Reduction in *Fusarium* Populations in Soil by Oilseed Meal Amendments

Michael A. Zakaria and John L. Lockwood

Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824. Present address of M. A. Zakaria: Department of Botany, Bogor Agricultural University, Bogor, Java, Indonesia.
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ABSTRACT

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Natural loamy sand, artificially infested with *Fusarium oxysporum* and *F. solani* was amended with 10 kinds of plant or animal residues, each at 1% (w/w), and incubated in closed plastic containers. Linseed, cottonseed, and soybean meals reduced *Fusarium* chlamydospore populations from $\sim 10^5$ per gram of soil to 10^2 or fewer per gram in 4–5 wk. Crab shell reduced populations to 10^4 per gram, but six other amendments were ineffective. The oilseed meals were effective at concentrations at least as low as 0.25% (w/w). Soybean meal reduced *Fusarium* populations about equally in soil held at 15, 30, 50, or 100% of water-holding capacity (WHC) (approximately equivalent to −3.0, −0.2, −0.03, and 0 bars water potential, respectively). Linseed and cottonseed meals were most effective at 100% of WHC. Once reduced, *Fusarium* populations remained low for at least 4 wk. Total numbers of fungi, actinomycetes, and bacteria were not reduced as greatly by the oilseed meal amendments as were the fusaria. Severity of pea root rot in soil amended with oilseed meal was proportional to the surviving population of *F. solani* f. sp. *pisi*. Soil treated with linseed or cottonseed meal was phytotoxic to pea and soybean, whereas soil treated with soybean meal was not. The oilseed meals also reduced *Fusarium* populations in Capac loam, but not in Brookston loam. The rate of reduction in Capac loam was slower than that in loamy sand.

Additional key words: biological control.

Fusarium oxysporum Schlect. and *F. solani* Mart. often are associated with a seed, seedling, and root rot of soybean grown in light-textured soils in southwest Michigan. The first evidence of the disease is poor emergence over either restricted or extensive (several hectares) areas in the field. Symptoms include seed rot, curling and enlargement of seedling hypocotyls, and necrotic lesions. The disease may be similar to other *Fusarium*-caused diseases of soybean reported previously (2,5,6,8).

The purpose of this study was to investigate whether selected organic amendments in soil would reduce populations of *Fusarium* associated with the *Fusarium* disease of soybean in Michigan, and to investigate various parameters influencing the effectiveness of the amendments.

MATERIALS AND METHODS

Preparation of inocula. *Fusarium solani* Mart., *F. oxysporum* Schlect., and *F. solani* (Mart.) Appel & Wr. f. sp. *pisi* (F. R. Jones) Snyd. & Hans., were used in the experiments. *F. oxysporum* and *F. solani* were isolated from diseased soybean seedlings and *F. solani* f. sp. *pisi* was isolated from pea. The fungi were maintained on potato-dextrose agar (PDA) slants at 4 C. For production of inocula, the fungi were grown on wheat bran-sand medium prepared by mixing 200 g of wheat bran, 500 g of white silica sand, and 500 ml of distilled water, and steaming the mixture for 60 min. The wheat bran was first ground in a Wiley mill and passed through a 0.85-mm (20-mesh) sieve. The steamed mixture was placed in Erlenmeyer flasks and autoclaved. The three fungi were individually grown in the medium for 4–6 wk at 24 C before use.

Soil preparation and infestation. Unless otherwise specified, Oshtemo-Boyer sandy loam (sand : silt : clay, 73:18:9; pH 5.5; organic matter, 2.02%; water-holding capacity [WHC], 250 ml/kg) collected from St. Joseph County, MI, was used in all experiments.

Soil was infested with *F. oxysporum, F. solani,* and *F. solani* f. sp. *pisi,* by mixing the wheat bran-sand cultures (previously passed through a 1.7-mm [10-mesh] sieve) with the soil at a rate of 10% (w/w). The infested soil was moistened to about 50% of WHC and incubated for 8 wk, when a constant chlamydospore concentration of 10^4–10^6/g was achieved, as determined by plate counts and microscopic examination. Unless otherwise specified, soils were infested with a mixture of *F. oxysporum* and *F. solani.*

Incorporation of organic residues into infested 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 sugarbeet pulp, alfalfa meal, linseed meal, cottonseed meal, and soybean meal; and ground crab shells (supplied by H. Komada, Japan). The first five materials were ground and passed through a 1.7-mm (10-mesh) sieve before being used. Unless otherwise specified, these materials were mixed with air-dried *Fusarium*-infested soil at a rate of 1% (w/w). The moisture content was then adjusted to 30–35% of WHC (approximately −0.2 bar) by adding tap water. The soil (200–300 g per container) was incubated in 550-ml plastic containers 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 before being incorporated into the 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 (approximately −0.2 bars) and incubated in sealed (Parafilm M, American Can Co., Greenwich, CT 06830) 500-ml Erlenmeyer flasks for 4, 6, and 10 wk at 28 C. During incubation, the mixtures were stirred weekly with a spatula. The composted materials were air-dried for 24 hr before being used.

Estimation of soil microbial populations. Soil microbial populations were estimated by making serial dilutions of soils from different treatments prepared in 0.1% water agar, and plating them on selective media. For *Fusarium,* a selective medium developed by Komada (11) was used. The efficiency of this medium in supporting germination of chlamydospores and growth of *Fusarium* spp. from soil is greater than 80% (11). For total fungi, the medium of Steiner and Watson (16) was modified to contain 0.25 g of chloramphenicol per liter. The medium of Farley and Lockwood (7) was used for bacteria, and that of Hsu and Lockwood (9) for actinomycetes. Plates were incubated for 1 wk at 24 C for fungi and bacteria, and 1–2 wk for actinomycetes before colonies were counted.

Field experiments. Soil either was artificially infested with wheat

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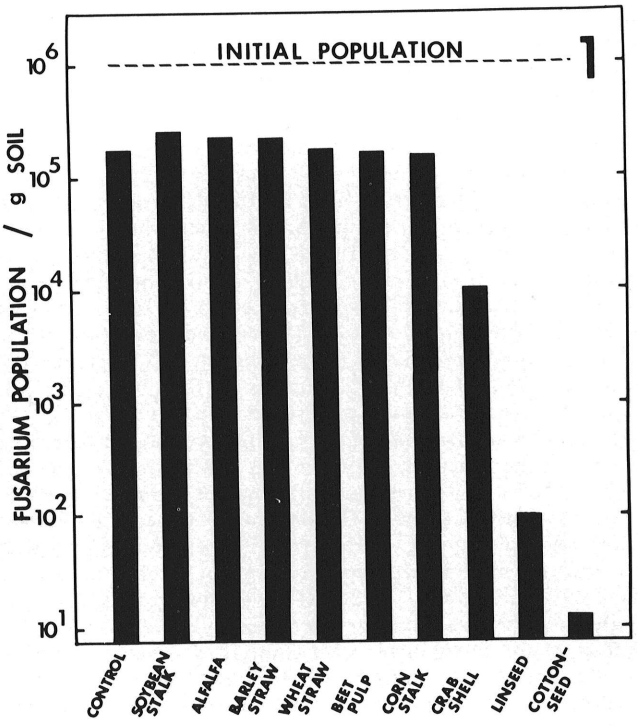
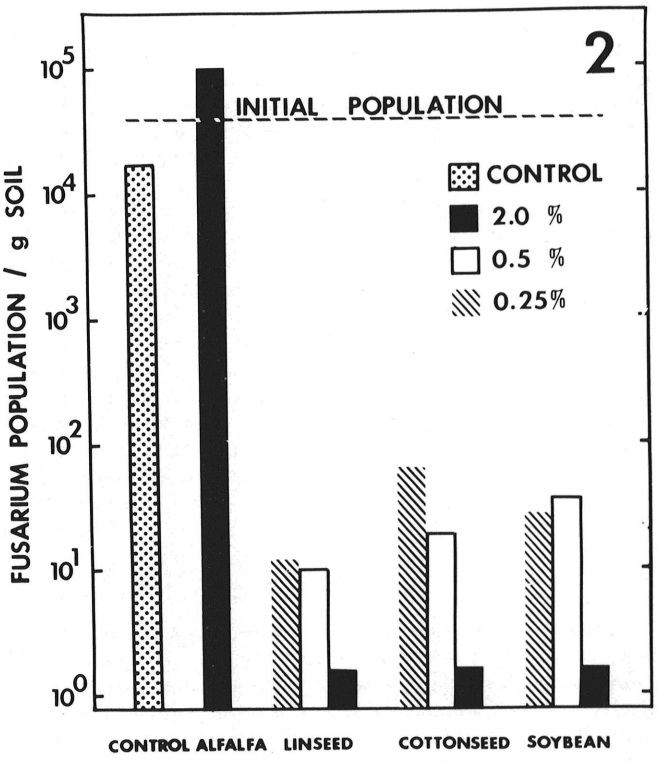
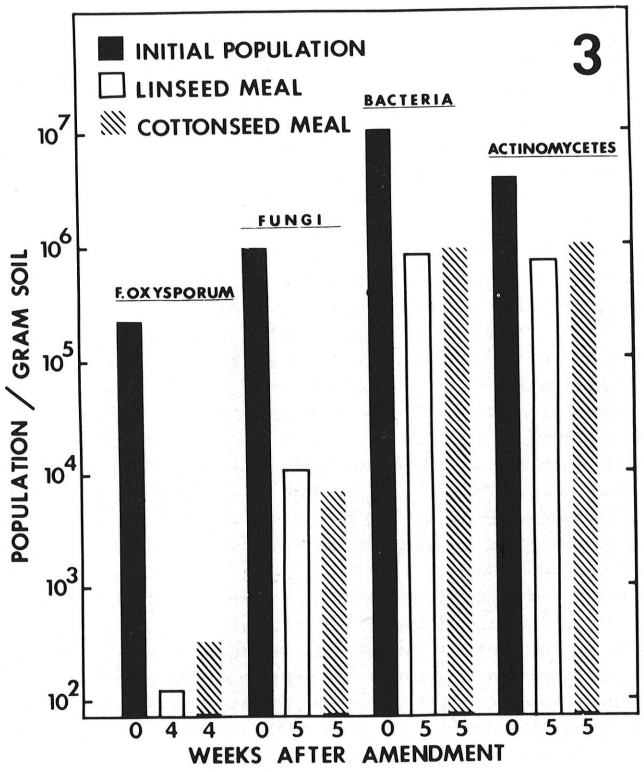
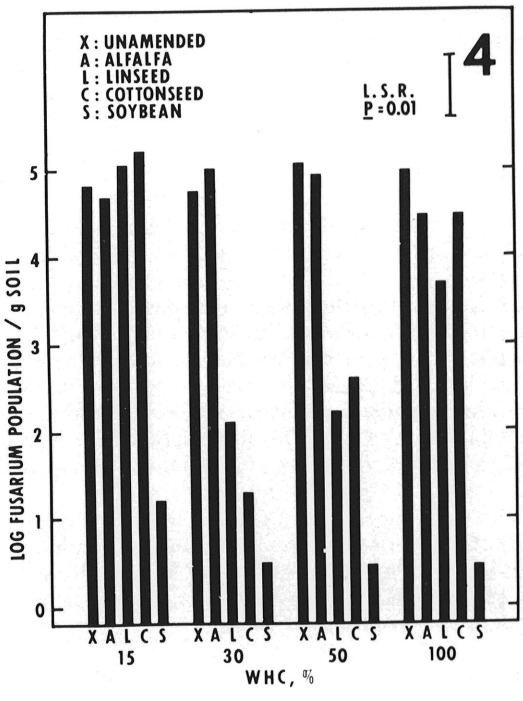
bran-sand cultures of *F. oxysporum* and *F. solani*, or was used without artificial infestation. Infestation was done by rototilling cultures at a rate of approximately 1% (w/w) into the soil 15 cm deep. Two weeks later, soil samples were collected for estimation of the initial *Fusarium* populations. At the same time, the organic amendments were applied to the soil surface in a band 15 cm wide and 3 m long. These were incorporated ~15 cm deep by rototilling, to give a concentration of ~1% (w/w). Changes in *Fusarium* populations were followed by plating pooled soil samples composed of four to six random ~10-g subsamples taken









Figs. 1–4. 1, *Fusarium* populations in soils incubated 4 wk in closed containers after amendment with 1% (w/w) ground plant and animal residues. Least significant range for log populations was 1.50 according to Tukey's *w* procedure ($P = 0.01$). **2,** *Fusarium* populations in soils incubated 5 wk in closed containers after amendment with three concentrations (w/w) of ground plant residues. Least significant range using Tukey's *w* procedure was 0.64 ($P = 0.01$). **3,** Soil microbial populations 4 or 5 wk after amendment with 1% (w/w) linseed and cottonseed meals, determined by culturing on selective media. Least significant ranges obtained using Tukey's *w* procedure for *Fusarium*, total fungi, actinomycetes, and bacteria were 1.84, 0.35, 0.47, and 0.44, respectively ($P = 0.01$). **4,** Effect of different soil moisture contents on reduction of *Fusarium* populations 4 wk after amendment with 1% (w/w) alfalfa and oilseed meals. Initial log population was 5.25. Least significant range by Tukey's *w* procedure ($P = 0.01$) for the soil treatment × soil moisture interaction was 0.68. Indicated soil moisture contents are approximately equivalent to −1.5, −0.1, −0.02, and 0 bars water potential, respectively, for nonamended soils, and −3.0, −0.2, −0.03, and 0 bars water potential for amended soils.

3–10 cm below the surface of each plot every 2 wk. Treatments were randomized within three replications.

Bioassay with pea (*Pisum sativum* L.) as host plant. To determine whether reductions in *Fusarium* populations were correlated with severity of root rot, soil previously infested with *F. solani* f. sp. *pisi* was amended with 1% (w/w) alfalfa or oilseed meals, or was left unamended. After 4 wk of incubation in closed containers, treated soils were spread on plastic trays and air-dried for 1 wk at 24 C. After air-drying, the soils were individually remoistened to about 30% of WHC and four 200-g portions of each soil were transferred into 200-ml styrofoam cups. Peas (*P. sativum* 'Miragreen') were planted, six in each cup, and watered every day by adding 20 ml of tap water per cup. The cups were incubated in a growth chamber at 27 C, a photoperiod of 12 hr/day, and a light intensity of ~20,000 lux.

Three weeks after planting, the plants were uprooted and washed. Root rot was assessed on a scale of 0–6, with 0 indicating a disease-free root system and 6, most severely diseased plants.

Statistical analysis. In experiments conducted by using the dilution plate method, data are based upon two or three replications, each consisting of three to six plates. Analysis of variance was done using data transformed to $\log_{10}(X + 1)$ to maintain homogeneity of the variances of different treatments (15). In the other experiments, three replications were used, and data were analyzed without transformation. Experiments were repeated at least once to verify the results. Significant differences among treatments were estimated according to least significant ranges obtained by using Tukey's *w* procedure.

RESULTS

Effect of soil amendments on *Fusarium* populations. Three-hundred-gram portions of soil infested with *F. oxysporum* and *F. solani* were individually amended with nine different kinds of ground plant and animal residues. *Fusarium* populations densities were estimated at the beginning of the experiment and at weekly intervals for 4 wk. Significant changes in *Fusarium* populations did not occur until the 3rd wk, when those in linseed meal and cottonseed meal-amended soils were reduced to less than 10^3 propagules per gram. After 4 wk, populations were reduced to 10^1–10^2/g by using these amendments, and to 10^3–10^4/g with crabshell, compared with more than 10^5/g in the control and other treatments (Fig. 1). Since the *Fusarium* population in soil amended with ground crabshell was higher than that in soil amended with linseed and cottonseed meals, crabshell was not used in further work. Later it was found that soybean meal reduced *Fusarium* populations at least as effectively as did linseed and cottonseed meals.

Effectiveness of different rates of oilseed amendments. Soils were amended with oilseed meals at rates of 2, 0.5, and 0.25% (w/w), and incubated in closed containers. Nonamended control soil and soil amended with 2% alfalfa meal were included for comparison. *Fusarium* population densities were reduced to near zero after 5 wk in soils amended with 2% oilseed meals (Fig. 2). Rates of 0.5 and 0.25% reduced *Fusarium* populations to less than 10^2 propagules per gram compared with more than 10^4 propagules per gram in the nonamended and alfalfa meal-amended soils.

When the experiment was completed, the soils containing 2% amendments were incubated for another 4 wk without covers; moisture contents were adjusted weekly by weighing. During this period *Fusarium* populations in the oilseed meal-amended soils remained near 10 propagules per gram, whereas *Fusarium* populations in the alfalfa meal-amended and nonamended soils remained at 10^4/g or more.

Effect of amendments on soil microorganisms. Three-hundred-gram portions of soil, either nonamended or amended with 1% linseed or cottonseed meal were incubated in closed containers after the moisture content was adjusted to 30% of WHC (approximately −0.2 bar). Soil microbial populations were estimated at intervals by using dilution plates containing media selective for *Fusarium*, fungi in general, bacteria, or actinomycetes. After 4 wk, *Fusarium* population densities on oilseed meal-amended soils were reduced to 0.1% or less of the initial population (Fig. 3) or those in nonamended controls. After 5 wk the total fungal population in the amended soils was reduced to about 1% of the original population density ($P = 0.01$). The most frequently isolated fungi were from the order Mucorales and from the genus *Trichoderma*. The numbers of actinomycetes and bacteria in the amended soils showed relatively less (but still significant, $P = 0.01$) reduction, compared with those at the beginning of the experiment. Data for nonamended soil after 4–5 wk are not shown in Fig. 3. However, they differed only slightly from those of the initial population, except for bacteria which decreased from 1.9×10^7 to 2.5×10^6. Similar results also were obtained with soybean meal in other experiments.

Effect of soil amendment with composted organic materials. Three-hundred-gram portions of *Fusarium*-infested soil were amended individually with 1% (w/w) composted oilseed meals to observe whether composting would enhance or hasten reduction in the populations. Soil amended with composted alfalfa meal and nonamended soil were included for comparison.

Only slight reduction in *Fusarium* population densities was observed during the first 10–14 days after amendment. *Fusarium* populations were drastically reduced 4 wk after amendment with oilseed meals composted for 4, 6, and 10 wk. Four-week-old composts reduced *Fusarium* populations to 0.01%, and six- and 10-wk old composts to 0.1–1.0%, of that in nonamended soil ($P = 0.01$). Soil amendment with 4- and 6-wk-old alfalfa composts had no effect on *Fusarium* populations, but soil amended with 10-wk-old alfalfa compost had 10× greater numbers than did the nonamended control. These results indicated that composting neither hastened nor enhanced the reduction of *Fusarium* populations by oilseed meal amendments.

Effect of soil moisture content. Three-hundred-gram portions of *Fusarium*-infested soil either were amended with 1% oilseed meal, alfalfa meal, or were untreated. The moisture contents of the soils were adjusted to 15, 30, 50, and 100% of WHC before incubation in closed containers at 24 C. These soil moisture contents corresponded to −1.5, −0.1, −0.02, and 0 bars water potential for the nonamended soils, and −3.0, −0.2, −0.03, and 0 bars for the amended soils.

Four weeks after amendment, *Fusarium* populations were lowest in soil amended with soybean meal; this amendment was effective at all moisture contents tested (Fig. 4). Linseed and cottonseed meals were most effective with soil moistures maintained at 30 and 50% of WHC; there was little or no reduction by these amendments at 15 or 100% of WHC. In the alfalfa-amended soils, *Fusarium* populations were not reduced in soil maintained at any soil moisture level.

Relation of soil Fusarium populations to root rot severity. Because of the difficulty in consistently obtaining root disease symptoms with soybean, *Fusarium* root rot of peas was used in this work. Pea root rot severity was related to the *Fusarium* population. *Fusarium solani* f. sp. *pisi* population per gram of nonamended soil and of soils amended with 1% (w/w) alfalfa, linseed, cottonseed, and soybean meals were 7.9×10^3, 5.8×10^3, 1×10^2, 5.0×10^1, and 2.5×10^1, respectively. The corresponding disease indices of peas

TABLE 1. Fusarium populations in natural and artificially infested loamy sand in two fields 4 wk (field A) and 5 wk (field B) after amendment with 1% (w/w) alfalfa and oilseed meals

	Log Fusarium population per gram of soil		
	Field A[a]	Field B[b]	
Soil treatment	Naturally infested	Naturally infested	Artificially infested
Nonamended control	3.45	3.32	4.08
Alfalfa meal	5.18	4.28	4.53
Linseed meal	5.20	4.34	4.20
Cottonseed meal	5.04	4.25	4.40

[a]Initial log Fusarium population was 3.28.
[b]Log Fusarium populations at time of soil amendment in natural and artificially infested soils were 3.60 and 4.84, respectively.

after a 3-wk period were 3.0, 2.7, 2.1, 1.6, and 0.9; however, statistically significant differences occurred only with soybean meal-amended soil, as compared to the alfalfa meal-amended ($P = 0.05$) and the nonamended control ($P = 0.01$) soils. Phytotoxicity induced by linseed and cottonseed meal complicated disease evaluation with these amendments. No phytotoxicity was observed in plants grown in soybean meal-amended soils.

Field experiments. Two experiments were conducted in a loamy sand soil in southwest Michigan. Natural soil and soil artificially infested with wheat bran-sand cultures of *F. oxysporum* and *F. solani* were amended with alfalfa and oilseed meals at rates of 1%, based on the weight of soil in rows 3 m long, 15 cm wide, and 15 cm deep. Nonamended controls were included. All treatments had *Fusarium* populations equivalent to or higher than those in the nonamended controls after 4–5 wk (Table 1). After 9–12 wk, *Fusarium* populations in all amended soils were higher than those in the nonamended soils. Therefore, the results obtained from these field experiments did not support those obtained under laboratory conditions.

Effect of different soils. Capac loam (pH 7.8, sand:silt:clay = 50:24:26, 2.96% organic matter, WHC = 280 ml/kg) and Brookston loam (pH 7.5, sand:silt:clay = 48:33:19, 6.46% organic matter, WHC = 480 ml/kg) soils were artificially infested with wheat bran-sand cultures of *F. solani* f. sp. *pisi*. Three-hundred-gram portions of each soil were individually amended with 1% alfalfa, soybean, cottonseed, or linseed meal and incubated in closed containers.

No reduction in *Fusarium* population density occurred in Brookston loam soil. Even after 10 wk of incubation, the *Fusarium* populations in all treatments remained in the range of $5–7 \times 10^5$ propagules per gram of soil. *Fusarium* populations were slightly reduced in oilseed meal-amended Capac loam soil after 6 wk of incubation; after 8 wk, population densities were still in the order of about 10^3 propagules/g soil. After 10 wk, the *Fusarium* populations in oilseed meal-amended soils were reduced to $3–6 \times 10^1$ propagules per gram of soil. The *Fusarium* populations in the alfalfa meal-amended soil remained about 5×10^5 propagules per gram throughout the experiment.

DISCUSSION

Soil amendments, including chitin (2,10), plant residues (12–14), sugars (14), urea (14), amino acids (4), and spent coffee grounds (1) previously have been evaluated for effects on populations of various species of *Fusarium*. Some reduced populations (1,3,10,14), whereas others did not (1,12,13). Sequeira (14) obtained elimination of *Fusarium oxysporum* f. *cubense* from soils supplemented with high concentrations of glucose, sucrose, or chopped leaf and stem tissues of sugar cane, when the soils were held at 60% of WHC or higher. We have found no previous reports of the use of oilseed meals as soil amendments to reduce *Fusarium* populations. These amendments reduced *Fusarium* population densities to 0.001 or less of the original in 4–6 wk. They were effective at concentrations as low as 0.25%, (w/w), and at soil moisture contents down to at least 30% of WHC (−0.2 bar); soybean meal was effective at 15% of WHC (−3.0 bars).

Reduction of viable population of pathogenic *Fusarium* in soybean meal-amended soil was correlated with reduction in root rot of pea. Soil amended with soybean meal was not phytotoxic following 1 wk of air-drying, whereas root stunting and necrosis were associated with linseed and cottonseed meal, after this period.

Although the oilseed meals efficiently eradicated *Fusarium oxysporum* and *F. solani* from field soil confined in closed containers in the laboratory, attempts to reduce populations by these materials failed in the field, possibly due to unfavorable soil moisture, inadequate mixing with the soil, or escape of volatile products of microbial degradation. The oilseed amendments were most effective at 30 and 50% of WHC (equivalent to −0.2 and −0.03 bars water potential, respectively); they were less effective in saturated soil (0 bars), and only soybean meal was effective at 15% of WHC (−3.0 bars). Water potentials in the field could have been this low or lower. Of three soils tested in the laboratory only the loamy sand favored drastic declines in *Fusarium* populations, following amendment with oilseed meals. Population reductions were less in Capac loam, and did not occur in Brookston loam. The order of responsiveness to the amendments was inversely related to the organic matter content of the soils. Thus, practical applications of oilseed meals, if any, may be restricted to specific soil types, and may require sealing the soil surface with plastic or other tarping for four weeks. Whether *Fusarium* populations can be reduced in treated soils under plastic in the field is not yet known.

Subsequent work (Zakaria, Lockwood, and Filonow, *unpublished*) has shown that toxic volatile substances, principally ammonia, are produced during decomposition of the oilseed meals in soil. This work will be published in a separate communication.

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