Effect of Nontreated Field Soil on Sporulation of Vesicular-Arbuscular Mycorrhizal Fungi Associated with Soybean

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ABSTRACT

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Experiments were conducted in which cultures of *Glomus macrocarpus* var. *geosporus* or *Gigaspora gigantea* were established on soybean plants growing in steamed or fumigated soil or sand amended with various amounts of nontreated soil or organic matter extracted from nontreated soil. Spore counts were made after 3–4 mo and expressed as number per gram of root. Amendments of 1 and 5% nontreated soil to steamed soil reduced *Glomus* sporulation almost three and six fold, respectively, compared with sporulation in steamed soil. Average chlamydospore size of *Glomus* was significantly less in soil amended with nontreated soil compared with that in steamed soil and was positively correlated with spore numbers. Additions of 5% nontreated soil decreased azygospore production of *G. gigantea* by 93%. Separation of cultures of *Glomus* in steamed soil from nontreated soil by means of a polycarbonate membrane (pore size of 0.2 μm) prevented sporulation suppression. Soybean seedling roots grown in nontreated soil for 1 wk did not transmit the sporulation suppressor when transplanted without soil into steamed soil. Organic matter extracted from nontreated soil manifested sporulation suppression similar to that of nontreated soil; the suppressive activity was eliminated when organic matter was heated for 15 min at 65–75 C. Additions of small amounts of nontreated soil or organic matter to *Glomus* cultures in quartz sand induced 2–3 and 7–8-fold increases in sporulation, respectively, over that obtained with the same additions that had been steamed. Additions of cultures of actinomycetes isolated from nonsterile soil to cultures of *Glomus* or *Gigaspora* in steamed soil had little or no effect on sporulation. Additions of 4.6% by volume of nonfumigated soils, of four different classifications, to soil in plots which had been fumigated with methyl bromide also caused significant reductions in sporulation of mycorrhizal fungi. A clay soil induced a greater reduction of total *Glomus* sporulation than the three loam soils. The results of these studies explain in part why infection and sporulation on soybean roots in the field are only a fraction of those found in experiments with sterile soils.

Additional key words: Glomus, Gigaspora

The ubiquitous nature of vesicular-arbuscular (V-A) endomycorrhizal fungi usually requires that the natural mycorrhizal fungal populations be eliminated from soil so that experiments with regulated inocula of selected species may be done. Such initial treatments virtually eliminate many organisms from the soil and create an entirely artificial soil environment. Recently the role of hyperparasites and competition between species of endomycorrhizal fungi were reported (11); however, other factors may be involved that affect survival of populations of these fungi.

The presence of factors in certain soils that suppress certain soilborne pathogens has been well documented (1,3); however, explanations of these phenomena are often fragmentary and theoretical. Although root infection by mycorrhizal fungi in most cases is not considered pathological, the distinction between pathological and symbiotic may be moot, and under certain conditions V-A mycorrhizal relationships may appear quite pathological (12). The microbial relationships in certain soils that suppress soilborne pathogens and control root disease may find a parallel in the ecological factors affecting endomycorrhizal fungi (1).

Soon after research was initiated on the endomycorrhizal relationships of soybean (10), soil samples from soybean fields were found to contain root fragments with small numbers of exceptionally small chlamydospores (<70 μm) presumably of *Glomus macrocarpus* Tul. & Tul. var. *geosporus* (Nicol. & Gerd.) Gerd. & Trappe. This was in contrast to the larger chlamydospores found in greenhouse cultures initiated in steamed soil or field plots previously fumigated with methyl bromide and subsequently inoculated with chlamydospores. When these small chlamydospores were selected to initiate cultures on soybean in steamed or methyl bromide-treated soil in pots in the greenhouse, only a small percentage of the cultures were successfully established, and in these the endophyte developed large chlamydospores, many 130–150 μm in diameter. These observations led to a series of greenhouse and small plot experiments to confirm and to explain more fully the differences between cultures of V-A mycorrhizal fungi in treated and field soil.

MATERIALS AND METHODS

Inocula (spores) of *G. macrocarpus* var. *geosporus* and *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe were produced on soybean (*Glycine max* [L.] Merr.) roots grown in steam-sterilized soil in the greenhouse. Cultures were initiated with healthy-appearing spores selected individually under a dissecting microscope. Chlamydospores of *G. macrocarpus* were obtained from 90- to 120-day-old plants by blending roots washed free of soil, passing the root blend through a 230-μm sieve, and retaining the spores on a 53-μm sieve. In some experiments spores used for inoculum were cleaned from much of the root debris by centrifugation in 50% sucrose for 3 min in a clinical centrifuge at 1,500 rpm. *G. gigantea* azygospores were obtained from greenhouse pot cultures by suspending the soil and roots in water and decanting the spores through a 595-μm sieve, to remove coarse debris and roots, onto a 200-μm sieve. Spores were subjected to additional sedimentation and decanting for further isolation. All inocula and amendments were mixed with the soil before filling the pots and incorporated into plot soil with the aid of a small concrete

mixer.

Heat treatment was accomplished by steaming soil at 82 C for 30 min in 10-cm-deep wooden flats. Soil fumigation was performed using methyl bromide at the rate of 80 cm^3/m^2 3 wk prior to use.

Assays for root infection and sporulation. Soil samples were obtained from pots in the greenhouse with a cork borer (1 cm in diameter) and from plots with a soil sampling tube (2.5 cm in diameter). The number of soil borings taken varied from three per pot to eight per plot. Roots were washed from the soil, cut into fragments 1–2 cm long, cleared, and stained in 0.1% Trypan blue (10). Approximately 25–35 randomly selected stained fibrous root segments were placed in rows on a microscope slide and each piece was observed under the dissecting microscope for total length, length infected, and number of vesicles.

Spore numbers were determined from soil samples taken 3–4 mo after planting. Chlamydospores of *G. macrocarpus* were recovered from within root tissue, on the root surface, and in the soil by first gently washing the roots to remove the soil and allowing the mycelium and spores to remain attached to the roots. The surface spores were removed by forcefully spraying the roots supported on a 595-μm sieve and retaining the spores on a nested 53-μm sieve. Roots were separated from debris by flotation and manual selection, blotted to remove excess water, weighed fresh, blended in water for 30 sec, passed through a 200-μm sieve to remove coarse debris, and the chlamydospores were retained on a 53-μm sieve. Azygospores of *G. gigantea* were recovered by gently washing the soil sample through a 595-μm sieve to collect roots free of soil. The azygospores attached to the roots by mycelia were removed by forcefully spraying the roots and retained on a 200-μm sieve; roots were cleaned and weighed as described above. Spore counts are expressed as numbers per gram of root tissue except where indicated.

Sizes of chlamydospores were determined either by measuring 50 randomly selected spores or by determining the percentage of total spores that could pass through a 74-μm or 105-μm sieve when vigorously sprayed.

In all experiments a suspension of *Rhizobium* inoculum was added to each pot or plot at planting. No fertilizer was used unless stated.

RESULTS

Dilution of steamed soil with nontreated soil. The effect of mixing 0, 5, 20, 50, and 100% of nontreated soil (Portsmouth sandy loam [Typic Umbraquult; fine-loamy, siliceous, thermic]) with steamed soil on the sporulation of *G. macrocarpus* and *G. gigantea* was studied. Two seedlings of soybean cultivar Davis were grown in each of four replicate 1,500-cm^3 pots for each soil mixture - fungus treatment combination. Half of the pots in each soil mixture treatment were not inoculated and the other half were inoculated with either 1,200 *G. macrocarpus* chlamydospores or 650 *G. gigantea* azygospores per pot. Root samples were taken 96 days after planting to determine infection and sporulation.

Chlamydospore density of *G. macrocarpus* within roots growing in soil to which chlamydospores were added was 52% less in the 5% nontreated soil mixture and was 94% less in 100% nontreated soil compared with that in roots growing in 100% steamed soil (Table 1). Within each soil mixture, addition of chlamydospores as inoculum resulted in increased chlamydospore formation in the roots; however, this increase was significant only in the 5% nontreated soil mixture. Chlamydospore production in roots growing in 5% nontreated soil mixture without chlamydospore inoculum was three to four times greater than that in other soil mixtures with greater percentages of nontreated soil.

Chlamydospores formed in treatments with nontreated soil were smaller than those formed in steamed soil (Table 1); the percentage of spores smaller than 74 μm was not markedly affected by increasing percentages of nonsteamed soil over 20%. The number of spores smaller than 74 μm was negatively correlated with spore number ($r = -0.8766$). No differences in plant growth appeared among the various treatments.

Sporulation of *G. gigantea* in treatments with additions of 5% or more nontreated soil was reduced to less than 7% of that in 100% steamed soil (Table 1). Sporulation differences among treatments in which nontreated soil was added were nonsignificant. Counts of spores with contents not uniform or "normal"-appearing indicated that addition of nonsteamed soil significantly increased the percent of these abnormal spores.

Role of diffusible substances. An experiment was designed to assess the nature of the suppressive sporulation factor(s) by determining whether membrane-permeable substances were involved. Bags of polycarbonate membranes with a pore size of 0.2 μm were made by glueing the edges of a 20 × 25-cm sheet together with Elmer's fabric cement. Each bag (open at the top) was placed inside of and also lined with nylon cloth bags to protect the membranes during the process of setting up the experiment.

The nylon cloth bags with or without the polycarbonate membrane were filled with steamed Portsmouth sandy loam plus 320 chlamydospores of *G. macrocarpus* and placed into nontreated or steamed soil contained in a 20-cm-diameter pot. There were two or three replications per treatment. Each bag held 700 g of soil and was immersed in the pot so that the rim of the bag was about 2 cm above the soil surface. The soil was carefully irrigated during the experiment so the soil was moistened throughout, but there was no surface water movement from outside to inside the polycarbonate bags. A germinated soybean seed was planted in the soil in each bag, and plants were grown for 103 days.

When the bags were removed from the pots, root penetration of the polycarbonate bags into the soil surrounding the bags had occurred only at the seams and this occurred infrequently. Soil and root samples from within the bags were processed for spore numbers in the previously described manner. The average number

TABLE 1. Effect of growing soybean for 96 days in various proportions of field soil mixed with steamed soil on chlamydospore production by *Glomus macrocarpus* var. *geosporus* and azygospore production by *Gigaspora gigantea*

	G. macrocarpus			*G. gigantea*		
Percent field soil	Chlamydospores added[a]	Spores recovered per gram of root	Percent spores <74 μm[b]	Azygospores added[c]	Spores recovered per gram of root	Percent abnormal spores
0	+	3,900	15	+	1,700	16
	−	0	−	−	0	−
5	+	1,880	37	+	120	51
	−	1,120	32	−	30	30
20	+	360	59	+	80	31
	−	350	63	−	40	37
50	+	530	38	+	80	29
	−	330	54	−	70	28
100	+	220	67	+	70	34
	−	130	71	−	70	30
	LSD (.05)	422			150	8

[a] 1,200 chlamydospores/1,500 ml soil.
[b] Percentage spores passed through sieve with 74 μm openings.
[c] 650 azygospores/1,500 ml soil.

of spores developed per g of root inside and outside the roots, respectively, were: 2,290 and 4,100 (6,390 total) from roots within the membrane, in pots with nontreated soil outside; 280 and 500 (780 total) from roots within the nylon bag, nontreated soil outside; 1,130 and 5,630 (6,760 total) within membrane, steamed soil outside.

Association with roots. The nature of the suppression of sporulation was studied further in an experiment designed to reveal whether the suppressor was associated with roots. Each of five soybean seedlings, in the primary leaf stage, was transplanted from sand into nontreated field soil plus 280 *G. macrocarpus* chlamydospores. After 1 wk, the roots of each plant were thoroughly washed to remove as much soil as possible with minimal root injury; the plants were transplanted to fumigated soil in 10-cm-diameter pots and allowed to grow for 3 mo before soil samples were taken and chlamydospore assays performed. The average number of spores formed per g of root tissue for plants that had grown for 7 days in nontreated soil was 4,260 compared with 3,740 for plants grown only in steamed soil. The difference was not statistically significant.

Effect of high soil dilutions. The magnitude and potential of the sporulation suppression was determined by mixing enough nontreated soil with steamed soil to give 1, 2, and 5% nontreated soil in the final mixture. Also included were 100% steamed and 100% nontreated soil. Four hundred chlamydospores of *Glomus* were added to each 1-L pot and each treatment was replicated five times. One germinated soybean seed was planted per pot.

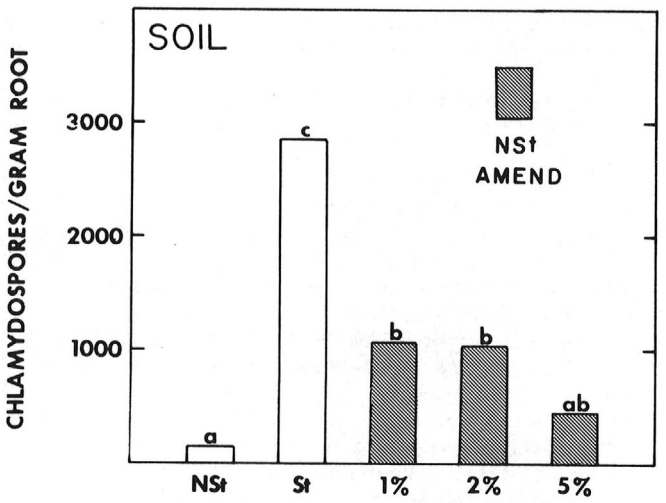

Fig. 1. Effect of adding nonsteamed soil (NSt) to steamed soil (St) in ratios of 1, 2, or 5% on the number of chlamydospores of *Glomus macrocarpus* var. *geosporus* per gram of soybean roots after 84 days. Bars with the same letter are not significantly different, $P = 0.05$.

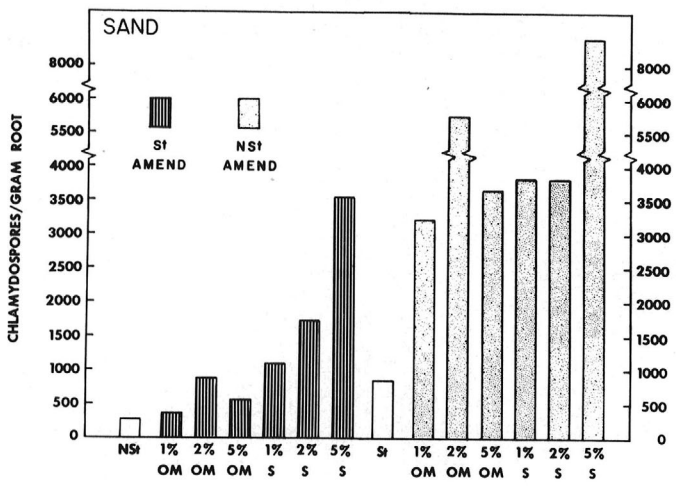

Fig. 2. Effect of adding to sand different amounts of nonsteamed (NSt) or steamed (St) soil (S) or the organic fraction (OM) recovered from these amounts of nonsteamed or steamed soil on the number of chlamydospores of *Glomus macrocarpus* var. *geosporus* per gram of soybean root after 90 days.

Chlamydospores collected after 84 days from inside and outside the roots were counted and combined. Three-fold fewer chlamydospores were produced in 1% nontreated soil than in the 100% steamed soil, and sporulation was 50% less in the 5% nontreated soil treatment than that in the 1 and 2% treatments. (Fig. 1).

Effect of soil organic matter in white quartz sand culture. Since a preliminary experiment indicated that addition of organic matter extracted from nontreated soil had the same suppressive effect on *G. macrocarpus* sporulation as did nontreated soil, an experiment was performed to compare the sporulation suppressor residing in the soil organic fraction with that found in whole soil. The organic fractions extracted from 10, 20, and 50 ml of nontreated and steamed Portsmouth sandy loam were used to supply the 1, 2, and 5% organic matter treatments, respectively. These organic fractions were obtained by suspending each soil volume in water and decanting the floating material three times through an 840 μm sieve to remove coarse debris, onto a nested 53-μm sieve. Treatments that involved organic fractions were replicated three times. Other amendments included nontreated and steamed soil volumes of 10, 20, and 50 ml; these treatments were replicated five times. This experiment was carried out by growing the plants in white quartz sand (230 μm [65-mesh]) in 1-L containers and supplying nutrients weekly in the form of a nutrient solution containing essential elements except phosphorus. Four times during the experiment a suspension containing 0.15 g of tribasic calcium phosphate was injected with a syringe at various depths into sand in each container. Each organic fraction or soil volume was mixed along with 400 chlamydospores into 1-L of steamed sand. Controls of steamed and nontreated sand plus chlamydospores were included. Chlamydospore assays were performed 90 days after planting and included spores within and those attached to the outside of the root samples.

Additions of nontreated organic matter caused 7- to 8-fold increases in spore production over the same percent additions of steamed organic matter whereas spore populations in the steamed organic matter treatments were virtually of the same magnitude as populations in the steamed sand controls (Fig. 2). Sporulation in treatments which received nontreated soil was two to three times greater than that in treatments that received the same percent additions of steamed soil. Within the treatments receiving either steamed or nontreated soil, spore populations in treatments receiving 5% soil were double those in which 2% soil was added. Spore populations in treatments receiving 5% organic matter did not show this trend.

Effect of high temperature on inactivation of suppressor. Since both organic matter and soil affected sporulation similarly, an experiment was performed in which 5-ml slurries of the organic matter (~1.5 g dry wt) extracted from 50 g of nontreated Portsmouth sandy loam were exposed to various temperatures in thin-walled glass tubes in a water bath for 15 min. Two tubes were used at each temperature. The heated organic matter from each tube was mixed with 1-L of steamed soil and 400 *G. macrocarpus* chlamydospores. One germinated soybean seed was planted in each 1-L pot and grown for 4 mo. The average number of spores recovered per gram of root for steamed soil (SS), SS+ nonsteamed organic matter (NO), SS+NO heated to 45 C, SS+NO heated to 65C, SS + NO heated to 75 C, and SS + NO heated to 85 C were 11,700, 2,140, 1,090, 4,780, 9,690, and 8,050, respectively.

Role of actinomycetes. Preliminary isolations for fungi, bacteria, and actinomycetes from dilutions of soil from some of the experiments indicated that in soil with high chlamydospore populations after 3 mo, actinomycete populations were very low. A greenhouse experiment was conducted to determine if actinomycetes influenced sporulation of *G. macrocarpus* and *G. gigantea*. Actinomycetes were isolated from dilutions of nontreated soil on plates of either arginine-glycerol-salt agar (4) or 0.2% chitin agar (7). Twenty such isolates were each grown in 100 ml of glucose-

peptone broth (10 g glucose, 10 g peptone, 1L H_2O) in shake culture for 1 wk. The culture medium was filtered off, the mycelia combined and homogenized in a blender for 30 sec. This homogenate was used as inoculum.

Inocula consisting of 2,000 *G. macrocarpus* chlamydospores or 10,000 *G. gigantea* azygospores were mixed along with the actinomycete inoculum with Portsmouth sandy loam to fill each 20-cm-diameter pot. For each V-A fungus there were four treatments: steamed soil; steamed soil mixed with high level actinomycete mixtures (5.2 g dry wt per pot); steamed soil mixed with the low level actinomycete mixture (0.6 g/pot); nontreated soil. Each treatment was replicated four times. After two soybean plants per pot were grown for 70 days, soil samples were taken from each *Glomus*-infested pot and roots were stained and examined for infection. Four months after planting, soil samples were taken from each pot for spore population determinations, and actinomycete population levels were determined by plating out in duplicate 0.5 ml of a 1:10,000 soil dilution on agar containing 2 g of colloidal chitin per liter (7). Plates were incubated for 8 days at 28 C. Two weeks after the sampling for spores, soil was removed from all pots of each treatment, bulked and mixed, and coarse roots were removed. The soil was replaced in the pots, soybeans were planted and grown for an additional 3 mo, and soil samples were taken for spore population analyses.

The treatments had little if any effect on the percent root infection by either fungus; the only significant reduction in sporulation compared with the steamed-soil control at either sampling time was in the nontreated soil (Table 2). The reduction in number of *Glomus* chlamydospores between either treatment with actinomycetes and the sterile soil was approximately 20% (not statistically significant) at both sampling times and did not approach the 97% reduction found in the untreated soil. Sporulation of *G. gigantea* was affected less by the actinomycetes than was that of *G. macrocarpus*; the former was 89–97% less in nontreated soil than it was in the other treatments.

Actinomycete population levels in soil infested with the high inoculum level was five to eight times that found in the nontreated soil or soil infested with the low inoculum level. No actinomycete colonies were observed in assays of the steamed soil.

Effect of four soil types in small plots. To assess the effect on sporulation of the two V-A fungi, nontreated field soil from four different locations were added to Norfolk sandy loam in microplots (8); each of the 48 207-L plots were fumigated with 20 cm^3 of methyl bromide and allowed to aerate for 3 wk. The soil in each plot was mixed with 9.5 L of either fumigated or nontreated soil of either Portsmouth fine sandy loam, Cecil clay, Norfolk sandy loam, or Bladen loam. At incorporation, inocula consisting of either 5,000 *G. macrocarpus* chlamydospores or 2,800 *G. gigantea* azygospores were mixed along with 10 g of KCl. Each treatment was replicated three times. On 5 June, seed of soybean cv Bedford was planted, and 3 wk later seedlings were thinned to nine per plot. Plots were irrigated as required.

On 19 September (106 days) all *Glomus* inoculated plots were sampled and the soil and roots were assayed for spores. Chlamydospore population densities inside roots from plots amended with nontreated soil were markedly less than those from plots amended with fumigated soils (Fig. 3A); the percentage of chlamydospores greater than 105 μm was also reduced in plots amended with the nontreated soils (Fig. 3). This smaller spore size was most evident with spores formed inside the roots growing in plots amended with nonfumigated Bladen or Cecil soils; the percentage of spores greater than 105 μm in diameter from plots amended with nonfumigated Bladen soil was significantly less (0.05 level) than this percentage of spores from plots amended with nontreated Portsmouth and Norfolk soils.

Roots extracted from soil samples taken on 27 August (83 days) from *Glomus* inoculated plots showed no difference among the different soil types with respect to percent of roots infected with V-A fungi. In roots from plots to which nontreated soil had been added, infection averaged 75% (73–81%) and roots growing in plots amended with fumigated soils averaged 78% (73–83%). Nontreated Norfolk and Bladen soils had a greater effect on reducing vesicle formation in roots than nontreated Portsmouth or Cecil soils; vesicle numbers were less in all root samples from plots amended with nontreated soil than in those from plots amended with the same soil that had been fumigated (Fig. 3A).

The effect of adding nontreated soils on *Glomus* chlamydospore production outside the roots was similar to that on chlamydospores formed inside the roots (Fig. 3B). The percentage of spores greater than 105-μm in diameter varied from 60–66% in plots that had received fumigated soil and varied from 16–32% in plots to which nontreated soil had been added.

In plots amended with nontreated soils, total *Glomus* sporulation was significantly less in plots amended with Cecil clay than in plots amended with other soils (Table 3). This was mainly due to the relatively small number of spores formed outside the roots in plots amended with Cecil clay. The total *Glomus* sporulation in plots amended with nontreated soils was 87% less than that in plots amended with fumigated soils.

Soil samples were taken in early December for *Gigaspora* azygospore assays. Because roots had decayed considerably by this time, spore populations were expressed per 100 ml of soil. Azygospore populations in plots that received fumigated soils were three to five times greater than those in plots amended with nontreated soils (Table 3). Azygospore numbers were not significantly different amont treatments which received fumigated soils; however, plots amended with nontreated Norfolk sandy loam had twice as many azygospores as the other three treatments receiving nonfumigated soils.

DISCUSSION

Results from these experiments demonstrate that biotic factors exist in nontreated agricultural field soils that markedly reduce the ecological position of *G. macrocarpus* and *G. gigantea* by limiting sporulation to only a fraction of that which potentially could occur after treatment of soil by heat or methyl bromide. Sparse sporulation in nontreated soil probably results in low inoculum density for the subsequent growing season and hence mycorrhizal associations of soybean in nontreated soil do not attain levels commonly found in experiments using fumigated or heat-treated soils.

TABLE 2. Effect of actinomycetes on root infection and spore production of *Glomus macrocarpus* var. *geosporus* and *Gigaspora gigantea* on soybean

Treatment	Mycorrhizal fungus	Actinomycete inoculum level	Root infection after 70 days (%)	Spores per g of root		Actinomycetes (colonies/plate[b])
				After 4 mo	After 8 mo[a]	
Steamed soil	*G. macrocarpus*	0	90	26,600	43,000	0
	G. gigantea	0	75	18,275	9,500	0
Steamed soil	*G. macrocarpus*	High	94	20,900	34,300	16
	G. gigantea	High	76	19,850	9,000	25
Steamed soil	*G. macrocarpus*	Low	87	18,600	33,100	2
	G. gigantea	Low	86	17,775	7,200	11
Nonsteamed soil	*G. macrocarpus*	0	88	775	330	2
	G. gigantea	0	84	1,925	206	5

[a] Following repotting and replanting in the same soil after 18 wk.
[b] After 4 mo, at a 1:10,000 soil dilution.

The magnitude of the dilution effect on the sporulation-suppressing factors, as shown in the soil mixture experiment, was manifest even though increasingly greater amounts of natural *Glomus* inoculum were present with increasing percentages of nonsteamed soil. The reduction in sporulation in the 20, 50, and 100% field soil treatments compared with that in the 5% nonsteamed mixture indicates that the dilution effect was greater on the sporulation-suppressing factors than on initial inoculum levels. Because spore size and number were positively correlated, in this and other experiments, the same factors may suppress sporulation and reduce spore size.

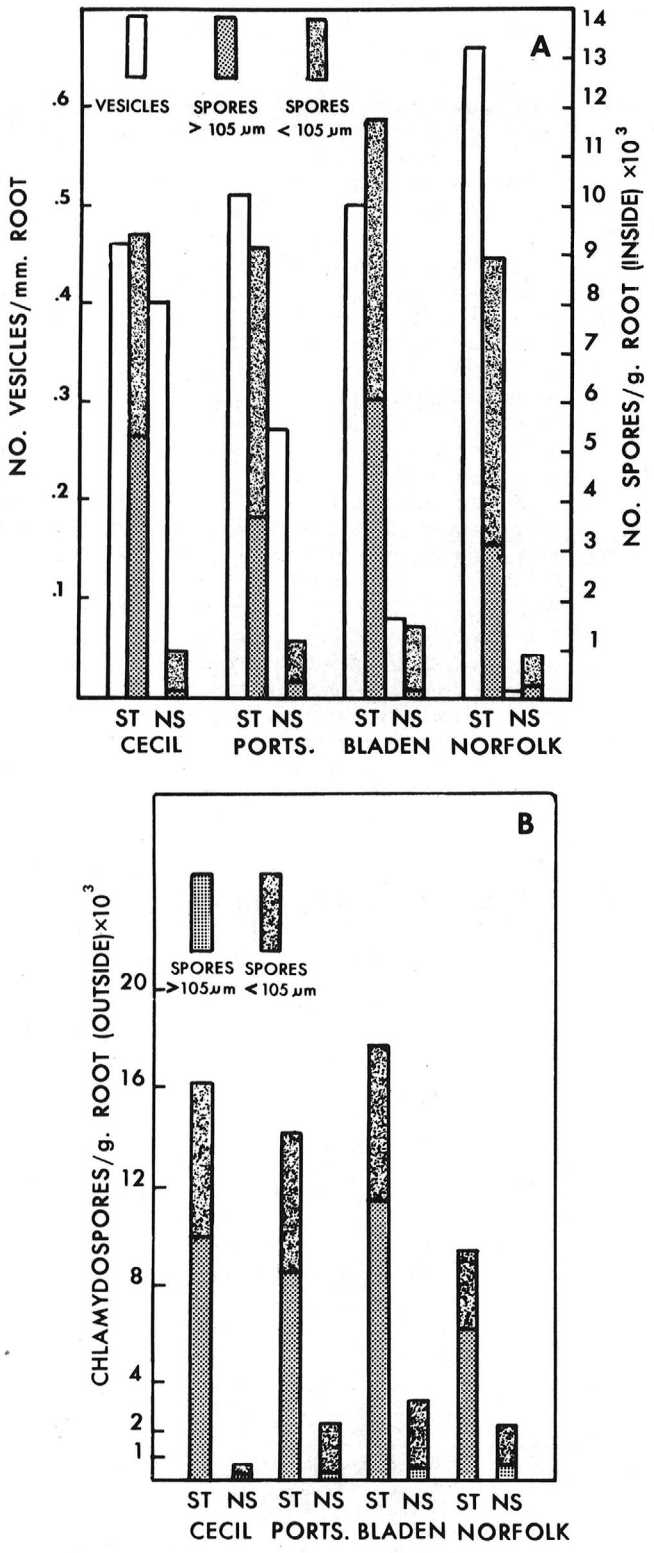

Fig. 3. Effect on vesicle formation and sporulation of *Glomus macrocarpus* var. *geosporus* of incorporating into fumigated microplots 6.5% by volume of methyl bromide-treated soil (ST) or nontreated (NS) soil of four different soil types: Cecil clay, Portsmouth sandy loam, Bladen loam, and Norfolk sandy loam. Vesicle counts were made from samples taken on 27 August; spore counts were made from samples taken 19 September. **A,** Vesicle formation and sporulation within soybean roots. **B,** Sporulation outside soybean roots.

Although *Gigaspora* sporulation was reduced more than that of *Glomus* by the addition of 5% nonsteamed soil in the soil mixture experiment, the similar reaction of *Gigaspora* and *Glomus* sporulation to higher proportions of field soil could mean that the suppressive factors are acting in a similar manner on both fungi.

The role of diffusible substances appears minimal because the suppressive factor(s) did not readily penetrate the membrane with 0.2-µm pores. This may imply that the inhibitory mechanism does not involve highly diffusible compounds produced by organisms, but rather involves an action of organisms in close proximity to mycorrhizal structures. The 0.2-µm pore size of the membrane is sufficiently small to prevent most organisms from passing through. The 88% reduction of chlamydospore numbers inside the nylon cloth bag compared with the populations inside the membrane bag indicates that the suppressive factor can move through the steamed soil and exert its action in areas originally free of the factor. Failure to transfer the suppressive factor in the roots of soybean after they had grown for 1 wk in nontreated soil is evidence that the factor does not invade the roots, and results of the high soil dilution experiment indicate that the suppressive factor(s) apparently increases or multiplies in steamed soil and suggests that it is associated with living organisms. Thermal inactivation of the suppressive factor(s) (~65 C for 15 min) approximates the temperature found by other workers to eliminate antagonists of *Phytophthora cinnamomi* (2). A combined view of these results suggests that the suppressive sporulation factors are associated with soil microorganisms directly rather than with diffusible materials.

The stimulation of *G. macrocarpus* sporulation by small additions either of organic matter from nontreated soil or of nontreated soil alone to sand culturing soybean may reveal a relationship between the action of the sporulation suppressor and the media or soil type. If organic matter is required for suppressor levels to increase, then rooting media very low in organic matter (sand) may allow only small increases of the factor(s), which in turn may stimulate sporulation; minute amounts of toxic or inhibitory materials commonly exhibit stimulatory biological effects. Addition of 5% soil to the quartz sand cultures gave large increases in the spore production over the addition of 2% soil, whether the added soil was steamed or not. The 5% soil may have provided additional nutrients which may have been limiting production of chlamydospores.

Results of the actinomycete experiment indicate that actinomycetes do not strikingly depress sporulation. No attempt was made, however, to classify the actinomycetes used, and vast differences could exist between the effect of one species and another; furthermore, actinomycetes could interact with other organisms (bacteria or fungi) to cause significant effects on sporulation of V-A fungi. However, since *G. gigaspora* sporulation was not affected by additions of actinomycetes, and actinomycete

TABLE 3. Effect of adding different fumigated or nonfumigated soil types to fumigated microplots on total chlamydospore production by *Glomus macrocarpus* var. *geosporus* and on azygospore production by *Gigaspora gigantea* on soybean roots

	G. macrocarpus		*G. gigantea*	
	Chlamydospores/g root		Azygospores/100 cc soil	
Soil type	Fumigated	Nonfumigated	Fumigated	Nonfumigated
Cecil clay	25,900	1,800[a]	6.2	1.4
Portsmouth sandy loam	23,490	3,760	4.4	1.3
Bladen loam	29,490	4,680	7.5	1.4
Norfolk sandy loam	18,290	3,010	6.3	2.8[a]

[a]Significantly different from other nonfumigated treatments, $P = 0.05$.

populations in the high inoculum treatment were ten times those found in nonsteamed soil, the actinomycetes used are probably not primarily involved in the sporulation repression of these V-A fungi.

The suppressive effect of the nontreated soils on *G. macrocarpus* sporulation in the microplots was much greater than that on *G. gigantea* sporulation which indicates that *G. macrocarpus* is more sensitive to the suppressive factors in nonfumigated soils. This is also supported by the large reduction of vesicle formation in roots and the chlamydospore size exerted by the different nontreated soils. This greater sensitivity of *G. macrocarpus* sporulation is apparently supported by results of former studies (11) where, for two successive seasons in initially fumigated soil, *G. macrocarpus* sporulation on soybean and peanut was high in the first season but dropped by the end of the second growing season. Sporulation of *G. gigantea* showed an opposite trend. The slow increase of the suppressive factors in fumigated soil during the first season and the high levels subsequently reached in the second season could explain the reduction of *Glomus* sporulation during the second season. The concomitant increase of the *Gigaspora* sporulation may indicate that the latter may not be as sensitive to the suppressive factors.

Because the vesicle of *Glomus* is considered the precursor structure of the chlamydospore (5), the lack of correlation between number of vesicles per millimeter of root and the number of spores subsequently found within the roots, especially from plots amended with nontreated soil, was unusual. In roots the number of vesicles that develop thick walls and become chlamydospores may be more closely related to factors in the soil than to number of vesicles.

That total spore formation was significantly less in plots amended with nontreated Cecil clay than in plots amended with the other nontreated soils indicates that certain agricultural soils may contain greater sporulation suppressors than others. The low number of spores formed outside the roots in the soil amended with nontreated Cecil clay compared with that in other treatments was not reflected in the number of spores formed inside the roots and may indicate that the suppressor is more active in the soil than within root tissue. This is supported by the data that indicated that the suppressive factors apparently did not enter the root. However, *Glomus* spore formation within the roots is also markedly suppressed by factors in nontreated soil.

Preliminary tests with small *Glomus* chlamydospores (<75 μm) have shown that their germination is less than that of large (>105 μm) chlamydospores. Because both numbers and sizes of spores are reduced by the factors in nontreated soil, it is reasonable to believe that both factors play a vital role in limiting development of mycorrhizae on soybean by *Glomus*.

The results presented here could be explained by the existence of a "mycosphere" surrounding the mycelium of the mycorrhizal fungus in the soil. Metabolites from organisms within this "mycosphere" could readily be absorbed by the mycelium and transported back to the root (as is phosphate). Accumulation of these metabolites either within the fungus and/or root could act as an inhibitory mechanism to reduce sporulation of the mycorrhizal fungus both within and outside of the root as recorded in these studies.

The relatively sparse sporulation of V-A mycorrhizal fungi on soybean in field soil probably is one of the direct causes of its low infection maintenance level under field conditions compared with that observed in heat-treated or fumigated soils. The magnitude of the mycorrhizal benefit to soybean growth and yields under field conditions may be vastly less than that measured by researchers using fumigated or steamed soils (10). Moreover, variations in spore numbers (6) may in reality be under control or influence of the sporulation-suppressive factors measured in the research data presented herein. If inoculum potential of indigenous V-A fungi is considered of prime importance in determining the benefit derived by host plants (8), then factors controlling or limiting sporulation require elucidation. Future research should be directed towards identifying these limiting factors and determining whether they play a role in other mycorrhizal relationships.

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