means of survival, one mechanism of which is the capacity of rapid change morphologically, as well as physiologically, to a new environment.

Some pathogenic Fusarium species are capable of colonizing root systems of a wide range of non-host plants. For example, weeds belonging to the genera Oryzopsis, Digitaria, Amaranthus, and Malva have been known to harbor the tomato wilt pathogen, F. oxysporum f. sp. lycopersici without showing any symptoms (76). Crop plants such as cotton, soybean, and cowpea were also found to carry the sweet potato wilt Fusarium without showing any symptoms (7).

Dormant stages of Fusarium in soil may be encountered in the form of mycelium in rotted tissues or humus particles and as chlamydospores (167). The chlamydospores may be formed by the direct conversion of macroconidial cells, on the germ tubes of germinated macroconidia, or from mycelia (40, 41, 43, 45, 68, 112).

While Fusarium macroconidia are relatively insensitive to soil fungistasis, the chlamydospores are more sensitive (4, 156).

Germination of the latter spore type occurs only if nutrients are available (29, 138, 142, 163). Carbon and nitrogen sources are required for germination, although carbon exerts a greater limitation than nitrogen. Forty-two percent chlamydospore germination was obtained in the presence of glucose and $(NH_4)_2SO_4$ while only 14-23% germinated in the presence of glucose alone. In the absence of glucose, $(NH_4)_2SO_4$ did not stimulate any germination. Sterilized soil infested with Fusarium also showed a much higher respiration rate when amended with sucrose and NH_4NO_3 than with sucrose alone (38). Respiration rate in soil amended with NH_4NO_3 alone was about the same as that in unamended soil.

During germination the chlamydospores produce germ tubes which may grow extensively and form new chlamydospores, or may undergo extensive lysis before new chlamydospores were formed (30, 46, 159, 163). Lysis of germ tubes occurs in the presence of C, if N is adequate. When N is low, lysis occurs very slowly, replacement chlamydospores are formed, and the population will remain steady or slightly increase.

Taxonomy. The genus Fusarium was described by Link in 1809 for species having fusiform non-septate spores borne on a stroma or sporodochium, and was based on Fusarium roseum (15). It was validated by Fries in 1821. According to the Sacardo system of classification, this genus belongs to the family Tuberculariaceae of the order Moniliales (13, 50). However, with the development of single-spore culture methods for Fusarium identification, the presence of a stroma or sporodochium is no longer accepted as an essential character of the genus.

All modern systems of Fusarium classification are based on the work of Appel and Wollenweber as summarized by Wollenweber and

Reinking (172). In their system 143 species, varieties, and forms were grouped in 16 sections mainly based on shape, septation, size, color, and growth of macro- and microconidia; presence or absence of sporodochia and pionnotes; production of chlamydospores, whether intercalary or terminal, singly, in clusters or in chains; and presence or absence of sclerotia. The 16 sections were Arachnites, Arthrosporiella, Discolor, Elegans, Eupionnotes, Gibbosum, Lateritium, Liseola, Macroconia, Martiella, Pseudomicrocera, Roseum, Spicarioides, Sporotrichiella, Submicrocera, and Ventricosum. Assignments of species to sections were to some extent related to the discovery of the perfect stages which consisted of the genera Calonectria, Gibberella, Hypomyces, Micronectriella, and Nectria. All of these genera belong to a single order, Hypocreales.

Snyder and his co-workers (148, 149, 150, 151, 154) suggested that only stable morphologic characters be used to delimit species and all higher taxonomic categories. Shape of macroconidia, presence and kind of microconidia and chlamydospores were suggested to be suitable characters for speciation. Size, septation, and pigmentation of conidia have been found to vary with media and subculturing. Single-spore subcultures originating from one perithecium may show natural variability with respect to colony color, configuration, growth rate, relative abundance of conidia, presence or absence of sporodochia and of sclerotia, spore size, septation, and even their pathogenicity. Even progenies of a single conidium have shown variations with respect to presence or absence of sporodochia and size of macroconidia. They also suggested that speciation should be based on similarities among different individuals rather than on differences. Based on this

concept, all the 143 members of the genus Fusarium in the Wollenweber and Reinking system were dramatically reduced to 9 species. For example, all 40 members of the section Elegans which were separated on the basis of the presence or absence of sporodochia and the width of macroconidia were grouped into a single species, Fusarium oxysporum. The other eight species comprising this nine-species system are: F. episphaeria, F. lateritium, F. moniliforme, F. nivale, F. rigidiusculum, F. roseum, F. solani, and F. tricinctum. This nine-species system has been widely accepted, especially by plant pathologists, for over three decades. A pictorial guide to the identification of Fusarium species, based on this nine-species system, was published by Toussoun and Nelson (160).

To differentiate the pathogenic forms from the saprophytic forms within a Fusarium species appropriate designations (formae speciales) were added to species names. For example, F. oxysporum f. sp. lycopersici is actually a F. oxysporum which is pathogenic to tomatoes, and F. oxysporum f. sp. pisi is pathogenic to peas. At first it was believed that these physiological strains (formae speciales) were host specific, but it has since been determined by cross-inoculation studies that this is not necessarily the case (6, 7, 16, 114). For example, F. oxysporum f. sp. apii, a celery wilt pathogen, has been known to attack sunflower and F. oxysporum f. sp. batatas was also isolated from naturally infected corn. More than one pathogenic race may also exist within a forma specialis.

Forms with transitory or minor variations in conidial morphology which were too unstable to find a place in botanical classification were labelled as cultivars; e.g., F. roseum f. sp. cerealis cultivar

'Culmorum' if pathogenic, or merely F. roseum 'Culmorum' if saprophytic (152, 161).

Recent work in the taxonomy of the imperfect fungi is based in large part on the studies of Mason as regards the importance of the conidiogenous cell and the ontegony of the conidium. Production of phialides with phialospores (micro-and macroconidia) and occasionally, blastospores is characteristic of the genus Fusarium. This genus is also characterized by the presence of a foot cell at the base of the macroconidium. The recent taxonomic treatment of Fusarium by Booth (15) recognizes 12 sections and 44 species and follows the concepts of Mason. As a result, the sections Sporotrichiella, Arthrosporiella, and Gibbosum of the Wollenweber and Reinking system were modified and section Roseum discarded. Two species with polyblastic conidiogenous cells from section Sporotrichiella were transferred to section Arthrosporiella in which this spore formation type is the unifying character. F. avenaceum from section Roseum was transferred to section Arthrosporiella for the same reason. The remaining species in section Roseum, having simple phialidic conidiogenous cells, were transferred to section Gibbosum. Species differentiation was based on 1) microconidia: presence or absence, borne in chains or not, shape; 2) macroconidia: shape, morphology of foot cells, size, septation; 3) conidiophore: growth, whether polyblastic or simple- or poly-phialidic, borne in sporodochia or not; 4) chlamydospores: presence or absence, borne terminal or intercalary or both; 5) cultures (based on potato-sucrose agar): growth rate, color, presence or absence of aerial mycelium. Keys to the sections and species, and a laboratory guide to the identification of the major species of Fusarium were published (15, 17).

Booth (15, 16), realizing the capacity of the genus Fusarium for rapid change under different environmental conditions, proposed that single-spore cultures and a standardized culture medium be used in species identification. In his method, potato-sucrose agar with a pH of 6.5-7.0 is used for growing the fungus. Growth is maintained at 22°-25°C, 10-14 inches below a daylight fluorescent tube with a photoperiod of 12 hours/day. For microscopic studies the spores are mounted in erythrosin disolved in 10% NH_4OH; the coverslip is sealed with two layers of nail varnish.

Biological control using soil amendments

Soil fungistasis, its importance for survival. Most fungi survive in soil in the form of resting spores, hyphae, or sclerotia, which are intimately associated with the phenomenon of soil fungistasis (54). The importance of exogenous and endogenous nutrients on the germination of spores has been discussed in detail by Lockwood and his coworkers (19, 67, 84, 99, 100, 101, 156, 157). The static state of spores which is of a widespread occurrence in soil may be attributed to lack of essential exogenous nutrients or to rapid loss of essential endogenous nutrients by competing microorganisms. Although the presence of a low level of diffusible inhibitory substances has been claimed to contribute to soil fungistasis, the involvement of those substances occurred especially in alkaline(62, 63, 64, 81, 82, 83, 130) or limed soils (65, 66) and the fungistatic condition may or may not be annulled by appropriate nutrients (11, 62, 130).

A pathogenic propagule is apt to survive longest in soil under non-lethal conditions least favorable for germination (10, 35). While

continued lack of activity of a population of resting stages may lead to a gradual decline in numbers through senescence, there is a converse situation that reversion to activity under the wrong sort of conditions may lead to an even more rapid disappearance (121). Stimulation of a pathogenic propagule into activity in the absence of its host may waste its reserves and decrease its population (2, 58, 119, 145, 147).

Increased soil fungistatic level as a means of control. Control of soil-borne plant pathogens can be achieved by modifying the biological equilibrium to the detriment of the pathogens or their activities (20, 70, 124, 168). This can often be attained by increasing the activities of microflora through the addition of crop residues. Reduction in disease may or may not be associated with reduction in pathogen numbers. Reduction in root diseases due to increased fungistatic level of amended soils have been reported (1, 2, 3, 91). Fusarium root rot of beans in soil amended with 0.5-1.0% (w/w) spent coffee was reduced to about 30% that of unamended control soil, although the Fusarium population in the amended soil was increased by about 50%. Increased fungistasis was also obtained when cellulose was used to control the disease. The fungistatic level of both the amended and unamended soils was determined by adding certain amounts of glucose and by observing their effect on chlamydospore germination or by measuring the decomposition rate of the anthrone-positive substances. Gilpatrick (52) found that production of sporangia by Phytophthora cinnamomi at the surface of inoculated avocado roots was prevented in alfalfa-amended soil, while similar roots showed abundant production of sporangia in unamended soil. When inoculated roots from the alfalfa-amended

soil were used as inoculum in natural, uninfested soil in which an avocado seedling was growing, infection was delayed by 20 days. This delay was overcome when the surface of the inoculated root was disinfected using $HgCl_2$. Microorganisms on the root surface were therefore suggested as being responsible for the prevention of sporangial production. West and Hildebrand (171) reported a shift in bacterial populations in strawberry root rot soils caused by different amendments. About 40 X more pathogenic isolates were obtained from unamended and red clover-amended soils than from soybean-amended soil. They suggested that incorporation of soybean plants into the soil resulted in a pronounced selective stimulation of non-pathogenic bacteria and inhibition of the pathogenic types. Ko (80) successfully untilized the soil fungistatic phenomenon in the control of seedling root rot of papaya caused by Phytophthora palmivora. He used pathogen-free soil to replace a core of pathogen-containing soil in the field as a growth medium for the papaya during the susceptible stage. The pathogen was excluded due to the fungistatic effect of the pathogen-free soil.

Increased antagonism as a means of control. Reduction in disease may be associated with increased populations of antagonists to a particular pathogen as a result of soil amendment. Field soil amended with 200-500 lb/acre of finely ground chitin showed 30-50% reduction in bean root rot caused by Fusarium solani f. sp. phaseoli as compared to unamended soil (108, 109). After two weeks of amendment, chitinase producing microorganisms increased to five times that of the unamended soil, 70% of which were actinomycetes and 30% bacteria. Complete control of radish yellows caused by F. oxysporum f. sp. raphani was also obtained in chitin-amended soil (73). Control of the disease was

associated with an increased population of antagonistic actinomycetes. A similar relationship was shown in the reduction of pea wilt caused by F. oxysporum f. sp. pisi (21, 77) and in reduced sclerotial germinability of Sclerotium rolfsii in chitin-amended soils (60).

Beside chitin, plant materials have been used as soil amendments to control diseases. Reduction in Rhizoctonia disease of beans was obtained in soil amended with 1% (w/w) immature buckwheat, corn, oat, snapbean, and Sudan grass (118). Corn and oat amendments greatly stimulated soil and rhizosphere streptomycetes antagonistic to R. solani. By seven weeks after amendment the populations of the antagonists in the amended soils were about six times that of the unamended soil. Weinhold and Bowman (169) found that amendment of soil with green soybean residues prevented build up of common scab of potatoes, whereas incorporation of barley residues increased disease. Both amendments resulted in three-fold increase in the number of antagonists, which were predominantly Bacillus subtilis, as compared to the unamended soil. However, when soybean and barley extracts were used as sources of nutrients for antibiotic production, the former extract showed the higher antagonism, indicating that it was a more suitable medium for antibiotic production.

The effect of soil amendments or antagonists on the control of soil-borne diseases could be augmented by application of the antagonists together with the amendments. Menzies (106) was able to suppress potato scab to about 25% of untreated soil by using a combined amendments containing 10% suppressive soil and 1% alfalfa meal; neither of the constituents was consistently effective alone. Soil amended with 1.5% (w/w) antagonist-inoculated chitin has also been shown to reduce

scab to less then 20% of that in unamended soil and in soil amended with the antagonists alone (165). Suppression of Fusarium root rot of beans was greater in soil amended with barley straw which had been inoculated with a culture suspension of Chaetomium sp., than in soil amended with barley straw alone (103). Fusarium wilt of cucumber was also effectively controlled in soil amended with bark-sawdust compost which had been fortified previously with antagonistic actinomycetes (88). This method is now being commercially used in controlling Fusarium wilt of greenhouse tomatoes in Japan (H. Komada, personal communication). Backman and Rodriguez-Kabana (9) found that diatomaceous earth granules impregnated with a 10% molasses solution were suitable for growth and delivery of Trichoderma harzianum to peanut fields. Using this amendment, significant reductions in damage caused by Sclerotium rolfsii and increases in yield were obtained over a three year test period. An amendment rate of 140 kg/ha gave equivalent control to 10% PCNB treatment at a rate of 112 kg/ha.

Germination-lysis as a mechanism of control. One mechanism which may be involved in controlling soil-borne diseases using organic amendments is propagule germination followed by lysis of the germ tube and death of the propagule (10). Organic residues supply nutrients that stimulate germination of pathogenic propagules, but these nutrients are also quickly utilized by other microorganisms, leading to starvation of the pathogenic germlings, which in turn may cause (endolysis) autolysis and death (85, 98). Soybean meal and ascorbic acid were found to stimulate germination of Helminthosporium sativum conidia in soil during the first 20 hours of amendment, but germination was soon followed by lysis of the germ tube, leading to the eradication of the conidia

(23, 24, 25). Alfalfa hay has been reported to control bean root rot caused by Thielaviopsis basicola when incorporated into soil (117). The alfalfa hay as well as its hexane- and water-soluble extracts were stimulatory to germination of the fungus chlamydospores during the first two days of amendment, but germination was followed by lysis without formation of secondary endoconidia or chlamydospores (2, 119, 145).

A similar mechanism has been shown to be responsible for the reduction of Fusarium chlamydospore populations in amended soil. For example, sugarcane amendment stimulated germination of F. oxysporum f. sp. cubense chlamydospores which was soon followed by lysis of germ tubes (136). Chitin amendment was stimulatory to germination of F. solani f. sp. cucurbitae macroconidia but inhibitory to the formation of chlamydospores (131, 134). The general absence of Fusarium spp. in conifer forest soils may also be explained by the germination-lysis mechanism (57, 159). Germination of Fusarium chlamydospores was stimulated twice as much by a water extract of conifer litter as by 1% glucose and 2.5% asparagine. Shikimic and quinic acids that occur in pine needles were suggested to play a major role in stimulating the germination of chlamydospores. In all cases, germination was followed by lysis before new chlamydospores could be formed.

Effect of carbon to nitrogen ratio. Carbon to nitrogen ratio associated with soil amendment has frequently been suggested to function in disease suppression. Control of root diseases using organic materials of high C/N has been reported (92, 102, 120, 153). Organic materials of high C/N, such as mature barley, wheat, corn, soybean, and oat residues were effective in controlling Fusarium root rot of beans, while green barley, soybean, and alfalfa residues that have low

C/N were not effective (92, 153). Suppression of disease was not necessarily associated with reduction in inoculum density of the pathogen; for example, oat straw, glucose, maltose, and dextran, although effective in reducing disease, were found to increase inoculum density (55, 116). It was suggested that control of root rot was due to increased antagonism and to increased competition for limiting N by soil microorganisms. The effectiveness of high C/N amendments could be negated by addition of supplemental inorganic N, especially ammonium-N (104, 170).

High C/N by itself, however, could not be used to predict the effectiveness of soil amendments in reducing diseases. Low C/N amendments have also been found effective in controlling certain diseases. For example, control of Phytophthora root rot of avocado was achieved using alfalfa meal, the C/N of which was low (174). Alfalfa hay (C/N = 17) was also effective in suppressing take-all of wheat, and even greater suppression could be obtained by addition of ammonium-N (139). Oat straw (C/N = 83) when incorporated into the soil at a rate of 1% (w/w) reduced activity of Rhizoctonia solani by 50%, but so did soybean hay (C/N = 29) (33). Suppression of Rhizoctonia activity was also obtained using cellulose enriched with NH_4NO_3 (C/N = 5); suppression was associated with a decrease in soil pH from 6.1 to 4.0.

The germination-lysis mechanism which was involved in the reduction of Fusarium chlamydospores in soil was enhanced by availability of adequate N sources (30). Mixon and Curl (110), working with Sclerotium rolfsii, reported that addition of nitrate-N to oat residue amendment decreased germination of sclerotia in soil to half that caused by oat residue alone.

Effect of inorganic N. Inorganic N sources like NH_4NO_3, $(NH_4)_2SO_4$, and $NaNO_3$ have been known to suppress saprophytic activity of sclerotia in soil, and NH_4NO_3 at a rate of 2000 ppm N was toxic to the sclerotia (8). Huber and Watson (71) suggested that amendments modify disease severity through their effect on nitrification, which in turn determines the form of N available. For example, accumulation of ammonium-N decreased root rot caused by Pythium, Phymatotrichum, and Ophiobolus, while accumulation of nitrate-N decreased root rot caused by Rhizoctonia, Fusarium, and Aphanomyces. Urea has also been known to suppress Fusarium disease of banana through accumulation of nitrite (137). Toxicity of nitrite to Phytophthora cinnamomi was demonstrated in the form of complete inhibition of zoospore germination (175).

Effect on plant parasitic nematodes. Incorporation of certain plant residues has been found to reduce populations of plant parasitic nematodes in soil. Chopped oat straw at a rate of 1% (w/w) resulted in 80% reduction of root knot of tomatoes caused by Meliodogyne incognita when incorporated into soil (74). Stems and leaves of alfalfa and soybean were found to inhibit hatching of M. incognita eggs in soil (75). Linford et al (97) reported that incorporation of Panicum barbinode and pineapple residues into Heterodera marioni-infested soils resulted in more than 95% reduction of gall formation as compared to unamended soil. Reduction in disease was associated with rapid increase in saprophytic nematode populations.

Other beneficial effects. Beside stimulating microbial activities, soil amendment with organic materials improves soil structure and physical condition, and may increase productivity of the soil (39, 45). More than 50% increase in yield of snap beans was obtained by

incorporation of 1-2% (w/w) mature or green buckwheat into soil (32). Highly significant increases in potato yields and percent of No. 1 tubers have resulted from incorporation of barley straw from the previous year into the soil, compared to baling off or removing the straw from the soil (166). Hildebrand and West (61) obtained more than 50% increase in aggregate weights of strawberry roots grown in soils amended with green soybean tissues, corn, or barnyard manure as compared to those grown in unamended soil. Control of take-all of wheat has been obtained in soil when the N and P levels were maintained high, notwithstanding the presence of the pathogen. Improved nutrition of the suscept was considered to augment its resistance to the pathogen (26, 158).

Influence of volatile substances on soil microorganisms

Volatile substances in soil may be of abiotic or of biotic origin and their influence on soil microorganisms may be a stimulatory or an inhibitory one. To microorganisms which have virtually every single cell exposed to the surrounding medium and where consequently the surface to volume ratio is very high, the influence of volatile substances may be of a great significance (44).

As mentioned earlier, the presence of a low level of diffusible inhibitory substances in soil has been claimed to be involved in soil fungistasis, especially in alkaline and limed soil. Liming autoclaved soil with $Ca(OH)_2$ and $CaCO_3$ at rates of 0.5% (w/w) suppressed germination of conidia of Trichoderma viride and sporangiospores of Zygorhynchus vuilleminii conidia by more than 50% as compared with unlimed sterilized soil, suggesting that volatile inhibitors could be produced

abiotically (65). Ko and his co-workers (83) found that ammonia was released from limed soils known to produce volatile fungistatic factors.

All living things produce metabolites which are volatile at the normal temperatures of their environments, and most produce at least one that can have a significant effect on other members in the community (72). Acetaldehyde has been suggested as being produced by different Trichoderma species grown on 2% malt extract agar. When incubated together with the Trichoderma cultures, linear growth of Rhizoctonia solani, Fomes annosus, and Fusarium oxysporum was inhibited by 49%, 75%, and 19%, respectively (34). Linear growth and sporulation of different fungal species were also inhibited by volatiles produced by different bacteria grown on nutrient agar (111). For example, when the fungal cultures were incubated for six days on a bent glass rod placed on the surface of nutrient agar inoculated with the bacteria, 6-85% inhibition in linear growth and 8-26% reduction in number of spores/sample were obtained. Similar inhibition of linear growth of Gelasinospora cerealis was shown by the bacteria, and production of perithecia was inhibited over 90%. Inhibition of linear growth and sporulation was accompanied by an increase in pH of the fungal media from 6.0 to 8.2-8.6, although pH 8.6 itself was not inhibitory.

Ethylene has been reported to be involved in soil fungistasis (12.), especially in water-logged soil with high N and organic matter content (141). More than 90% inhibition in germination of sclerotia of Sclerotium rolfsii and conidia of Helminthosporium sativum was shown on non-aerated soil known to produce ethylene as compared to an aerated one. Bacteria isolated from water-logged soil were capable

of producing ethylene when grown on culture medium (129).

Involvement of actinomycetes in the production of volatile substances inhibitory to spore germination was demonstrated in sterilized soils recolonized by mixed soil actinomycetes (63). Inhibition was more prominent on soils with pH 7-8 than on those with pH 5.4-6.0. Streptomyces spp. which produce earthy-smelling compounds have been found to be abundant in soil between pH 6.5-8.0, and were favored by liming (65).

Amendment of soils with certain organic materials may result in production of volatile substances inhibitory to fungi. Cabbage and other crucifers have been known to produce sulfur-containing volatiles such as methanethiol, dimethyl sulfide, and dimethyl disulfide when incorporated into soil (89). These compounds were inhibitory to hyphal growth and to zoospore formation, motility, and germination in Aphanomyces euteiches (90). Production of volatile inhibitory compounds in chitin-amended soil has also been reported. Reduction in saprophytic growth and pathogenicity of Rhizoctonia solani was observed in natural, chitin-amended soil within 42 days of incubation (144, 146). In another case, germination of Aspergillus flavus and Fusarium solani f. sp. cucurbitae conidia was reduced by more than 40% in chitin-amended soil as compared to germination in water (132). The volatile inhibitory factor was completely absorbed by boric acid solution and when the conidia were incubated on NH_4Cl solution, similar inhibition in germination was obtained, suggesting that ammonia might at least be a component of the volatile inhibitory factor (133). A concentration of 60-197 ppm ammonia was also found in soil one week after amendment with 5% (w/w) alfalfa meal (51). Complete elimination of

Phytophthora cinnomomi within avocado root tissues was obtained when the infected roots were incubated for three to four days in a one-week-old alfalfa-amended soil.

Ammonia has been known to be effective in reducing plant pathogenic fungi and nematodes in soil (14, 37, 59, 60, 66, 113, 140). For example, reduction in populations of Fusarium spp. to zero, or nearly so, within the ammonia retention zone was obtained under field conditions 14 days after injection with anhydrous ammonia at a rate of 730 mg ammonia-N (140). Inside the retention zone with a radius of about 5 cm, Fusarium populations remained low or undetectable even after 225 days of injection. Complete loss in germinability of Sclerotium rolfsii sclerotia was also observed in soil amended with 0.2% ammonia (60). Reduction in germinability was correlated with increased numbers of antagonistic actinomycetes and with increase in soil pH. Birchfield et al (14) reported that more than 80% reduction in populations of Rotylenchus reniformis was obtained in soil within the ammonia retention zone following injection with about 20 ppm ammonia-N.

As mentioned earlier, volatile substances, especially at lower concentrations, may have stimulatory effects on soil microorganisms. Coley-Smith and his co-workers (27, 28, 78, 79) found that germination of sclerotia of Sclerotium cepivorum was stimulated by volatile substances evolved from growing Allium species. Germination of sclerotia in soil through which air was drawn after bubbling through Allium extracts was five times that when air was bubbled through water. A number of n-propyl and alkyl sulphides were identified from vapors of chopped garlic and onion bulbs and their extracts. Volatile alkyl sulphides, which were produced when alkyl cystein sulphoxides

originating from Allium roots were metabolized by soil bacteria, were stimulatory to Sclerotium cepivorum.

Gilbert and his co-workers (47, 48, 49, 94, 95, 96, 107, 115) found that volatiles from alfalfa hay and its distillate induced an immediate rise in respiration rate of soil microflora. The duration and magnitude of respiration were dependent on the volume of the distillate, one ml of which was yielded from 50 g alfalfa hay. The optimum volume for stimulation was 4 ml; a distillate volume as high as 16 ml was inhibitory. In addition to stimulating the respiration of soil microflora in general, volatiles from the distillate were also stimulatory to germination and growth of Verticillium dahliae and Sclerotium rolfsii sclerotia. At optimum concentration, two to five fold increase in populations of V. dahliae were obtained, while at higher concentrations decrease in populations occurred.

Detrimental effect of decomposing plant residues on plants

Decomposing plant residues have many and diverse effects upon soil, soil microflora, and living plants (45, 124). Although in general the beneficial effects far outweigh the detrimental ones, the latter may, under certain conditions, be of a considerable importance.

Compounds toxic to living plants may be produced in soil amended with plant residues especially under heavy, poorly aerated, waterlogged soils and relatively low temperature (125). The compounds represent a great variety of chemical classes such as acids, bases, esters, alcohols, aldehydes, nitrogenous compounds, and alkaloids (56, 105, 125). Soil amendment with plant residues, especially under the aforementioned conditions, may result in depletion of O_2 and N, and in

excessive production of CO_2, NH_3, and other gases (124). Free ammonia is known as a cell toxin; toxic effects on plants may be expected if its concentration becomes sufficiently high (105). Ammonia was found in soil at a concentration of 60-197 ppm after one week of amendment with 5% (w/w) alfalfa meal (51). At a concentration of 10^{-3} M or more, ammonia caused severe root and top stunting of avocado seedlings.

Extracts of soil amended with different plant residues have been found toxic to seedlings. For example, extracts of soil amended with timothy, rye, tobacco, and corn at a rate of 6% (w/w) for 15-20 days were toxic to tobacco, timothy, and barley seedlings (122). Phytotoxicity was also exerted by barley-amended soil on lettuce and bean seedlings during the first three weeks of amendment (126, 164). Phytotoxicity was associated with inhibition of seed germination and respiration, root stunting, necrosis, and death of apical meristems.

Enhancement of soil-borne diseases as a result of phytotoxic effects has also been reported (93, 123, 162). Exposure of tobacco plants to phytotoxic extracts from decomposing rye and timothy residues for 10 minutes increased their susceptibility to *Thielaviopsis basicola* root rot (123). Up to 50% of the root system of a wild *Nicotiana* sp., known to be highly resistant, became diseased when inoculated with *T. basicola* spores after exposure to the toxic extracts. Infection of bean stem segments by *Fusarium solani* f. sp. *phaseoli* was also enhanced after exposure to water-soluble extracts from barley, rye, wheat, timothy, broccoli, and broadbean residues (162). Chromatographic analysis showed that exudation of ninhydrin-positive substances was greatly increased from areas of the stem in contact with the various extracts and in the absence of the pathogen, suggesting

that the permeability of the exposed cells had been altered by the extracts.

In burying fresh plant materials in soil there is always some danger that such materials could be suitable substrates for non-obligate pathogens (45). The semi-linging plant materials will selectively favor colonization by these pathogens and retard colonization by obligate saprophytes. Fresh papaya residues in soil were readily colonized by Pythium aphanidermatum and Phytophthora parasitica, leading to a seedling root-rot problem in papaya nurseries. Living weeds buried under soil surface also served as food bases for Sclerotium rolfsii in peanut fields.

MATERIALS AND METHODS

Preparation of inocula

The species of Fusarium used in the experiments were recognized as in Snyder and Hansen system of classification (149, 150, 160). They were F. lateritium Link, F. moniliforme Sheld., F. oxysporum Schlecht., F. rigidiusculum (Brick) Snyd. & Hnas. [= F. decemcellulare Brick], F. roseum Snyd. & Hans. [=F. concolor Reinking], F. solani (Mart.) Sacc., F. solani f. sp. pisi (F.R. Jones) Snyd. & Hans., and F. tricinctum (Corda) Sacc. F. oxysporum and F. solani were isolated from diseased soybean seedlings, F. solani f. sp. pisi was isolated from pea, and the remaining five Fusarium species were obtained from Dr. T. Kommedahl of the University of Minnesota. The fungi were maintained as stock cultures on potato-dextrose agar (PDA) slants at 4°C. For production of inocula, the fungi were grown either on wheat bran-sand medium or in potato-maltose broth.

The wheat bran-sand medium was prepared by mixing 200 g ground wheat bran, 500 g, white silica sand, and 500 ml distilled water, followed by 60 minutes of steaming. The wheat bran was ground in a Wiley mill and passed through a 0.85 mm (20-mesh) sieve. The steamed mixture was broken up with a fork before putting it into Erlenmeyer flasks for steam sterilization. F. solani, F. solani f. sp. pisi, and F. oxysporum were individually grown in the medium for 4-6 weeks at 24°C before use. The medium was inoculated using spore suspensions of individual

species at a rate of about 10^5 spores/g. The wheat bran-sand cultures were vigroously shaken every other day during the first week of growth to obtain an even distribution of growth.

Potato-maltose broth was prepared by substituting maltose (20 g/l) for dextrose in the potato-dextrose broth. One hundred ml portions of the broth were poured into 250 ml Erlenmeyer flasks and autoclaved. F. rigidiusculum, F. roseum, F. moniliforme, F. tricinctum, and F. lateritium were individually grown in the medium for four weeks at 24°C with continuous shaking.

Soil preparation and infestation

Unless otherwise specified, Oshtemo-Boyer sandy loam soil (sand: sile:clay = 73:18:9, pH 5.5, 2.02% organic matter, water-holding capacity (WHC) = 287 ml/kg), collected from St. Joseph county, Michigan, was used throughout the experiments.

Soil was infested with F. solani, F. solani f. sp. pisi, and F. oxysporum by mixing the wheat bran-sand cultures, previously passed through a 1.7 mm (10-mesh) sieve, with the soil at an approximate rate of 10% (w/w). The soil was moistened to about 50% WHC and incubated for eight weeks, during which time a constant chlamydospore concentration of 10^4-10^6/g was achieved.

With the other Fusarium species, 100 ml portions of potato-maltose broth cultures, previously homogenized in a Servall Omni Mixer (Ivan Sorvall, Inc.) at a rheostat setting of 50 for 5 minutes, were mixed with 500 g soil in plastic bags, followed by incubation at 24°C for eight weeks. After the eight week incubation period, the infested soils were air-dried and kept at 4°C until further use.

Individual WHC of soils was determined using a standard method (128). A metal container with a porous bottom (diameter: 5cm, height: 3 cm) in which a moist filter paper was placed, was used. After placing 10 g of air-dried soil in the container, it was placed on a tray containing water deep enough to touch the first 1 mm layer of the soil. After two minutes on the tray the soil had absorbed an excessive amount of water. The container was then transferred to a moist chamber, placed on a piece of filter paper for about 10 minutes to drain the excessive water. Care was taken so that the soil in the container did not touch the filter paper sheet. The soil was then reweighed, the increase in weight from the initial indicating the WHC of the soil.

Soil pH, where necessary, was measured using a glass electrode (127). Twenty g of soil was mixed with 20 ml distilled water in a 50 ml beaker; the mixture was stirred at intervals for 30 minutes and let stand for one hour before pH measurement.

Incorporation of organic residues into soil

Ten different kinds of plant and animal residues were tested: mature stems of barley, wheat, corn, and soybean collected from the Michigan State University farm; commercial beet pulp, alfalfa meal, linseed meal, cottonseed meal, and soybean meal; and ground crab shells (supplied by H. Komada from Japan). The first five residues were ground and passed through a 1.7 mm (10-mesh) sieve before used. Unless otherwise specified, these materials were mixed with air-dried *Fusarium*-infested soil at a rate of 1% (w/w) by shaking in a plastic bag for 30 seconds. The moisture content was then adjusted to 30-35% WHC using tap water, and the moistened soil was further shaken for

another 30 seconds to obtain an even moisture distribution. The soil was then transferred into a 550 ml plastic container (200-300 g per container), covered with a sheet of polyethylene and secured with a rubber band. The treated soils were incubated at 24°C on a laboratory bench.

In some experiments, the organic materials were composted for different lengths of time before incorporation into soil. Composting was done by mixing individual organic materials with 20% (w/w) soil and 1% (w/w) urea. Two hundred g portions of the mixtures were moistened to about 30% WHC and incubated in sealed (Parafilm 'M', American Can Co.) 500 ml Erlenmeyer flasks for 4, 6, and 10 weeks at 28°C. During incubation, the mixtures were stirred weekly using a spatula. The composted materials were air-dried for 24 hrs before use.

Estimation of soil microbial populations

Soil microbial populations were estimated at intervals using serial dilutions of soils from different treatments in 0.1% water agar, and plating them on different selective media.

For Fusarium, a modified selective medium developed by Komada (86, 87) was used. The basal medium contained 1.0 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, 10.0 mg Fe-EDTA, 2.0 g asparagine, 20.0 g galactose, 15.0 g Difco agar, and 1000 ml distilled water. The antimicrobial supplement consisted of 1.0 g PCNB (75% wp), 0.5 g oxgall, 1.0 g $Na_2B_4O_7$. 10 H_2O, and 0.25 g chloramphenicol. This supplement was added after steaming the basal medium for one hour. Further steaming for another 30 minutes was required to completely dissolve the antimicrobial ingredients. The medium was then cooled to about 60°C in a

water bath before the pH was adjusted to 3.9 using 2 M H_3PO_4. After pouring onto sterile Petri plates (12 ml/plate) the medium was kept in plastic bags at 4°C for future use.

One-half ml of appropriate soil dilutions was pipetted into each plate, spread over the surface of the agar and incubated for one week at 24°C before Fusarium colonies were counted.

For fungi in general, PDA with 0.5 ml TMN detergent (Union Carbide Co.) and 0.25 g chloramphenicol per liter was prepared, poured in 5-7 ml portions into test tubes. After autoclaving for 15 minutes the agar medium was cooled to 45°C in a water bath and 0.5 ml of soil suspension was added, mixed for 5 seconds using a Vortex Jr. mixer, and plated. For bacteria a similar technique was employed except that a medium containing 100 ml soil extract, 1.0 g glucose, 0.5 g K_2HPO_4, 0.05 g PCNB (75% wp), 20 g Difco agar, and 900 ml distilled water was used. The soil extract was prepared by autoclaving 1 kg soil in 1 liter tap water, followed by repeated filtration until clear. For actinomycetes the selective medium contained 4.0 g colloidal chitin, 20 g Difco agar, and 1000 ml distilled water (69). Plates containing the three different media were incubated for one week at 24°C before colonies were counted.

Field experiments

Three separate experiments were conducted in the field using alfalfa and oilseed meals as organic amendments.

In the first two experiments, soil was either without supplemental infestation or was artificially infested with wheat bran-sand cultures of F. oxysporum and F. solani. In the third experiment, soil

was artificially infested with wheat bran-sand culture of F. solani f. sp. pisi. Infestation was done by incorporating each culture at a rate of ca. 1% (w/w) into the soil using a rototiller. Two weeks after infestation, soil samples were collected for estimation of the initial Fusarium populations. At the same time, the organic amendments were applied to the soil surface, and incorporated by rototilling. Changes in Fusarium populations were followed by collecting soil samples every two weeks.

Detection of volatile inhibitors

Several experiments were designed to determine if volatile inhibitors were involved in reducing Fusarium populations in the oilseed meal-amended soils. In one experiment, containers with or without polyethylene sheet covers were used for incubating treated soils. In another experiment, stainless steel planchets containing Fusarium-infested soil were incubated on the surfaces of treated soils in closed containers.

Possible presence of ammonia and/or amines in the volatiles was examined using 2% H_3BO_3 as a trapping solution (22,133) placed in glass vials and incubated on treated soils in closed containers. Periodically, the solutions were removed from the vials and titrated using 0.01 N H_2SO_4 to estimate the ammonia and/or amine equivalents (22). An indicator prepared by dissolving 0.33 g bromocresol green and 0.165 g methyl red in 500 ml ethyl alcohol was used in the titration.

Possible involvement of ammonia and/or amines in reducing Fusarium populations was tested by incubating Fusarium-infested soil in planchets together with H_3BO_3 solutions on treated soils in closed containers.

Fusarium populations were estimated at the beginning of appropriate experiments and bi-weekly thereafter.

Identification of volatile inhibitory substances

Four sets of 100 g portions of amended and unamended soils were prepared. The soils were maintained in plastic bags for four days, mixed every other day, before transferring to closed containers.

One set of the treated soils was used to verify that the volatile substances reduced Fusarium populations in soil. This was done by incubating planchets containing Fusarium-infested soil in the surfaces of amended and unamended soils in closed 500 ml containers.

The remaining three sets were utilized in the identification of the volatile substances using infra-red spectroscopy. The treated soils were incubated in 250 ml Erlenmeyer flasks sealed with rubber stoppers. Each stopper was equipped with a connecting glass tubing, 0.5 cm in diameter, which in turn was sealed at its terminus using a clamped tygon tubing (Figure 1). After three weeks of incubation, the gases evolved in each set of treated soils were handled in three different ways. In one, the gases were transferred directly to an evacuated infra-red cell. In the second and the third, the gasses were trapped by passing through a coiled glass tubing immersed in liquid nitrogen for a period of five minutes, either directly or indirectly via a column of anhydrous $CaSO_4$ (10 cm long, 1.7 cm diameter) (Figure 1). The two ends of the coiled glass tubing were then sealed using clamped tygon tubings, and the trapped gases were subsequently transferred to an evacuated infra-red cell for identification.

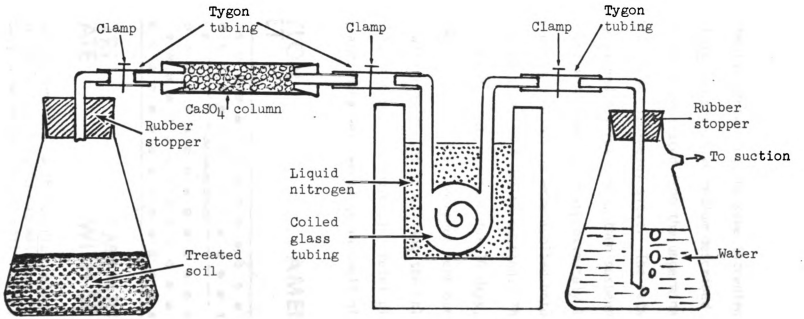

Figure 1. Schematic diagram of the system used in the collection of volatile substances evolved from treated soils for identification using infra-red spectroscopy. The flask containing water at the right end serves as a monitor for flow rate.

Observation on the fate of Fusarium chlamydospores in soil

Chlamydospores of F. solani were prepared according to the method of Hsu and Lockwood (68). Conidia were germinated for 16 hours in a shaking liquid medium containing the same ingredients as the basal portion of the Fusarium selective medium described earlier. The germlings were aseptically separated from the ungerminated conidia by filtration through 0.15 mm (100-mesh) sieve. The retained germlings were aseptically washed several times, then suspended in sterile distilled water to obtain a density of approximately 10^3 germlings/ml. Two ml portions of the suspension were applied onto Nuclepore membrane squares (1 cm^2, pore size: 0.4 um) by suction. The membranes were then floated on 0.03 M Na_2SO_4 solution for 10 days, during which time chlamydospores were formed. The membranes were buried in amended and unamended soils with a nylon net separating the chlamydospores from direct contact with the soils (Figure 2). Petri plates were used as the containers, and these were enclosed in small plastic bags.

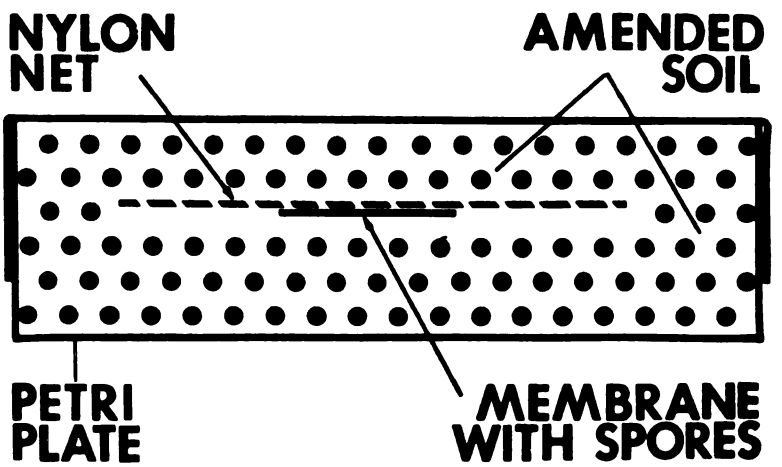

Figure 2. Nuclepore membrane with Fusarium chlamydospores on the upper surface. A nylon net was used to prevent direct contact between the treated soil and the chlamydospores.

The membranes were removed from the soils at intervals and the chlamydospores were transferred onto discs of the selective agar medium to observe germination (143).

Bioassay using soybeans (Glycine max (L.) Merr.) and peas (Pisum sativum L.) as host plants

To observe whether reduction in Fusarium populations would correlate with reduction in root rot, soils infested with either a mixture of F. oxysporum and F. solani (isolated from diseased soybean roots) or F. solani f. sp. pisi were amended with 1% (w/w) alfalfa and oilseed meals. Unamended but infested control soils were included for comparison. After four weeks of incubation in closed containers, all treated soils were spread on plastic trays to form a layer of about 1 cm thick and air-dried for one week at 24°C. After air-drying, the soils were individually remoistened to about 30% WHC and four 200 g-portions of each soil were transferred into 200 ml styrofoam cups. Soybeans (var. Hark) or peas (var. Miragreen) were planted, six in each cup, and watered every day using 20 ml tap water per cup. The cups were incubated in a growth chamber at 27°C, a photoperiod of 12 hrs/day, and a light intensity of about 20,000 lux.

Three weeks after planting, the plants were uprooted and the roots were washed with running tap water. Root rot was then assessed on a scale of 0-6, with 0 indicating a disease-free root system and 6, most severely diseased (173).

Statistical analysis

In experiments using the dilution plate method, data were presented as averages of 2-3 replications, each consisting of 3-6 plates.

Analysis of variance was done using data transformed to log (X + 1) to maintain homogeneity of the variances of different treatments (155). In the other experiments, 3 replications were used, and data were analyzed without transformation. Most experiments, where necessary, were repeated to verify the consistency in results. Significant differences among treatments were estimated using least significant ranges (L.S.R.) obtained from the Tukey's '_w_' procedure.

RESULTS

Effect of different soil amendments on Fusarium populations

Three hundred g portions of soil infested with F. oxysporum and F. solani were individually amended with nine different kinds of ground plant and animal residues, each at 1% (w/w) concentration. The soil moisture level was adjusted to 30% WHC and the treated soils were incubated in closed containers at 24°C. Estimation of Fusarium populations was done at the beginning of the experiment and at weekly intervals.

Linseed and cottonseed meal amendments were the most effective in reducing the Fusarium population (Figure 3). Reduction to 10^1-10^2/g was obtained using these amendments, and to 10^3-10^4/g with crabshell, as compared with more than 10^5/g in the control and other treatments. Fusarium population reductions by cottonseed meal, linseed meal, and ground crabshell were significant at the 1% level (Table 1). However, since the Fusarium population in soil amended with ground crabshell was still high, this amendment was not used further in the work. Later it was found that soybean meal was equally or more effective in reducing Fusarium populations.

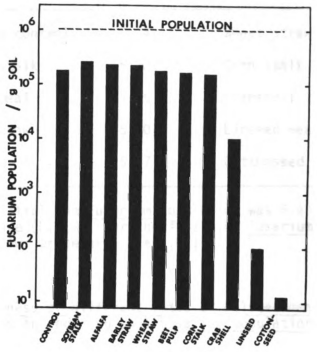

Figure 3. Fusarium populations in soils four weeks after amendment with 1% (w/w) ground plant and animal residues.

Table 1. *Fusarium* populations in soils four weeks after amendment with 1% (w/w) ground plant and animal residues.

Soil treatment	Log *Fusarium* population/ g soil[a]	Soil treatment	Log *Fusarium* populations/ g soil[a]
Unamended control	5.74	Wheat straw	5.76
Soybean stalk	5.92	Corn stalk	5.64
Alfalfa meal	5.85	Crabshell	3.85
Barley straw	5.80	Linseed meal	2.14
Beet pulp	5.77	Cottonseed meal	1.57

[a]Initial log *Fusarium* population was 6.49/g soil. Least Significant Range (L.S.R.) for the final log *Fusarium* populations was 1.51 (Tukey's '*w*' procedure, *P* = 0.01).

Effectiveness of different rates of oilseed meal amendments in reducing soil Fusarium populations

Soils were amended with oilseed meals at rates of (w/w) 1%, 0.50%, and 0.25%, and incubated in closed containers. Unamended control soil and soil amended with 1% (w/w) alfalfa meal were included for comparison.

Generally, soil *Fusarium* populations decreased with the increase in amendment rates and with time over a six week period (Figure 4). However, soybean meal applied at a rate as low as 0.25%(w/w) still reduced *Fusarium* populations to less than 0.1% of the unamended control after six weeks of incubation. More than 1000 fold reduction was also obtained in soils amended with linseed and cottonseed meals at a rate as low as 0.50% (w/w).

When the experiment was completed, the soils containing 1% (w/w)

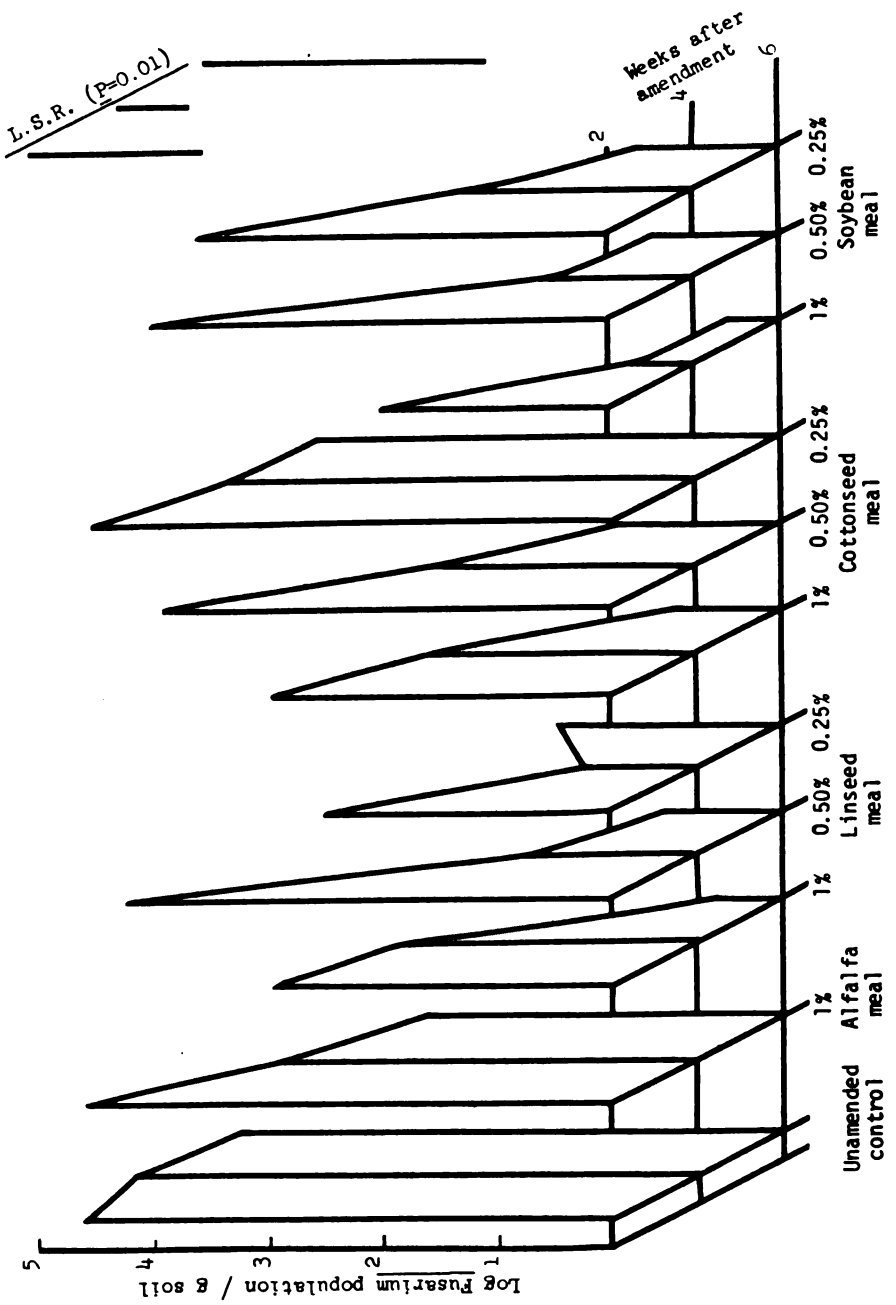

Figure 4. Fusarium populations in soils amended with different concentrations (%, w/w) of alfalfa or oilseed meals after two to six weeks of incubation. Least Significant Range (L.S.R.) values were obtained using the Tukey's 'w' procedure.

amendments were incubated for another four weeks without covers; moisture contents were adjusted weekly by weighing. During this period, Fusarium populations in the oilseed meal-amended soils remained in the order of 10^1 propagules/g. Fusarium populations in the alfalfa meal-amended and unamended soils were still more than 10^4 propagules/g at the end of the experiment. This result suggested that the oilseed meal-amended soils maintained their fungistatic properties against recolonization by the Fusarium.

Effect of soil amendments on soil microorganisms

Three hundred g portions of soil, either unamended or amended with 1% (w/w) linseed and cottonseed meals were incubated in closed containers after the moisture content had been adjusted to 30% WHC. Estimation of soil microbial populations was done by employing four different media selective to either Fusarium, fungi in general, bacteria, or actinomycetes. Soil microbial populations were estimated at intervals using dilution plates containing the selective media.

After four weeks of incubation, Fusarium populations in oilseed meal-amended soils were reduced to 0.1% or less of the unamended control (Table 2). After five weeks of incubation, the total fungal populations in the amended soils were also highly significantly reduced, but less than that of Fusarium; the densities were still in the order of about 10^4 propagules/g compared to about 10^6 propagules/g in the initial population. The most frequently isolated fungi were from the order Mucorales and from the genus Trichoderma. Similar reductions were observed in soybean meal-amended soil. The numbers of actinomycetes and bacteria were only slightly (but significantly) lower in the amended soils than in unamended control.

Table 2. Soil microbial populations four to five weeks after amendment with 1% (w/w) linseed and cottonseed meals, as determined using selective media.

Microorganism	Initial log populations/ g soil	Log populations/g soil			L.S.R.[a]	
		Unamended control	Linseed meal	Cottonseed meal	$P = 0.05$	$P = 0.01$
Fusarium spp.[b]	5.53	5.45	2.41	2.46		1.84
Total fungi[c]	5.98	5.68	4.13	3.94		0.35
Bacteria[c]	7.29	6.40	5.98	5.99	0.26	
Actinomycetes[c]	6.85	6.75	5.98	6.01		0.44

[a]Least Significant Range (L.S.R.) values were obtained using the Tukey's '*w*' procedure.

[b]Populations were estimated four weeks after amendment.

[c]Populations were estimated five weeks after amendment.

Effect of increased soil pH on reduction in Fusarium populations

Oilseed meal-amended soils consistently showed an increase in pH up to 2.3 units as compared to alfalfa meal-amended and unamended soils. Therefore, an experiment was carried out to test whether artificially raising the soil pH would enhance the effectiveness of oilseed meal amendments.

Artificially infested soil was treated with 0.17% (w/w) $Ca(OH)_2$ to increase the natural pH (5.5) to 8.1. Three hundred g portions of this soil were amended with 1% (w/w) alfalfa and oilseed meals. Infested soils with natural pH were similarly amended for comparison. After adjusting the moisture content to 30% WHC, the treated soils were incubated in closed containers.

Linseed and cottonseed meal-amended soils with increased pH showed less reduction in Fusarium populations than those with natural pH after four weeks of incubation. However, soybean meal-amended soil with increased pH showed as much reduction as that with natural pH. After six weeks of incubation, highly significant reduction in Fusarium populations was obtained in oilseed meal-amended soils whether or not treated with $Ca(OH)_2$, as compared to the alfalfa meal-amended soil (Table 3). Except for soybean meal, however, Fusarium populations were always higher in the $Ca(OH)_2$-treated soils than in soils with natural pH. The pH of the natural, but oilseed-meal amended soils, was increased by 1.2-2.7 units as compared to that of unamended soil; the pH of the alfalfa meal-amended soil remained nearly constant (Table 3). In the $Ca(OH)_2$-treated soil, the pH of unamended soil and of soils amended with alfalfa, linseed, and cottonseed meals dropped

from 0.7-1.2 units (from pH 8.1 at the start of incubation). In contrast, soybean meal-amended soil showed an increase of 1.3 pH units.

Table 3. Effect of increased soil pH on reduction in *Fusarium* populations in soil six weeks after amendment with 1% (w/w) alfalfa and oilseed meals.

Soil treatment	Log *Fusarium* populations[a]/g soil		Final pH[b]	
	Natural soil	$Ca(OH)_2$-treated soil	Natural soil	$Ca(OH)_2$-treated soil
Alfalfa meal	3.85	4.13	5.3	6.9
Linseed meal	1.04	2.38	6.5	7.4
Cottonseed meal	0.82	1.58	7.0	7.0
Soybean meal	0.48	0.48	8.1	9.4

[a]Initial log *Fusarium* population was 4.7/g soil. Least Significant Range for the final log *Fusarium* populations was 1.70 (Tukey's '*w*' procedure, *P* = 0.01).

[b]Soil was treated with 0.17% (w/w) $Ca(OH)_2$ to raise the pH from 5.5 to 8.1. The final pH of the natural and the $Ca(OH)_2$-treated control soils were 5.4 and 7.4, respectively.

Effect of soil amendment with composted organic materials on Fusarium populations

Three hundred g portions of *Fusarium*-infested soil were individually amended with 1% (w/w) composted oilseed meals to observe whether reduction in the populations could be hastened. Soil amended with composted alfalfa meal and unamended soil were included for comparison. Incubation of the treated soils was done in closed containers.

Only slight reduction in *Fusarium* populations was obtained during

the first 10-14 days of amendment. Fusarium populations were drastically reduced by four weeks after amendment with oilseed meal composts 4, 6, and 10 weeks old (Table 4). However, significantly less reduction occurred in soils amended with 6 and 10 week old cottonseed composts and 10 week old soybean compost. Soil amendement with 4 and 6 week old alfalfa composts exerted no effect on Fusarium populations, but soil amended with 10 week old alfalfa compost had significant higher Fusarium populations than the unamended control. These results indicated that composting neither hastened nor enhanced the effectiveness of the oilseed meal amendments in reducing Fusarium populations.

Field experiments using uncovered plots

To test whether the oilseed meal amendments effective under laboratory conditions would also be effective in the field, two experiments were carried out in St. Joseph county, S.W. Michigan, in Spring and early Summer of 1976.

One experiment was done near Nottawa at a site where Fusarium seed and seedling disease of soybeans was found extensively during the previous year. Natural soil and soil artificially infested with wheat bran-sand cultures of F. oxysporum and F. solani were amended with alfalfa, linseed, and cottonseed meals at rates of 1%, 0.5%, and 0.25% based on the weight of soil in rows, 3 meters long, 15 cm wide, and 15 cm deep. Unamended controls were included, and each treatment was replicated three times.

The other experiment was done near Burr Oak where soybean seedlings failed to emerge after the Spring planting and Fusarium was associated with the root rot symptoms. The treatments consisted of

Table 4. Log *Fusarium* populations from soils after four weeks of amendment using 1% (w/w) composted alfalfa and oilseed meals.

Compost age[a]	Log *Fusarium* population/g soil					L.S.R.[b] ($P = 0.01$)
	Unamended control	Alfalfa	Linseed	Cottonseed	Soybean	
4 weeks	5.55	5.37	1.15	1.25	1.15	0.39
6 weeks	4.53	4.12	1.41	2.34	1.07	0.62
10 weeks	4.17	5.14	1.07	2.46	2.20	0.48

[a]Composting was done by mixing individual organic material with soil at a weight ratio of 80:20, plus 1% (w/w) urea. The mixture was moistened to about 30% WHC, incubated in 500 ml Erlenmeyer flasks at 28°C, and stirred weekly. The composted materials were air-dried before use.

[b]Least Significant Range (L.S.R.) values were obtained using the Tukey's '*w*' procedure.

1% (w/w) amendments of alfalfa meal, linseed meal, cottonseed meal, and unamended control; each was replicated three times. The plot size for each treatment was the same as that in the earlier experiment.

Fusarium populations were estimated immediately before soil amendment and bi-weekly thereafter. Most of the amended plots showed higher Fusarium populations after 4-5 weeks as compared to the unamended controls (Tables 5 and 6). By 9-12 weeks after amendment, Fusarium populations in all amended soils were higher than those in the unamended control soils. Therefore, the results obtained from these field experiments did not support those obtained under laboratory conditions. Under the laboratory conditions the treated soils were maintained at about 30% WHC and incubated in polyethylene sheet-covered containers. In the field, the soil moisture may fluctuate and the plots were not covered with polyethylene sheets.

Effect of soil moisture content on reduction in Fusarium populations

Three hundred g portions of artificially infested soil were either unamended or amended with 1% (w/w) alfalfa, linseed, and cottonseed meals. The moisture contents of the treated soils were adjusted to 15%, 30%, 50%, and 100% WHC before incubation in closed containers at 24°C. Fusarium populations were estimated at the beginning of the experiment and bi-weekly thereafter.

Four weeks after amendment reduction in Fusarium populations was greatest in linseed and cottonseed meal-amended soils with soil moisture maintained at 30% and 50% WHC; reduction was not as great at 100% WHC (Figure 5). Fusarium populations were increased slightly in those soils at 15% WHC. In the unamended soils, Fusarium populations were

Table 5. *Fusarium* populations in natural and artificially infested field soils five weeks after amendment with alfalfa and oilseed meals. The experiment was done near Nottawa in St. Joseph county, Michigan, in the Spring, 1976.

Soil treatment	Amendment, %(w/w)	Log *Fusarium* population/g soil[a]	
		Natural soil	Artificially infested
Unamended control		3.32	4.08
Alfalfa meal	1.00	4.28	4.53
	0.50	4.28	4.48
	0.25	3.97	4.20
Linseed meal	1.00	4.34	4.20
	0.50	4.08	4.20
	0.25	3.80	3.89
Cottonseed meal	1.00	4.25	4.40
	0.50	4.34	4.30
	0.25	3.96	4.30

[a]Log *Fusarium* populations at the time of soil amendment in natural and artificially infested soils were 3.60 and 4.84, respectively.

Table 6. *Fusarium* populations in naturally infested field soil four weeks after amendment with 1% (w/w) alfalfa and oilseed meals. The experiment was carried out near Burr Oak, St. Joseph county, Michigan, in early Summer, 1976.

Soil treatment	Log *Fusarium* population per g soil[a]
Unamended control	3.45
Alfalfa meal	5.18
Linseed meal	5.20
Cottonseed meal	5.04

[a]Initial log *Fusarium* population was 3.28.

affected only slightly by soil moisture.

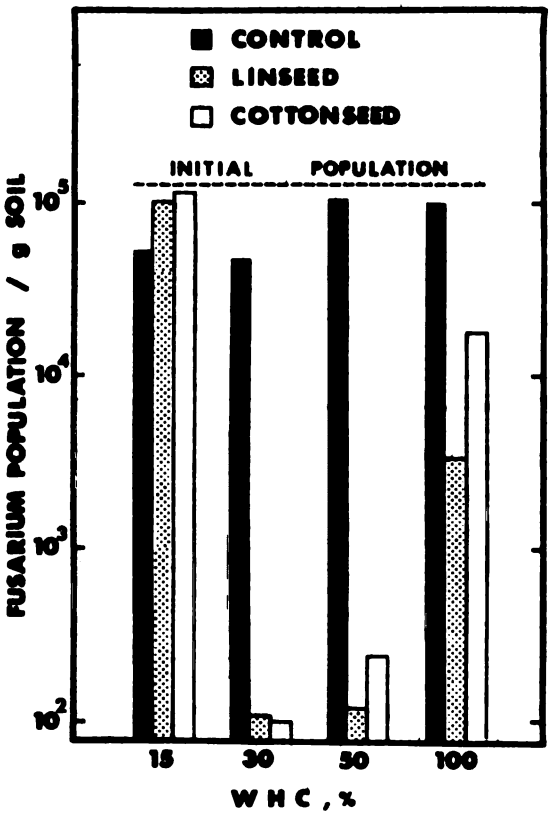

Figure 5. Fusarium populations in soils four weeks after amendment with 1% (w/w) linseed and cottonseed meals, as affected by different soil moisture contents in closed containers.

In another experiment, soybean and alfalfa meals were included. Similar trends in reduction of Fusarium populations were shown by linseed and cottonseed meal amendments. However, soybean meal was effective at all moisture levels tested, although reduction in Fusarium populations at 15% WHC was significantly less than that at other soil moisture levels (Table 7). Fusarium populations in the alfalfa meal-amended soil, like those in the unamended control soil, were not

much affected by soil moisture.

Table 7. Effect of different soil moisture contents on reduction of Fusarium populations four weeks after amendment with 1% (w/w) alfalfa and oilseed meals.

Soil treatment	Log Fusarium population/g soil at indicated soil moisture content (% WHC)[a]			
	15	30	50	100
Unamended control	4.86	4.78	5.08	5.00
Alfalfa meal	4.71	5.02	4.95	4.48
Linseed meal	5.08	2.15	2.23	3.76
Cottonseed meal	5.23	1.30	2.62	4.49
Soybean meal	1.21	0.52	0.52	0.52

[a]Initial log Fusarium population was 5.25. The Least Significant Range (Tukey's 'w' procedure, $P = 0.01$) for the soil treatment X soil moisture effect was 0.68.

Detection of volatile inhibitors

An experiment was done to compare the effect of oilseed meal-amended soils incubated in open and closed containers on Fusarium populations. Soils were either unamended or amended with 1% (w/w) alfalfa, linseed, and soybean meals; the moisture content was adjusted to 30% WHC. Two sets of such soil treatments were prepared; one was incubated in polyethylene sheet-covered containers, the other in open containers. The moisture contents of the soils in the open containers were maintained constant by addition of water every day. Fusarium populations were estimated at the beginning of the experiment and

bi-weekly thereafter.

Oilseed meal-amended soil kept in open containers showed less reduction in Fusarium populations than those kept in closed containers (Figure 6). Six weeks after amendment, Fusarium populations in the oilseed meal-amended soils in closed containers were reduced to about 10^2 propagules/g or less, as compared to more than 10^4 propagules/g in open containers. Fusarium populations in alfalfa meal-amended and in the unamended control soils showed little or no reduction in either type of containers. The much greater reduction in closed containers indicated that volatile substances might be involved.

To further test this possibility, 300 g portions of soil either unamended or amended with 1% (w/w) alfalfa and oilseed meals were prepared. Planchets, each containing 2 g of Fusarium-infested soil, were incubated on the surfaces of the treated soils in closed containers. Four planchets were incubated on each soil treatment. Fusarium populations were estimated initially and bi-weekly thereafter.

After four weeks of incubation on oilseed meal-amended soils Fusarium populations in soils from the planchets were reduced to less than 0.1% of those incubated on the unamended and alfalfa meal-amended soils (Figure 7). Fusarium populations from planchets incubated on the unamended and alfalfa meal-amended soils were not reduced. This result clearly showed that reduction in Fusarium populations was obtained in soil samples separated from direct contact with the amended soils, and strongly indicated the presence of volatile substances.

To test whether ammonia and/or amines were present in the volatiles, 2% of H_3BO_3 solution was used as the trapping agent. The solution was placed in glass vials and incubated on treated soils in

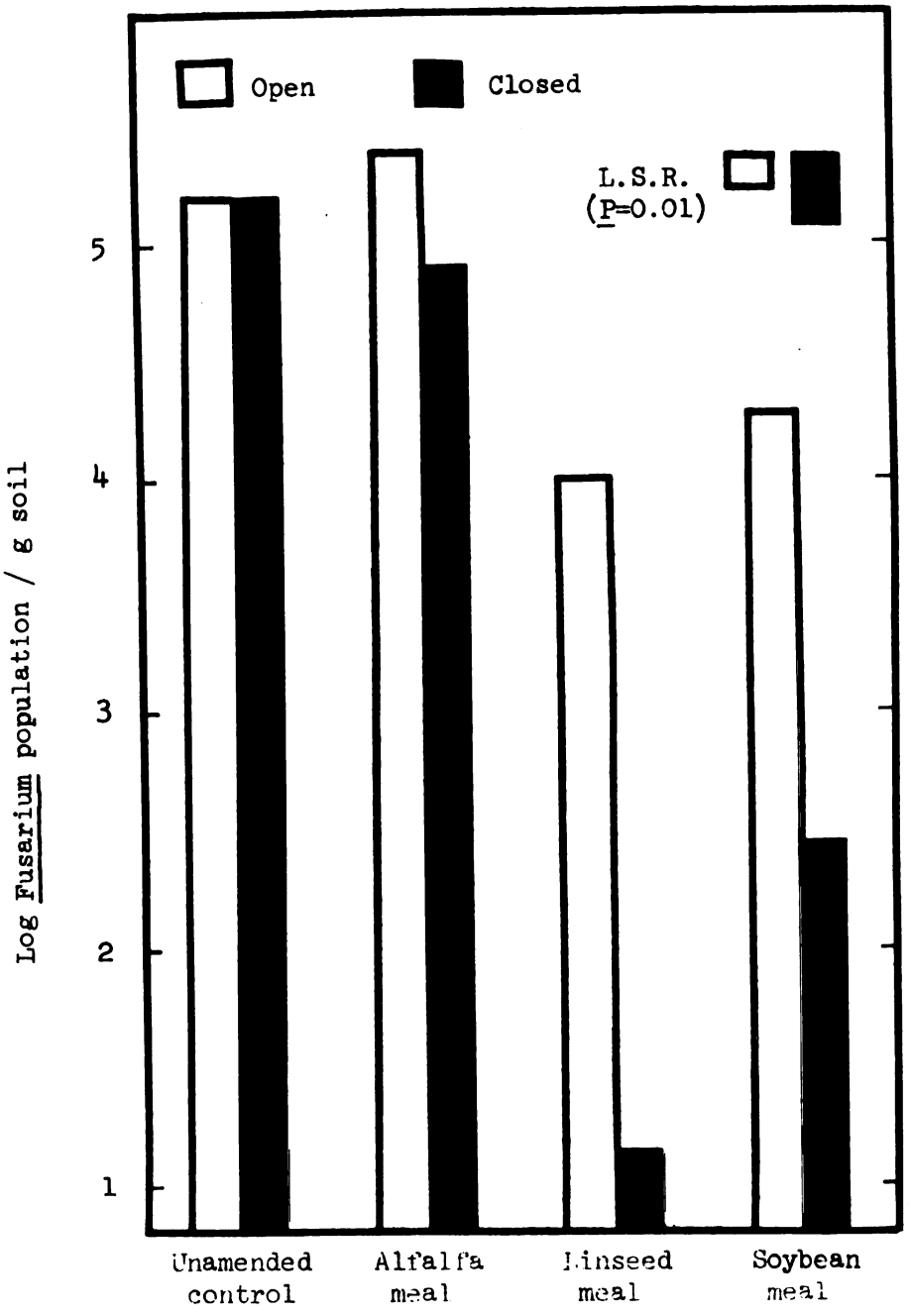

Figure 6. Fusarium populations in soil placed in open and closed containers 6 weeks after amendment with 1% (w/w) alfalfa or oilseed meals. Least Significant Range (L.S.R.) values were obtained using the Tukey's 'w' procedure.

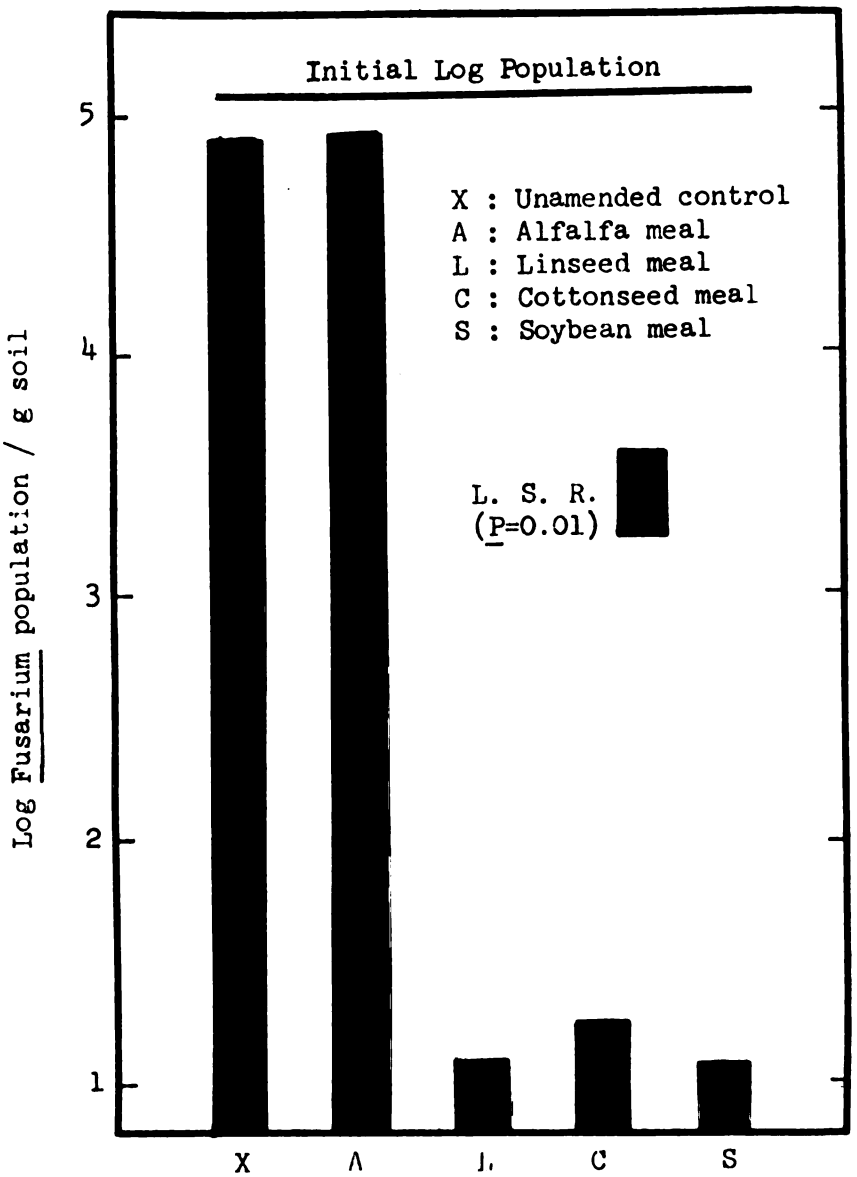

Figure 7. Fusarium populations from soil samples in planchets incubated on soil amended with 1% (w/w) alfalfa or oilseed meals in closed containers for four weeks. The Least Significant Range (L.S.R.) was obtained using Tukey's 'w' procedure.

closed containers. The soils were either unamended or amended with 1% (w/w) alfalfa and oilseed meals, and the moisture contents were adjusted to 30% WHC. At intervals the trapping solutions were removed from the vials and three drops of indicator were added. The solutions were then titrated with 0.01 N H_2SO_4 to estimate the ammonia or amine equivalents. The H_3BO_3 solutions were replenished immediately after each removal.

The lartest amount of titratable materials was derived from soil amended with soybean meal, followed by those amended with linseed and cottonseed meals (Figure 8). The H_3BO_3 solutions collected from the alfalfa meal-amended and the unamended control soils did not change the color of the added indicator.

An experiment was carried out to examine whether ammonia and/or amines trapped by the H_3BO_3 solutions contributed to reduction in Fusarium populations in soil. Four planchets, each containing 3 g of Fusarium-infested soil and four small Petri plates (5.5 cm diameter), each containing 10 ml of either 2% H_3BO_3, H_2O, or nothing at all, were incubated together on 500 g treated soils in closed containers. Square plastic containers, (19 X 19 X 16) cm^3, individually enclosed in polyethylene bags were used as the incubation chambers. The four planchets were placed in the center, and the Petri plates at the four corners of each containers. Fusarium populations were estimated at the beginning of the experiment and bi-weekly thereafter.

After four weeks of incubation, drastic reduction in Fusarium populations occurred in soils in planchets incubated on linseed and cottonseed meal-amended soils, regardless of the presence of the trapping solutions (Table 8). However, no reduction was obtained during

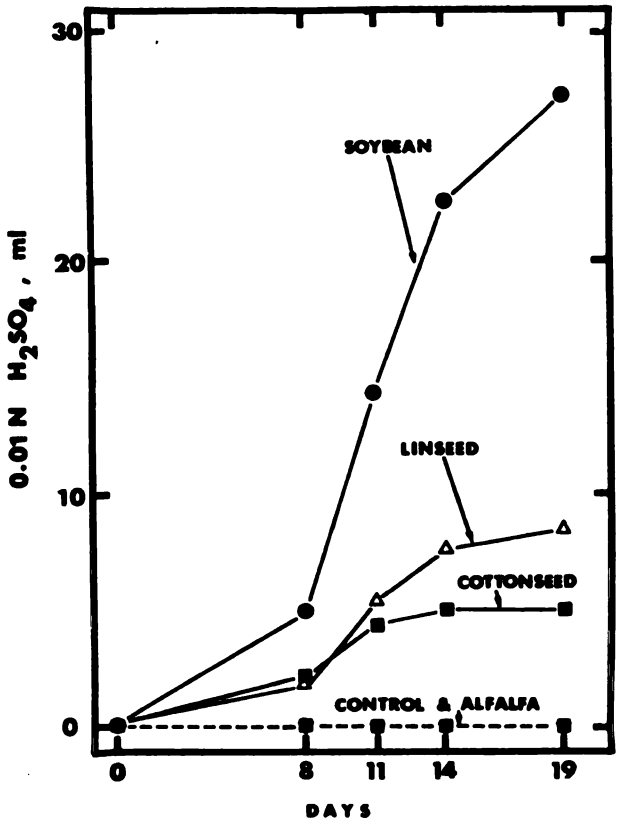

Figure 8. Titration curves for the estimation of ammonia and/or amines trapped by 2% H_3BO_3 solutions. The H_3BO_3 solutions were placed in glass vials and incubated on soils either unamended or amended with 1% (w/w) alfalfa and oilseed meals in closed containers. An indicator was added to the H_3BO_3 solutions before titrating with 0.01 N H_2SO_4.

incubation on soybean meal-amended soil in the presence of the trapping solutions, although drastic reduction occurred in their absence. Fusarium populations in soils incubated on the unamended control and the alfalfa meal-amended soils were not affected by the presence of the trapping solutions.

Table 8. Fusarium populations from soil samples in metal planchets placed on soil amended with 1% (w/w) alfalfa and oilseed meals. The soil samples were incubated in the presence of H_2O and H_3BO_3 as trapping solutions in closed containers for four weeks.

Trapping solution	Log Fusarium population[a]/g soil sample				
	Unamended control	Alfalfa meal	Linseed meal	Cottonseed meal	Soybean meal
None	4.90	4.43	2.24	2.02	1.48
H_2O	4.74	4.51	1.74	1.48	5.00
H_3BO_3	4.87	4.87	2.66	1.48	5.00

[a]Initial log Fusarium population was 5.30. The Least Significant Range (Tukey's 'w' procedure, P = 0.01) for the trapping solution X soil treatment effect was 1.38.

Identification of volatile inhibitory substances

Infra-red spectroscopy was used in attempting to identify the volatile inhibitory substances. Four sets of amended and unamended soils were prepared. In one set Fusarium-infested soil samples placed in planchets were incubated on the surfaces of treated soils in closed containers. After four weeks of incubation, Fusarium populations in soil incubated on oilseed meal-amended soils were reduced to less than

0.1% of those incubated on alfalfa meal-amended and unamended control soils, indicating that volatile substances inhibitory to the fungus were produced.

The other three sets of treated soils were incubated in sealed Erlenmeyer flasks. After three weeks of incubation the gases evolved in each soil were transferred to an evacuated infra-red cell, either directly or by first trapping them in liquid nitrogen. Gases originated from all amended soils showed formation of crystals upon passing through coiled glass tubings immersed in the liquid nitrogen. However, when the transferred gases were scanned with wavenumbers ranging from 850-2400 cm^{-1}, no indication of the presence of substances other than CO_2 (at 2360 cm^{-1}) was observed on the infra-red spectrograms. In view of the fact that oilseed meal amendments showed substances which were trapped in H_3BO_3 and titratable by 0.01 N H_2SO_4 (Figure 8), the method of obtaining samples of the volatile substances in the present experiment may not be an appropriate one.

The fate of Fusarium chlamydospores in the amended soils

To study the mode of reduction in Fusarium populations in the oilseed meal-amended soils, Fusarium chlamydospores were placed on membranes and buried in amended soils for different lengths of time. Ten membranes were buried in each 100 g portion of treated soil in a Petri plate. Two membranes were removed from individual soil treatments after each incubation period and the chlamydospores were transferred onto discs of a selective agar medium for microscopic observation on germination, either immediately or following a 24 hr incubation period on the agar discs. Cotton blue in lactophenol was used

to stain the chlamydospores and the germ tubes. Percent germination was estimated based on 100 counts from four different microscope fields on a single membrane.

In one experiment, the chlamydospores were buried at the same time as the soils were amended. When the membranes were taken out 24 hours after burial and immediately observed under a microscope, 95% or more of the chlamydospores from all amended soils had germinated, while none had germinated in the unamended soil. Production of conidia and some lysis of hyphae had occurred in all amended soils by 48 hours of burial. Lysis of hyphae was very extensive and production of new chlamydospores was frequently seen after three days burial in the amended soils. When membranes were removed from amended soils 7-21 days after burial, many new chlamydospores were present but none had germ tubes. No hyphae were present.

After seven days of incubation in the oilseed meal-amended soils, less than 30% of the chlamydospores germinated after 24 hour incubation on the agar discs, whereas the germinability of the chlamydospores buried in the unamended and the alfalfa meal-amended soils was more than 80% (Figure 9). After 21 days of incubation in the oilseed meal-amended soils, the germinability of the chlamydospores was completely lost, while in the unamended and the alfalfa meal-amended soils 65% or more of the chlamydospores were still viable.

In another experiment, the chlamydospores were buried seven days after soil amendment. After seven more days of burial in the oilseed meal-amended soils, the germinability of the chlamydospores was practically zero, whereas those buried in the unamended and the alfalfa meal-amended soils still showed 80% or more germination (Figure 10).

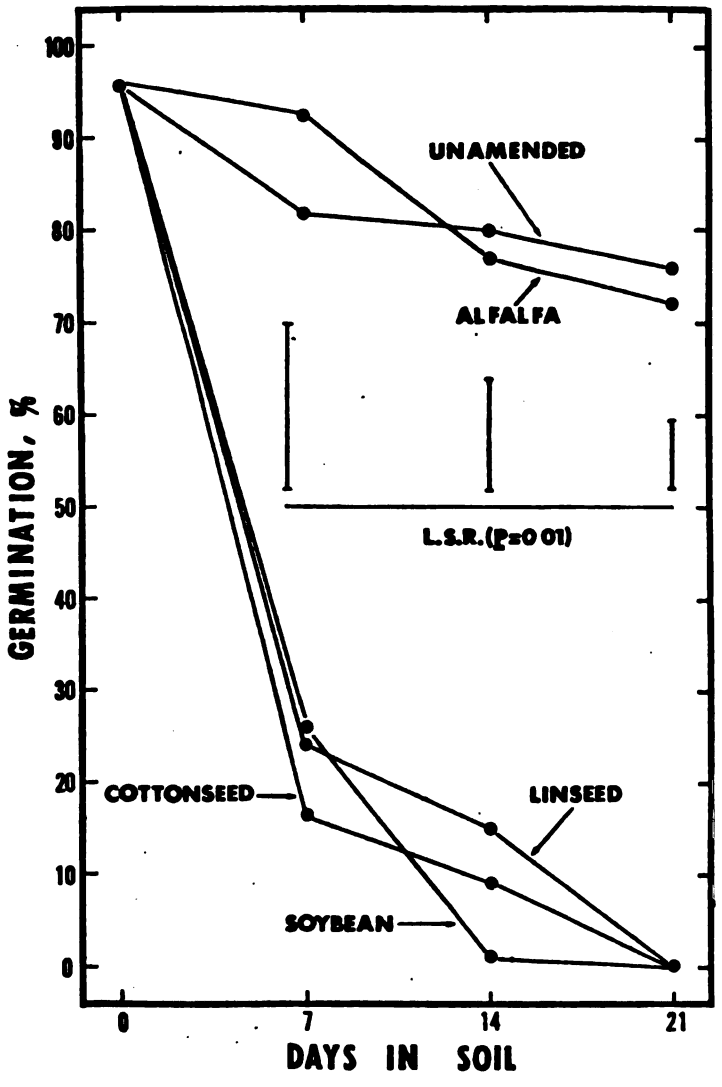

Figure 9. Viability of Fusarium chlamydospores as determined by the agar disc method. The chlamydospores prepared on Nuclepore membranes were incubated in soil amended with 1% (w/w) alfalfa and oilseed meals for different lengths of time. After each incubation period. membrane samples were removed and the chlamydospores were transferred onto discs of a selective agar medium for microscopic observation of germination either immediately or following a 24 hr incubation on the discs. Least Significant Range (L.S.R.) values were obtained using Tukey's 'w' procedure.

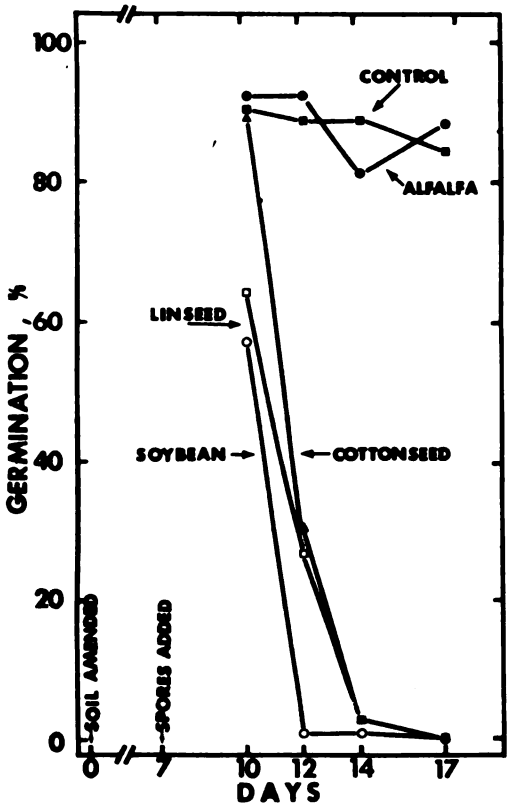

Figure 10. Viability of Fusarium chlamydospores as determined by the agar disc method. The chlamydospores were buried seven days after soil treatment. Technical descriptions for this experiment were as described in Figure 9. Least Significant Range (L.S.R.) values for 7 and 10 days of burial were 13.5 and 42.3, respectively (Tukey's 'w' procedure, P = 0.01).

Similar results were obtained when the membranes were removed from the treated soils after 10 days of burial.

Effect of oilseed meal amendments on different species of Fusarium

Two g portions of soil infested with either F. lateritium, F. moniliforme, F. rigidiusculum, F. roseum, or F. tricinctum were incubated on the surface of 500 g soil either unamended or amended with 1% (w/w) alfalfa and oilseed meals in closed containers, (19 X 19 X 6) cm^3.

After four weeks of incubation, all five Fusarium species tested were drastically reduced on the oilseed meal-amended soils (Table 9). None of the Fusarium species were reduced on the unamended and the alfalfa meal-amended soils.

Relation of soil Fusarium populations to amount of root rot

Oilseed meal-amended soils showed typically reduced Fusarium populations after four weeks. Using soybeans as host plants, a positive correlation between root rot and Fusarium populations/g soil was obtained. Soybean meal amendment seemed to be the most effective, as compared to linseed and cottonseed meal amendments, based on the fact that disease was reduced and no phytotoxic symptoms were observed. Phytotoxicity occurred in cottonseed meal- and linseed meal-amended soils; especially in the latter, germination of soybean seeds was completely inhibited. More root rot was associated with the unamended and alfalfa meal-amended soils. Because of the difficulty in obtaining root rot disease symptoms with soybean, due to apparently specific environmental requirements which are not yet completely worked out,

Table 9. Populations of different *Fusarium* species in soil samples placed in planchets after four weeks incubation on soils either unamended or amended with 1% (w/w) alfalfa and oilseed meals.

Fusarium spp.[a]	Log *Fusarium* population/g soil					L.S.R.[b] (*P* = 0.01)
	Unamended control	Alfalfa meal	Linseed meal	Cottonseed meal	Soybean meal	
F. lateritium	3.91	4.06	1.40	1.40	1.25	0.24
F. moniliforme	4.62	4.44	1.55	1.55	1.10	0.32
F. rigidiusculum	4.65	4.49	1.70	1.40	1.25	0.24
F. roseum	4.12	4.35	1.40	1.40	1.10	0.08
F. tricinctum	4.14	4.38	1.55	1.55	1.25	0.39

[a]Individual *Fusarium* spp. were grown in potato-maltose broth for four weeks, homogenized, and applied individually into soil. The infested soils were incubated for eight weeks before use.

[b]The Least Significant Range (L.S.R.) values were obtained using the Tukey's '*w*' procedure.

Fusarium root rot of peas was used in further work.

Reduction in root rot of peas was also associated with the decrease in Fusarium populations. Log F. solani f. sp. pisi populations/g of unamended soil and soils amended with 1% (w/w) alfalfa, linseed, cottonseed, and soybean meals were 3.90, 3.76, 2.00, 1.70, and 1.40, respectively; and the disease indices of peas after a three week growth were 3.0, 2.7, 2.1, 1.6, and 0.9, respectively (Table 10). However, significant reduction in root rot severity was only obtained in soybean meal-amended soil as compared to the alfalfa meal-amended ($P = 0.05$) and the unamended control ($P = 0.01$) soils.

Table 10. Severity of pea root rot caused by Fusarium solani f. sp. pisi as affected by reduced populations of the pathogenic propagules after four weeks of incubation in soil amended with 1% (w/w) oilseed meals.

Soil treatment	Log Fusarium population/g soil[a]	Disease index[b]
Unamended control	3.90	3.0
Alfalfa meal	3.76	2.7
Linseed meal	2.00	2.1
Cottonseed meal	1.70	1.6
Soybean meal	1.40	0.9

[a]Initial log Fusarium population/g soil was 4.35.

[b]Root rot was rated on a scale of 0-6, with 0 indicating no disease and 6, most severe disease. The Least Significant Range (Tukey's 'w' procedure) values for the disease indices were 1.5 ($P = 0.05$) and 2.0 ($P = 0.01$).

In this experiment, the least range required to show significant differences among treatments was relatively wide, presumably due to the great variations in root rot indices in the presence of linseed and cottonseed meal amendments. Both treatments caused some phytotoxic symptoms on the root systems, like root stunting and necrosis which, though not characteristic of the disease, complicated disease evaluation. Moreover, the disease indices for the four replications varied from 1.2-2.5 and 0.5-2.7 for cottonseed and linseed meal amendments, respectively. No phytotoxicity was observed in plants grown in soybean meal-amended soils.

A field experiment using covered plots

In Spring of 1977, an experiment was carried out at Michigan State University farm to examine whether the volatile inhibitory effect of oilseed meals could be maintained under field condition by covering the experimental plots with polyethylene sheets.

Field soil (Conover loam, pH 6.5, organic matter content: 31.5%, sand:silt:clay = 50:24:26, WHC: 390 ml/kg) artificially infested with Fusarium solani f. sp. pisi was either unamended or amended with about 1% (w/w) alfalfa and oilseed meals. Amendments were incorporated in rows 1.5 m long, 15 cm wide, and 15 cm deep. The rows were then covered individually with polyethylene sheets which extended 10 cm beyond the edges of the rows, and were secured by applying soil over them. Uncovered rows, amended with 1% (w/w) alfalfa and soybean meals were included for comparison. Assignment of treatments to rows was completely randomized, and each treatment was replicated three times.

No reduction in Fusarium populations was obtained in all amended

soils, whether or not they were covered with polyethylene sheets. By eight weeks after amendment, soil samples collected from all amended plots showed higher Fusarium populations as compared to those from unamended control plots (Table 11). When the pH of the soils in covered plots were measured, it was found that linseed, cottonseed, and soybean meal amendments reduced the natural pH of 6.5 to 4.5, 4.5, and 5.2, respectively. The pH of the unamended soil was 6.3 and that of soil amended with alfalfa meal was 5.8.

Table 11. Soil pH and Fusarium populations in artificially infested Conover loam soil eight weeks after amendment with 1% (w/w) alfalfa and oilseed meals. The experiment was done at Michigan State University farm in Spring of 1977.

Soil treatment	Log Fusarium population/ g soil	Soil pH[b]
Covered plots		
Unamended control	3.95	6.3
Alfalfa meal	4.73	5.8
Linseed meal	4.74	4.5
Cottonseed meal	4.62	4.5
Soybean meal	4.72	5.2
Non-covered plots		
Alfalfa meal	4.47	
Soybean meal	4.75	

[a]Log Fusarium population at the time of soil amendment was 4.25.

[b]Soil pH before soil amendment was 6.5.

Effect of different soils on effectiveness of oilseed meal amendments in reducing Fusarium populations

The failure of oilseed meal amendments to reduce Fusarium populations under covered field conditions in Conover loam soil led to an

experiment to test whether the effectiveness of those amendments could be affected by the types of soil used.

Conover loam and Brookston loam (pH 7.5, sand:silt:clay = 48: 33:19, 6.46% organic matter, WHC: 535 ml/kg) soils were artificially infested with wheat bran-sand cultures of F. solani f. sp. pisi. Three hundred g portions of each soil were individually amended with 1% (w/w) alfalfa and oilseed meals. After adjusting the moisture level to 30% WHC, the amended soils were incubated in closed containers.

No reduction in Fusarium populations was obtained in Brookston loam soil. Even after 10 weeks of incubation, the Fusarium populations in the alfalfa and the oilseed meal-amended soils remained in the order of about 10^5 propagules/g soil (Table 12). In Conover loam soil, slight reduction in Fusarium populations were shown in the oilseed meal-amended soils by six weeks of incubation. However, by eight weeks of incubation Fusarium populations in such soils were still in the order of about 10^3 propagules/g soil. After 10 weeks of incubation the Fusarium populations in oilseed meal-amended soils were reduced to less than 10^2 propagules/g (Table 12). The Fusarium populations in the alfalfa meal-amended soil remained in the order of about 10^5 propagules/g throughout the experiment.

When the soil pH was measured after 10 weeks of incubation, all amended soils showed a reduction of 1.3-2.0 pH units from the original in Conover loam soil, and 1.4-1.8 pH units in Brookston loam soil. A reduction of 1.9-2.1 pH units from the originals was also obtained when unamended Conover loam and Brookston loam soils were incubated for 10 weeks in closed containers.

Table 12. Soil pH and *Fusarium* populations in artificially infested Conover loam and Brookston loam soils 10 weeks after amendement with 1% (w/w) alfalfa and oilseed meals.

Soil treatment	Conover loam soil		Brookston loam soil	
	Log *Fusarium* pop. per g soil[a]	Final soil pH[b]	Log *Fusarium* pop. per g soil	Final soil pH[b]
Alfalfa meal	5.61	4.5	5.84	6.1
Linseed meal	1.48	4.5	5.61	5.7
Cottonseed meal	1.76	4.5	5.76	5.6
Soybean meal	1.45	5.2	5.48	5.6

[a]Least Significant Range (Tukey's '*w*' procedure) = 1.89 (*P* = 0.01).

[b]Initial pH of the Conover loam and Brookston loam soils were 6.5 and 7.5, respectively. Unamended soil pH after 10 weeks were 4.4 for Conover loam soil and 5.6 for Brookston loam soil.

DISCUSSION

Estimation of Fusarium populations in soil was based mainly on the use of a selective medium. The efficiency of the medium in supporting germination and growth of Fusarium appears to be very high. Direct microscopic observation of soils naturally infested with F. oxysporum f. sp. lycopersici revealed that two or more chlamydospores in soil were often seen aggregated and adhering to soil particles (87). When each such aggregate was considered as a unit, this medium showed a plating efficiency of more than 80%. Regardless of the plating efficiency of the medium, appropriate controls were always included in the experiments, and plate counts were correlated with spore viability as determined microscopically and with a disease bioassay, as will be discussed in the following.

Linseed, cottonseed, and soybean meals incorporated into a sandy loam soil at a rate of 1% (w/w) were effective in drastically reducing Fusarium populations in closed containers in the laboratory. These amendments, however, did not reduce Fusarium populations under natural field conditions (Tables 5 and 6). Further work in the laboratory indicated that a much greater reduction in Fusarium populations was obtained in oilseed meal-amended soils incubated in closed containers than in those incubated in open containers (Figure 6). Reduction was also obtained in soil placed in planchets and incubated on the surfaces of oilseed meal-amended soils in closed containers

(Figure 7). These results provide strong evidence that volatile inhibitory substances were involved.

Volatile amines and/or ammonia were apparently produced by the oilseed meal-amended soils, as indicated by the titration curves of H_3BO_3 solutions using 0.01 N H_2SO_4 (22, 133, Figure 8). The fact that oilseed meal-amended soils showed phytotoxicity and an increase in pH up to 2.6 units also is consistent with the possible production of ammonia (5, 105). In soybean meal-amended soil, the volatile substances trapped in the H_3BO_3 solutions and water were apparently solely responsible for the reduction in Fusarium populations, at least insofar as volatile activity is concerned, since their effects were nullified by the presence of those solutions (Table 8). The fungitoxic effect of linseed and cottonseed meal amendments, however, were not nullified when similarly treated, suggesting that other volatile toxic substances may also have evolved from the linseed and cottonseed meal amendments.

Attempts to identify the volatile inhibitory substances using infra-red spectroscopy have not been successful. Except for CO_2, the presence of other volatile substances was not detected. This was presumably due to very low equilibrium concentrations present in the gas phase at any given time over the soils. CO_2 could not be responsible for the reduction in Fusarium populations, because even in alfalfa meal-amended soils high concentrations of CO_2 were produced.

The fact that reduction in Fusarium populations in oilseed meal-amended soils was governed by production of volatile inhibitory substances may explain why those amendments were not effective in the field experiments. Moreover, the effectiveness of the amendments,

especially linseed and cottonseed meals, was affected by soil moisture levels (Figure 5, Table 7). Laboratory experiments in closed containers showed that soil types also influenced the effectiveness of the oilseed meal amendments. Reduction in Fusarium populations occurred at a much slower rate in the Conover loam soil than in the Oshtemo-Boyer sandy loam soil; no reduction was obtained in the Brookston loam soil (Table 12). The Brookston loam soil contained twice as much organic matter as the Conover loam, and three times as much as the sandy loam soils. The reduced or nullified effectiveness of the oilseed meal amendments in the two loam soils may be due to immobilization of the inhibitory substances by the soil organic matter and clay minerals, the concentrations of which were high in these soils, and also to microbial degradation (18). These considerations also may explain why no reduction in Fusarium populations was detected within eight weeks in the field experiment using Conover loam soil, even though the plots were covered (Table 11).

Activities and growth of nitrifying bacteria are known to be favored by neutral to alkaline soil environments (5). In acid soils nitrification proceeds very slowly even in the presence of adequate nutrients and the responsible species are rare or may be totally absent. Oilseed meal amendments in the sandy loam soil (pH 5.5) resulted in an increase in pH (to pH 8.1 in case of soybean meal); in the two loam soils (pH 6.5-7.5), however, these amendments decreased the soil pH by 1.3 units or more after 10 weeks of incubation. This decrease in pH is probably due to nitrification of nitrogenous compounds originated from the organic amendments, and from the indigenous organic matter in the case of unamended loam soils. It would be of interest to

investigate how the soil pH would change and how Fusarium populations would be affected if 2-chloro-6-(trichloro-methyl)pyridine (N-serve) is applied together with the amendments in these soils. This compound is an inhibitor of nitrification of ammonium-N (53).

The volatility of the toxic substances may limit the practicability of the oilseed meal amendments. However, this may be compensated in part by their broad range of effectiveness against different species of Fusarium and the magnitude of the reduction achieved (Table 9). Moreover, drastic reduction in Fusarium populations was obtained at amendment rates as low as 0.25% (w/w) for soybean meal and 0.50% (w/w) for linseed and cottonseed meals after six weeks of incubation (Figure 4). These results are important not only from the economic standpoint, but also in minimizing the hazard of phytotoxicity. Whether or not the oilseed meal amendments would be effective in reducing populations of other pathogenic fungi is still to be looked into.

Another positive feature of the oilseed meal amendments was their selective action against different groups of soil microorganisms. In spite of their strong inhibitory effects against Fusarium spp., they affected the actinomycete and bacterial populations relatively slightly (Table 2). Fungi from the order Mucorales and Trichoderma spp. were also found plentiful in the oilseed meal-amended soils. The presence of these microorganisms will maintain the fungistatic levels of the soils (99, 100, 101) which will in turn minimize recolonization of the soil by Fusarium.

Raising the pH of Oshtemo-Boyer sandy loam soil prior to amendment with oilseed meals did not enhance reduction in Fusarium populations (Table 3). In fact, linseed and cottonseed meal-amended soils

with increased pH showed less reduction in Fusarium populations than those with natural pH; effectiveness of soybean meal was not affected by the soil pH. This approach, therefore, would not appear to be a useful means of enhancing the effectiveness of the amendments. The identities of the volatile substances evolved from the first two oilseed meal amendments need to be known before the effect of pH can be explained.

Composting the oilseed meals prior to incorporation into the soil offered no advantages over non-composted materials in reducing Fusarium. Decreased reduction in Fusarium populations occurred with the increase in composting periods (Table 4).

Amendment of soil with either alfalfa or oilseed meals temporarily nullified the fungistatic properties of the soil. In freshly amended soils 95% or more of Fusarium chlamydospores on buried membranes germinated after one day, while no germination occurred in unamended soils. Germination was followed by production of conidia, lysis of hyphae, and production of new chlamydospores during the first three days of burial. The fungistatic properties of the soils, however, were restored by seven days after amendments; no germination of chlamydospores was observed in soil after this period. Germination occurred only after incubating the chlamydospores on discs of the selective agar medium for 24 hours. Prolonged incubation in oilseed meal-amended soils led to a reduced viability of the chlamydospores (Figures 9 and 10). The viability of the chlamydospores in alfalfa meal-amended and unamended control soils remained above 60% even after 21 days of burial. These results indicated that the mechanism of population reduction was not associated with germination-lysis as has

been reported in several cases (3, 23, 24, 25, 119, 145), but rather a direct killing of the newly formed, but ungerminated chlamydospores by toxic substances produced during incubation in the oilseed meal-amended soils.

Reduction in viable populations of pathogenic Fusarium in oilseed meal-amended soils was correlated with reduction in root rot of soybeans and peas (Table 10). However, of the three oilseed meals used, soybean meal was the most effective in reducing root rot severity. It also showed no phytotoxicity following one week of air-drying, whereas root stunting and necrosis were associated with linseed and cottonseed meal amendments, even after this period. Phytotoxicity exerted by soil organic amendments has been known to enhance several soil-borne diseases (93, 123, 162).

In conclusion, non-specific and drastic reduction in Fusarium populations could be obtained in certain soils amended with linseed, cottonseed, and soybean meals, through production of volatile substances lethal to the chlamydospores of the fungus. This mode of reduction in Fusarium populations as a result of soil amendments using plant residues apparently has never been reported before. Reduction in pathogenic Fusarium populations was correlated with reduction in root rot, although with linseed and cottonseed meal amendments the severity of root rot was obscured by their residual phytotoxicity. Soybean meal seems to be the most promising among the three oilseed meals tested; it showed the greatest reduction in Fusarium populations, its phytotoxic effect in soil was completely removed within one week of air-drying, and its effectiveness could be maintained even at a rate as low as 0.25% (w/w). Reduction in Fusarium populations also

correlated with the results obtained from direct microscopic observations on chlamydospore viability. Therefore, the fact that oilseed meal amendments are effective in reducing Fusarium populations in soil is beyond any doubt. Because volatile substances are involved, if these amendments were to be used effectively in the field, the soil should be sealed and kept moist for a period of time, depending on the type of soil involved, enough time should be allowed for disappearance of phytotoxic residues of the amendments after the Fusarium populations have been reduced, before crops are planted.

LITERATURE CITED

LITERATURE CITED

1. Adams, P.B., J.A. Lewis, and G.C. Papavizas. 1968. Survival of root-infecting fungi in soil. IV. The nature of fungistasis in natural and cellulose-amended soil on chlamydospores of Fusarium solani f. sp. phaseoli. Phytopathology 58: 378-383.

2. Adams, P.B., J.A. Lweis, and G.C. Papavizas. 1968. Survival of root-infecting fungi in soil. IX. Mechanism of control of Fusarium root rot of bean with spent coffee grounds. Phytopathology 58: 1603-1608.

3. Adams, P.B. and G.C. Papavizas. 1969. Survival of root-infecting fungi in soil. X. Sensitivity of propagules of Thielaviopsis basicola to soil fungistasis in natural and alfalfa-amended soil. Phytopathology 59: 135-138.

4. Adams, P.B. and G.C. Papavizas, and J.A. Lewis. 1968. Survival of root-infecting fungi in soil. III. The effect of cellulose amendment on chlamydospore germination of Fusarium solani f. sp. phaseoli in soil. Phytopathology 58: 373-377.

5. Alexander, M. 1977. Introduction to soil microbiology. Second edition. John Wiley & Sons. New York. 467 pages.

6. Armstrong, G.M. and J.K. Armstrong. 1965. A wilt of soybean caused by a new form of Fusarium oxysporum. Phytopathology 55: 237-239.

7. Armstrong, G.M. and J.K. Armstrong. 1975. Reflections on the wild fusaria. Annu. Rev. Phytopathol. 13: 95-103.

8. Avizohar-Hershenzon, Z. and P. Shaked. 1969. Studies on the mode of action of inorganic nitrogenous amendments on Sclerotium rolfsii in soil. Phytopathology 59: 288-292.

9. Backman, P.A. and R. Rodriguez-Kabana. 1975. A system for the growth and delivery of biological control agents to the soil. Phytopathology 65: 819-821.

10. Baker, K.F. and R.J. Cook. 1974. Biological control of plant pathogens. W.H. Freeman and Co., San Francisco. 433 pages.

11. Baker, R.R. 1968. Mechanisms of biological control. Ann. Rev. Phytopathol. 6: 263-294.

12. Balis, C. and V. Kouyeas. 1968. Volatile inhibitors involved in soil mycostasis. Ann. Inst. Phytopathol. 8: 145-149.

13. Barron, G.L. 1968. The genera of Hypomycetes from soil. The Williams and Wilkins Co., Baltimore. 364 pages.

14. Birchfield, W., J.F. Parr, and S. Smith. 1969. Nematicidal and fungicidal activity of potassium azide in liquid anhydrous ammonia. Plant Dis. Rep. 53: 923-926.

15. Booth, C. 1971. The genus Fusarium. C.M.I., Kew, Surrey, England. 237 pages.

16. Booth, C. 1975. The present status of Fusarium taxonomy. Annu. Rev. Phytopathol. 13: 83-93.

17. Booth, C. 1977. Fusarium. Laboratory guide to the identification of the major species. C.M.I., Kew, Surrey, England. 57 pages.

18. Brian, P.W. 1957. The ecological significance of antibiotic production. pp. 168-188. In Microbial Ecology. (R.E.O. Williams and C.C. Spicer, eds.). Cambridge Univ. Press, London.

19. Bristow, P.R. and J.L. Lockwood. 1975. Soil fungistasis: role of spore exudates in the inhibition of nutrient-dependent propagules. J. Gen. Microbiol. 90: 140-146.

20. Burges, A. 1963. Some problems in soil microbiology. Trans. Brit. Mycol. Soc. 46: 1-14.

21. Buxton, E.W., O. Khalifa, and V. Ward. 1965. Effect of soil amendment with chitin on pea wilt caused by Fusarium oxysporum f. sp. pisi. Ann. Appl. Biol. 55: 83-88.

22. Cheng, H.H. and J.M. Bremner. 1965. Gaseous forms of nitrogen. pp. 1287-1323. In Methods of soil analysis. Part 2. Chemical and microbiological properties. (C.A. Black, D.D. Ewans, L.E. Ensminger, J.L. White, F.E. Clark, and R.C. Dinauer, eds.). Agronomy No. 9. Amer. Soc. Agronomy.

23. Chinn, S.H.F. and R.J. Ledingham. 1957. Studies on the influence of various substances on the germination of Helminthosporium sativum spores in soil. Can. J. Botany 35: 697-701.

24. Chin, S.H.F. and R.J. Ledingham. 1961. Mechanisms contributing to the eradication of spores of Helminthosporium sativum from amended soil. Can. J. Botany 39: 739-748.

25. Chinn, S.H.F., R.J. Ledingham, B.J. Sallans, and P.M. Simmonds. 1953. A mechanism for the control of common root rot of wheat. Phytopathology 43: 701.

26. Clark, F.E. 1942. Experiments toward the control of the take-all disease of wheat and the Phymatotrichum root rot of cotton. U.S.D.A. Tech. Bul. No. 835.

27. Coley-Smith, J.R. and R.C. Cooke. 1971. Survival and germination of fungal sclerotia. Annu. Rev. Phytopathol. 9: 65-92.

28. Coley-Smith, J.R. and J.E. King. 1969. The production by species of Allium of alkyl-sulphides and their effect on germination of sclerotia of Sclerotium cepivorum Berk. Ann. Appl. Biol. 54: 289-301.

29. Cook, R.J. and M.N. Schroth. 1965. Carbon and nitrogen compounds and germination of chlamydospores of Fusarium solani f. sp. phaseoli. Phytopathology 55: 254-256.

30. Cook, R.J. and W.C. Snyder. 1965. Influence of host exudates on growth and survival of germlings of Fusarium solani f. sp. phaseoli in soil. Phytopathology 55: 1021-1025.

31. Cromwell, R.O. 1917. Fusarium-blight, or wilt disease, of soybean. J. Agric. Res. 8: 421-439.

32. Davey, C.B. and G.C. Papavizas. 1959. Effect of organic soil amendments on the Rhizoctonia disease of snap beans. Agron. J. 51: 493-496.

33. Davey, C.B. and G.C. Papavizas. 1963. Saprophytic activity of Rhizoctonia as affected by the carbon-nitrogen balance of certain organic soil amendments. Proc. Soil Sci. Soc. Amer. 27: 164-167.

34. Dennis, C. and J. Webster. 1971. Antagonistic properties of species groups of Trichoderma. II. Production of volatile antibiotics. Trans. Brit. Mycol. Soc. 57: 41-48.

35. Dobbs, C.G. and W.H. Hinson. 1953. A widespread fungistasis in soils. Nature 172: 197-199.

36. Dunleavy, J. 1961. Fusarium blight of soybeans. Iowa Academy of Science 68: 106-113.

37. Eno, C.F., W.G. Blue, and J.M. Good, Jr. 1955. The effect of anhydrous ammonia on nematodes, fungi, bacteria, and nitrification in some Florida soils. Proc. Soil Sci. Soc. Amer. 19: 55-58.

38. Finstein, M.S. and M. Alexander. 1962. Competition for carbon and nitrogen between Fusarium and bacteria. Soil Sci. 94: 334-339.

39. Foth, H.D. and L.M. Turk. 1972. Fundamentals of soil science. John Wiley & Sons, Inc., N.Y. 454 pages.

40. Ford, E.J., A.H. Gold, and W.C. Snyder. 1970. Soil substances inducing chlamydospore formation by Fusarium. Phytopathology 60: 124-128.

41. Ford, E.J., A.H. Gold, and W.C. Snyder. 1970. Induction of chlamydospore formation in Fusarium solani by soil bacteria. Phytopathology 60: 479-484.

42. French, E.R. and B.W. Kennedy. 1963. The role of Fusarium in the root rot complex of soybean in Minnesota. Plant Dis. Rep. 47: 672-676.

43. French, E.R. and L.N. Nelson. 1966. Production of macroconidia of Fusarium oxysporum f. sp. batatas and their conversion to chlamydospores. Phytopathology 56: 1322-1323.

44. Fries, N. 1973. Effects of volatile organic compounds on the growth and development of fungi. Trans. Brit. Mycol. Soc. 60: 1-21.

45. Garrett, S.D. 1970. Pathogenic root-infecting fungi. Cambridge Univ. Press, England. 294 pages.

46. Garrett, S.D. 1975. Seed- and soil-borne pathogens: opening address. pp. 2-4. In Biology and control of soil-borne plant pathogens. (G.W. Breuhl, ed.). A.P.S., St. Paul, Minn.

47. Gilbert, R.G. and G.E. Griebel. 1969. Stimulation of soil respiration by volatiles from alfalfa. Proc. Soil Sci. Soc. Amer. 33: 270-273.

48. Gilbert, R.G. and G.E. Griebel. 1969. The influence of volatile substances from alfalfa on Verticillium dahliae in soil. Phytopathology 59: 1400-1403.

49. Gilbert, R.G., J.D. Menzies, and G.E. Griebel. 1969. The influence of volatiles from alfalfa upon growth and survival of soil microorganisms. Phytopathology 59: 992-995.

50. Gilman, J.C. 1957. A manual of soil fungi. Second Edition. The Iowa Univ. Press. 450 pages.

51. Gilpatrick, J.D. 1969. Role of ammonia in the control of avocado root rot with alfalfa meal soil amendment. Phytopathology 59: 973-978.

52. Gilpatrick, J.D. 1969. Effect of soil amendments upon inoculum survival and function in Phytophthora root rot of avocado. Phytopathology 59: 979-985.

53. Gray, T.R.G. and S.T. Williams. 1975. Soil microorganisms. Longman Group Ltd., London. 240 pages.

54. Griffin, D.M. 1972. Ecology of soil fungi. Chapman and Hall, London. 193 pages.

55. Griffin, G.J. 1964. Long term influence of soil amendments on germination of conidia. Can. J. Microbiol. 10: 605-612.

56. Guenzi, W.D. and T.M. McCalla. 1966. Phytotoxic substances extracted from soil. Proc. Soil Sci. Soc. Amer. 30: 214-216.

57. Hammerslag, F. and R.G. Linderman. 1975. Effect of five acids that occur in pine needles on Fusarium chlamydospore germination in non sterile soil. Phytopathology 65: 1120-1123.

58. Harrison, M.D. 1976. The effect of barley straw on the survival of Verticillium albo-atrum in naturally infested field soil. Amer. Potato J. 53: 385-394.

59. Henis, Y. and I. Chet. 1967. Mode of actionof ammonia on Sclerotium rolfsii. Phytopathology 57: 425-428.

60. Henis, Y. and I. Chet. 1968. The effect of nitrogenous amendments on the germination of sclerotia of Sclerotium rolfsii and on their accompanying microflora. Phytopathology 58: 209-211.

61. Hildebrand, A.A. and P.M. West. 1941. Strawberry root rot in relation to microbiological changes induced in root rot soil by the incorporation of certain cover crops. Can. J. Res. 19: 183-198.

62. Hora, T.S. and R. Baker. 1970. Volatile factor in soil fungistasis. Nature 225: 1071-1072.

63. Hora, T.S. and R. Baker. 1972. Soil sungistasis: microflora producing a volatile inhibitor. Trans. Brit. Mycol. Soc. 59: 491-500.

64. Hora, T.S. and R. Baker. 1972. Extraction of a volatile factor from soil inducing fungistasis. Phytopathology 62: 1475-1476.

65. Hora, T.S. and R. Baker. 1974. Abiotic generation of a volatile fungistatic factor in soil by liming. Phytopathology 64: 624-629.

66. Hora, T.S. and R. Baker. 1975. Humic substances involved in the formation of volatiles in fungistatic soils. Proc. Amer. Phytopathol. Soc. 2: 41 (Abstract).

67. Hsu, S.C. and J.L. Lockwood. 1973. Soil fungistasis: behavior of nutrient-independent spores and sclerotia in a model system. Phytopathology 63: 334-337.

68. Hsu, S.C. and J.L. Lockwood. 1973. Chlamydospore formation in Fusarium in sterile salt solutions. Phytopathology 63: 597-602.

69. Hsu, S.C. and J.L. Lockwood. 1975. Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. Appl. Microbiol. 29: 422-426.

70. Huber, D.M. and R.D. Watson. 1970. Effect of organic amendments on soil-borne plant pathogens. Phytopathology 60: 22-26.

71. Huber, D.M. and R.D. Watson. 1974. Nitrogen form and plant disease. Annu. Rev. Phytopathol. 12: 139-165.

72. Hutchinson, S.A. 1971. Presidential address: biological activity of volatile fungal metabolites. Trans. Brit. Mycol. Soc. 57: 185-200.

73. Inoue, Y., S. Takeuchi, and H. Komada. 1965. One of the ideas on the control of soil-borne diseases, especially on the biological control of radish yellows, by adding chitin to soil. Rev. Appl. Mycol. 44: 50 (Abstract).

74. Johnson, L.F. 1962. Effect of the addition of organic amendments to soil on root knot of tomatoes. II. Relation of soil temperature, moisture, and pH. Phytopathology 52: 410-413.

75. Johnson, L.F. and N.B. Shamiyeh. 1975. Effect of soil amendments on hatching of Meloidogyne incognita eggs. Phytopathology 65: 1178-1181.

76. Katan, J. 1971. Symptomless carriers of the tomato Fusarium wilt pathogen. Phytopathology 61: 1213-1217.

77. Khalifa, O. 1965. Biological control of Fusarium wilt of peas organic soil amendments. Ann. Appl. Biol. 56: 129-137.

78. King, J.E. and J.R. Coley-Smith. 1968. Effect of volatile products of Allium species and their extracts on germination of sclerotia of Sclerotium cepivorum Berk. Ann. Appl. Biol. 61: 407-414.

79. King, J.E. and J.R. Coley-Smith. 1969. Production of volatile alkyl sulphides by microbial degradation of synthetic alliin and alliin-like compounds, in relation to germination of sclerotia of Sclerotium cepivorum Berk. Ann. Appl. Biol. 64: 303-314.

80. Ko, W.H. 1971. Biological control of seedling root rot of papaya caused by Phytophthora palmivora. Phytopathology 61: 780-782.

81. Ko, W.H. and F.K. Hora. 1972. The nature of a volatile inhibitor from certain alkaline soils. Phytopathology 62: 573-574

82. Ko, W.H. and F.K. Hora. 1974. Factors affecting the activity of a volatile fungistatic substance in certain alkaline soils. Phytopathology 64: 1042-1043.

83. Ko, W.H., F.K. Hora, and E. Herlicska. 1974. Isolation and identification of a volatile fungistatic substance from alkaline soil. Phytopathology 64: 1398-1400.

84. Ko, W.H. and J.L. Lockwood. 1967. Soil fungistasis: relation to fungal spore nutrition. Phytopathology 57: 894-901.

85. Ko, W.H. and J.L. Lockwood. 1970. Mechanism of lysis of fungal mycelia in soil. Phytopathology 60: 148-154.

86. Kamada, H. 1972. Studies on the method for selective isolation of Fusarium oxysporum from natural soil. Bulletin of the Tokai-Kinki National Agriculture Exp. Sta. No. 23.

87. Komada, H. 1975. Development of a selective medium for quantitative isolation of Fusarium oxysporum from natural. Rev. Pl. Prot. Res. 8: 114-125.

88. Komada, H. and A. Ezuka. 1973. Biological control of Fusarium wilt in cucumber by addition of various kinds of organic matter. Proc. Kansai Pl. Prot. Soc. 15: 147-148 (in Japanese).

89. Lewis, J.A. and G.C. Papavizas. 1970. Evolution of sulfur-containing volatiles from decomposition of crucifers in soil. Soil Biol. Biochem. 2: 239-246.

90. Lewis, J.A. and G.C. Papavizas. 1971. Effect of sulfur-containing volatile compounds and vapors from cabbage decomposition on Aphanomyces euteiches. Phytopathology 61: 208-214.

91. Lewis, J.A. and G.C. Papavizas. 1975. Survival and multiplication of soil-borne plant pathogens as affected by plant tissue amendments. pp. 84-89. In Biology and control of soil-borne plant pathogens. (G.W. Breuhl, ed.). A.P.S., St. Paul, Minn.

92. Lewis, J.A. and G.C. Papavizas. 1977. Effect of plant residues on chlamydospore germination of Fusarium solani f. sp. phaseoli and Fusarium root rot of beans. Phytopathology 67: 925-929.

93. Linderman, R.G. 1970. Plant residue decomposition products and their effects on host roots and fungi pathogenic to roots. Phytopathology 60: 19-22.

94. Linderman, R.G. and R.G. Gilbert. 1969. Stimulation of Sclerotium rolfsii in soil by volatile components of alfalfa hay. Phytopathology. 59: 1366-1372.

95. Linderman, R.G. and R.G. Gilbert. 1973. Influence of volatile compounds from alfalfa hay on microbial activity in soil in relation to growth of Sclerotium rolfsii. Phytopathology 63: 359-362.

96. Linderman, R.G. and R.G. Gilbert. 1973. Behavior of sclerotia of Sclerotium rolfsii produced in soil or in culture regarding germination stimulation by volatiles, fungistasis, and sodium hypochlorite treatment. Phytopathology 63: 500-504.

97. Linford, M.B., F. Yap, and J.M. Oliviera. 1938. Reduction of soil populations of the root-knot nematodes during decomposition of organic matter. Soil Sci. 45: 127-140.

98. Lloyd, A.B. and J.L. Lockwood. 1966. Lysis of fungal hyphae in soil and its possible relation to autolysis. Phytopathology 56: 595-602.

99. Lockwood, J.L. 1964. Soil fungistasis. Annu. Rev. Phytopathol. 2: 341-362.

100. Lockwood, J.L. 1975. Quantitative evaluation of a leaching model system for soil fungistasis. Phytopathology 65: 460-464.

101. Lockwood, J.L. 1977. Fungistasis in soils. Biol. Rev. 52: 1-43.

102. Maier, C.R. 1959. Effect of certain crop residues on bean root-rot pathogens. Plant Dis. Rep. 43: 1027 (Abstract).

103. Maier, C.R. 1961. Selective effects of barley residue on fungi of the Pinto bean root-rot complex. Plant Dis. Rep. 45: 808-811.

104. Maurer, C.L. and R. Baker. 1965. Ecology of plant pathogens in soil. II. Influence of glucose, cellulose, and inorganic nitrogen amendments on development of bean root rot. Phytopathology 55: 69-72.

105. McCalla, T.M. and F.A. Haskins. 1964. Phytotoxic substances from soil microorganisms and crop residues. Bacteriol. Rev. 28: 181-207.

106. Menzies, J.D. 1959. Occurrence and transfer of a biological factor in soil that suppresses potato scab. Phytopathology 49: 648-652.

107. Menzies, J.D. and R.G. Gilbert. 1967. Responses of the soil microflora to volatile components in plant residues. Proc. Soil Sci. Amer. 31: 495-496.

108. Mitchell, R. and M. Alexander. 1961. The mycolytic phenomenon and biological control of Fusarium in soil. Nature 190: 109-110.

109. Mitchell, R. and M. Alexander. 1962. Microbiological processes associated with the use of chitin for biological control. Proc. Soil Sci. Soc. Amer. 26: 556-558.

110. Mixon, A.C. and E.A. Curl. 1967. Influence of plant residues on Sclerotium rolfsii and soil microorganisms. Crop Sci. 7: 641-644.

111. Moore-Landecker, E. and G. Stotzky. 1972. Inhibition of fungal growth and sporulation by volatile metabilites from bacteria. Can. J. Microbiol. 18: 957-962.

112. Nash, S.M., T. Christou, and W.C. Snyder. 1961. Existence of Fusarium solani f. sp. phaseoli as chlamydospores in soil. Phytopathology 51: 308-312.

113. Neal, D.C. 1935. Further studies on the effect of ammonia nitrogen on growth of the cotton-root-rot fungus, Phymatotrichum omnivorum, in field and laboratory experiments. Phytopathology 25: 967-968.

114. Oswald, J.W. 1949. Cultural variation, taxonomy, and pathogenicity of Fusarium species associated with cereal root rots. Phytopathology 39: 359-376.

115. Owens, L.D., R.G. Gilbert, G.E. Griebel, and J.D. Menzies. 1969. Identification of plant volatiles that stimulate microbial respiration and growth in soil. Phytopathology 59: 1468-1472.

116. Papavizas, G.C. 1963. Microbial antagonism in bean rhizosphere as affected by oat straw and supplemental nitrogen. Phytopathology 53: 1430-1435.

117. Papavizas, G.C. 1968. Survival of root-infecting fungi in soil. VI. Effect of amendments on bean root rot caused by Thielaviopsis basicola and on inoculum density of the causal organism. Phytopathology 58: 421-428.

118. Papavizas, G.C. and C.B. Davey. 1960. Rhizoctonia disease of bean as affected by decomposing green plant materials and associated microfloras. Phytopathology 50: 516-522.

119. Papavizas, G.C. and M.F. Kovacs, Jr. 1972. Stimulation of spore germination of Thielaviopsis basicola by fatty acids from rhizosphere soil. Phytopathology 62: 688-694.

120. Papavizas, G.C., J.A. Lewis, and P.B. Adams. 1968. Survival of root-infecting fungi in soil. II. Influence of amendment and soil carbon-to-nitrogen balance on Fusarium root rot of beans. Phytopathology 58: 365-372.

121. Park, D. 1965. Survival of microorganisms in soil. pp. 82-97. In Ecology of soil-borne plant pathogens. (K.F. Baker and W.C. Snyder, eds.). Univ. Calif. Press, Berkeley, California.

122. Patrick, Z.A. and L.W. Koch. 1958. Inhibition of respiration, germination, and growth by substances arising during the decomposition of certain plant residues in the soil. Can. J. Bot. 36: 621-647.

123. Patrick, Z.A. and L.W. Koch. 1963. The adverse influence of phytotoxic substances from decomposing plant residues on resistance of tobacco to black root rot. Can. J. Bot. 41: 747-758.

124. Patrick, Z.A. and T.A. Toussoun. 1965. Plant residues and organic amendments in relation to biological control. pp. 440-459. In Ecology of soil-borne plant pathogens. (K.F. Baker and W.C. Snyder, eds.). Univ. Calif. Press, Berkeley, California.

125. Partick, Z.A., T.A. Tousoun, and L.W. Koch. 1964. Effect of crop-residue decomposition products on plant roots. Annu. Rev. Phytopathol. 2: 267-292.

126. Patrick, Z.A., T.A. Toussoun, and W.C. Snyder. 1963. Phytotoxic substances in arable soils associated with the decomposition of plant residues. Phytopathology 53: 152-161.

127. Peech, M. 1965. Hydrogen ion activity. pp. 914-926. In Methods of soil analysis. Part 2. Chemical and microbiological properties. (C.A. Black, D.D. Ewans, L.E. Ensminger, J.L. White, F.E. Clark, and R.C. Dinauer, eds.). Agronomy No. 9. Amer. Soc. Agronomy.

128. Pramer, D. and E.L. Schmidt. 1964. Experimental soil microbiology. Burgess Publishing Co., Minn. 107 pages.

129. Primrose, S.B. and M.J. Dilworth. 1976. Ethylene production by bacteria. J. Gen. Microbiol. 93: 177-181.

130. Romine, M. and R. Baker. 1973. Soil fungistasis: evidence for an inhibitory factor. Phytopathology 63: 756-759.

131. Schippers, B. 1972. Reduced chlamydospore formation and lysis of macroconidia of Fusarium solani f. sp. cucurbitae in nitrogenamended soil. Neth. J. Pl. Pathol. 78: 189-197.

132. Schippers, B. and A. Bouman. 1973. Inhibition of germination and mycelial growth of Fusarium solani f. sp. cucurbitae and Aspergillus flavus by volatiles from soil. Acta Bot. Neerl. 22: 166 (Abstract).

133. Schippers, B. and L.C. Palm. 1973. Ammonia, a fungistatic volatile in chitin-amended soil. Neth. J. Pl. Pathol. 79: 279-281.

134. Schippers, B. and W.M.M.M. de Weyer. 1972. Chlamydospore formation and lysis of macroconidia of Fusarium solani f. sp. cucurbitae in chitin-amended soil. Neth. J. Pl. Pathol. 78: 45-54.

135. Schroth, M.N. and F.F. Hendrix. 1962. Influence of non-susceptible plants on the survival of Fusarium solani f. sp. phaseoli in soil. Phytopathology 52: 906-909.

136. Sequeira, L. 1962. Influence of organic amendments on survival of Fusarium oxysporum f. cubense in the soil. Phytopathology 52: 976-982.

137. Sequeira, L. 1963. Effect of urea applications on survival of Fusarium oxysporum f. cubense in soil. Phytopathology 53: 332-336.

138. Short, G.E. and M.L. Lacy. 1974. Germination of Fusarium solani f. sp. pisi chlamydospores in the spermosphere of pea. Phytopathology 64: 558-562.

139. Smiley, R.W. 1974. Take-all of wheat as influenced by organic amendments and nitrogen fertilizers. Phytopathology 64: 822-824.

140. Smiley, R.W., R.J. Cook, and R.I. Papendick. 1972. Fusarium root rot of wheat and peas as influenced by soil applications of anhydrous ammonia and ammonia-potassium azide solutions. Phytopathology 62: 86-91.

141. Smith, A.M. 1973. Ethylene as a cause of soil fungistasis. Nature 246: 311-313.

142. Smith, S.N. and W.C. Snyder. 1972. Germination of Fusarium oxysporum chlamydospores in soils favorable and unfavorable to wilt establishment. Phytopathology 62: 273-277.

143. Sneh, B. 1977. A method for observation and study of living fungal propagules incubated in soil. Soil Biol. Biochem. 9: 65-66.

144. Sneh, B. and Y. Henis. 1972. Production of antifungal substances active against Rhizoctonia solani in chitin-amended soil. Phytopathology 62: 595-600.

145. Sneh, B., B.F. Holdaway, G.R. Hooper, and J.L. Lockwood. 1976. Germination-lysis as a mechanism for biological control of Thielaviopsis basicola pathogenic to soybean. Can. J. Bot. 54: 1499-1508.

146. Sneh, B., J. Katan, and Y. Henis. 1971. Mode of inhibition of Rhizoctonia solani in chitin-amended soil. Phytopathology 61: 1113-1117.

147. Snyder, W.C. 1963. Root diseases biologically controlled. Science 141: 835-837.

148. Snyder, W.C. and H.N. Hansen. 1940. The species concept in Fusarium. Amer. J. Bot. 27: 64-67.

149. Snyder, W.C. and H.N. Hansen. 1941. The species concept in Fusarium with reference to section Martiella. Amer. J. Bot. 28: 738-742.

150. Snyder, W.C. and H.N. Hansen. 1945. The species concept in Fusarium with reference to Discolor and other sections. Amer. J. Bot. 32: 657-666.

151. Snyder, W.C. and H.N. Hansen. 1954. Variation and speciation in the genus Fusarium. N.Y. Acad. Sci. Ann. 60: 16-23.

152. Snyder, W.C., H.N. Hansen, and J.W. Oswald. 1957. Cultivars of the fungus Fusarium. J. Madras Univ. 27: 185-192.

153. Snyder, W.C., M.N. Schroth, and T. Christou. 1959. Effect of plant residues on root rot of bean. Phytopathology 49: 755-756.

154. Snyder, W.C. and T.A. Toussoun. 1965. Current status of taxonomy in Fusarium species and their perfect stages. Phytopathology 55: 833-837.

155. Steel, R.G.D. and J.H. Torrie. 1960. Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill Book Co., Inc., N.Y. 481 pages.

156. Steiner, G.W. and J.L. Lockwood. 1969. Soil fungistasis: sensitivity of spores in relation to germination time and size. Phytopathology 59: 1084-1092.

157. Steiner, G.W. and J.L. Lockwood. 1970. Soil fungistasis: mechanism in sterilized, reinoculated soil. Phytopathology 60: 89-91.

158. Stumbo, C.R., P.L. Gainey, and F.E. Clark. 1942. Microbiological and nutritional factors in the take-all disease of wheat. J. Agr. Res. 64: 653-665.

159. Toussoun, T.A., W. Menzinger, and R.S. Smith, Jr. 1969. Role of conifer litter in ecology of Fusarium: stimulation of germination in soil. Phytopathology 59: 1396-1399.

160. Toussoun, T.A. and P.E. Nelson. 1968. A pictorial guide to the identification of Fusarium species. The Penn. State Univ. Press. 51 pages.

161. Toussoun, T.A. and P.E. Nelson. 1975. Variations and speciation in the Fusaria. Annu. Rev. Phytopathol. 13: 71-82.

162. Toussoun, T.A. and Z.A. Patrick. 1963. Effect of phytotoxic substances from decomposing plant residues on root rot of bean. Phytopathology 53: 265-270.

163. Toussoun, T.A., Z.A. Patrick, and W.C. Snyder. 1963. Influence of crop residue decomposition products on the germination of Fusarium solani f. sp. phaseoli chlamydospores in soil. Nature 197: 1314-1316.

164. Toussoun, T.A., A.R. Weinhold, R.G. Linderman, and Z.A. Patrick. 1968. Nature of phytotoxic substances produced during plant residue decomposition in soil. Phytopathology 58: 41-45.

165. Vruggink, H. 1970. The effect of chitin amendment on actinomycetes in soil and on the infection of potato tubers by Streptomyces scabies. Neth. J. Pl. Pathol. 76: 293-295.

166. Walker, J.G. and M.D. Harrison. 1976. Effect of straw and nitrogen incorporation on soil productivity and growth of Russett Burbank potatoes in a barley-potato rotation. Amer. Potato J. 53: 401. (Abstract).

167. Warcup, J.H. 1955. On the origin of colonies of fungi developing on soil dilution plates. Trans. Brit. Mycol. Soc. 38: 298-301.

168. Warcup, J.H. 1965. Growth and reproduction of soil microorganisms in relation to substrate. pp. 52-68. In Ecology of soil-borne plant pathogens. (K.F. Baker and W.C. Snyder, eds.). Univ. Calif. Press, Berkeley, California.

169. Weinhold, A.R. and T. Bowman. 1968. Selective inhibition of the potato scab pathogen by antagonistic bacteria and substrate influence on antibiotic production. Plant Soil 28: 12-24.

170. Weinke, K.E. 1962. The influence of nitrogen on the root disease of bean caused by Fusarium solani f. sp. phaseoli. Phytopathology 52: 757. (Abstract).

171. West, P.M. and A.A. Hildebrand. 1941. The microbiological balance of strawberry root rot soil as related to the rhizosphere and decomposition effects of certain cover crops. Can. J. Res. 19: 199-210.

172. Wollenweber, H.W. and O.A. Reinking. 1935. Die Fusarien, ihre Beschreibung, Schadwirkung und Bekampfung. Paul Parey, Berlin. 355 pages.

173. Zakaria, M.A. 1975. Biological control of soybean root rot caused by Thielviopsis basicola (Berk. & Br.) Ferr. M.S. Thesis. Michigan State University. 70 pages.

174. Zentmyer, G.A. 1963. Biological control of Phytophthora root rot of avocado with alfalfa meal. Phytopathology 53: 1383-1387.

175. Zentmyer, G.A. and F.T. Bingham. 1956. The influence of nitrite on the development of Phytophthora root rot of avocado. Phytopathology 46: 121-124.