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The influence of crop rotation and soil fumigation on a mycorrhizal fungal community associated with soybean

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Abstract. Population densities of mycorrhizal fungal propagules in a western Kentucky field highly productive for soybean were measured by bioassay throughout a soybean production season. The primary experimental variables were crop rotation (soybeans in 1985, then 2 years in corn, milo, fescue, or soybean, then soybean in 1988 on all plots when populations of propagules were determined) and soil fumigation with 67% methyl bromide/33% chloropicrin. Of the 20 species in three genera found, *Glomus* predominated both in terms of number of species and population densities. Most species of *Glomus* occurred at higher population densities in rotated plots than in continuous soybean plots. In continuous soybean plots, species of *Gigaspora* made up a much higher proportion of the mycorrhizal fungal community than in rotated crops. Species richness and diversity were lower, and dominance and equitability higher, in nonfumigated continuous soybean plots than in rotated plots early in the season, but the differences were not present at the end of the season. Soil fumigation killed most propagules in the upper 15 cm of soil, but after production of a crop of soybeans, populations of total propagules and most *Glomus* spp. recovered to prefumigation densities. However, *Gigaspora margarita* and *Gigaspora gigantea* did not recover similarly. Fumigation reduced species richness and diversity and increased dominance, but the effects were ameliorated by the end of the season. Colonization of roots was low during vegetative growth but increased rapidly after the onset of soybean reproduction. There was no evidence for mutualism during the early half of the season, perhaps due to high soil P and low dependency of soybean. Fumigation increased soybean yields. A stable mycorrhizal fungal community appeared to become established with continuous soybean production, and both crop rotation and soil fumigation disrupted the community.

Key words: Endogonaceae—*Glycine max*—Soil ecology—Vesicular-arbuscular mycorrhizal fungi—Community analysis

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Introduction

Plants of most taxa are mycorrhizal with fungi of the order Glomales (= Endogonaceae, or vesicular-arbuscular fungi; Morton and Benny 1990), and soils supporting naturally-occurring or cultivated hosts harbor these fungi (Mosse 1973; Harley and Smith 1983). Knowledge of the relationships between mycorrhizal fungi and their hosts in natural habitats is limited. Mycorrhizal effects on plants growing in nonsterilized soil must be considered from the standpoint of the mycorrhizal fungal community, not just of individuals from the community, for pure cultures of Glomales fungi do not occur in nature. In agricultural or unmanaged habitats, over a dozen species are usually found (Schenck and Kinloch 1980; Rich and Schenk 1981; Hetrick and Bloom 1983; McGraw and Hendrix 1984, 1986; Modjo et al. 1987). At the site of the study reported here, An et al. (1990a) described propagules of 13 species in limited samplings, some occurring consistently and at high population densities, others occurring patchily.

Plants growing in unmanaged situations seldom occur in pure stands, and the site is often dominated by different plant species in different seasons. In contrast, nearly pure stands of agricultural crops are often obtained by cultivation and/or use of herbicides; however, crop rotation is essential for maintenance of high productivity. Annually rotated corn and soybean yielded 8 to 10% better than the continuous crops (Crookston et al. 1991).

Crop rotation is an essential component of low-input, sustainable cropping systems, and the influence of the mycorrhizal fungal community on the productivity effects of rotation should be considered. Hayman et al. (1975) reported that prior cropping of soil with a nonmycorrhizal plant such as swede inhibited mycorrhizal

development in seedlings of subsequently planted onion, which is mycorrhizal. Similar results have been reported by Iqbal and Ouereshi (1976), Hirrel et al. (1978), Powell (1982), and Harinikumar and Bagyaraj (1988). Smith (1980) found that spore abundance remained low under continuous wheat but recovered under rotation with pasture.

This present study examines the influence of crop rotation and soil fumigation on the mycorrhizal fungal community in a highly productive soybean soil. Because soil fumigants are lethal to mycorrhizal fungi (Menge et al. 1978, 1982; McGraw and Hendrix 1984, 1986; Modjo et al. 1987), soil fumigation was employed as a control treatment which disrupts the community of mycorrhizal fungi. The results reported are primarily from the final year of a 3-year experiment.

Materials and methods

Field experiment

This study was conducted in a field in McLean County, western Kentucky. The soil type is Melvin silt loam (fine silty, mixed, nonacid, mesic typic Fluvaquents). The site in the flood plain of the Green River is highly productive for soybean and is highly uniform pedologically. The pH varied from 6.5 to 6.9 and Mehlich III P content was high, averaging 233 kg/ha for all plots except those rotated with fescue. P content for the fescue plots was significantly lower statistically, 130 kg/ha, but still considered high for soybean production. In 1984 and 1985, prior to this experiment, the field was planted commercially to corn and soybean, respectively.

In 1986 and 1987, plots in the field were planted with either soybean (*Glycine max* (L.) Merr. cv. Douglas), tall fescue (*Festuca arundinacea* Schreb cv. Johnstone), milo (*Sorghum bicolor* (L.) Moench), or corn (*Zea mays* L.). In 1988, all plots were planted with soybean (cv. Douglas). The design was a split plot, randomized complete block with four replications. Each main plot was 18.3×61.0 m, containing 12 rows spaced 75 cm apart for all crops except fescue. A fumigated strip ca. 6.9 m wide was centered on half the plot. In 1986 and 1988, the same side of each plot was fumigated. In 1987, only the soybean plots were fumigated. The fumigant was 67% methyl bromide/33% chloropicrin (420 kg/ha), and was applied by the method described previously (An et al. 1990a). Briefly, the fumigant was injected into soil ca. 15 cm deep as three strips and covered by plastic with a tractor-mounted applicator which glued one side of the plastic cover to the previously applied plastic so that no soil was left untreated. In 1986, plots were fumigated on 3 April. On 7 April, the plastic was removed, and fescue plots were broadcast sown (48 kg/ha) with a hand cyclone seeder and firmed with a cultipacker. A good stand was obtained. Corn was planted on 24 April and soybean and milo on 21 May. Planting dates in 1987 were 26 April for corn and 22 May for milo and soybeans. Soybean plots were fumigated on 6 May. In 1988, fumigation was done on 3 May, and soybean was planted on 21 May. Each year, soybean seed were inoculated with commercial *Rhizobium* inoculum. Population densities of weeds were insignificant in any of the 3 years.

Soil samples from 0–15 cm deep in each plot were taken 7 times in 1988 (1 March, 3 May, 21 June, 25 July, 10 August, 9 September, and 16 October). Each sample consisted of a minimum of 25 2.5-cm-diameter cores. Cores were taken equidistantly from row 7 (one of the center rows) of each plot when rows were present, with no cores taken from the 3 m at each end. On the June through September sampling dates, samples were taken within the row, close to plants, and populations probably were influenced by the higher density of roots within the row compared to soil farther from the plants. On the March and May sampling dates, the soil was being prepared for planting by discing, and the location of rows of the 1987 crops could not be determined. On the October sampling date, the 1988 crop residue had been incorporated into the soil by discing, and samples were taken without regard to the rows; thus the sampling conditions for the October sampling were analogous to those for the March and May samplings.

Yields of soybeans were obtained from three areas 4.6×7.0 m within each plot, harvested with a Hege 125B small plot combine.

Laboratory procedures

The mycorrhizal fungal community was measured quantitatively by a "most probable number" bioassay involving dilutions of 1 part of the previous dilution:1 part of sterile sand, with five assay plants per dilution (An et al. 1990b). Soybean seeds (cv. Douglas) used in the bioassay were inoculated with commercial *Rhizobium* inoculum. The plants were watered daily and grown 9 weeks in a greenhouse. After the growth period, spores from each plant were wet-sieved (An et al. 1990b). The roots were stored in formaldehyde-acetic acid-ethanol. Roots were hand-picked from rooting medium, cleared in KOH, stained by a no-heat method (Kormanik and McGraw 1982) using 0.5 g trypan blue-lactophenol per liter of a mixture of lactic acid, phenol, glycerol, and deionized water in the ratio of 1:1:2:2, and observed for attached or intraradical spores. Spores present in sievings or roots were identified (Gerdemann and Trappe 1974; Hall and Fish 1979, Schenck and Perez 1988). The presence or absence of spores of each species was recorded as + or − for each assay plant at each dilution level, and the data were subjected to population density computations using an SAS (SAS Institute 1985a, b) translation of the "most probable number" procedure of Cochran (1950). Population densities of total mycorrhizal propagules are the sums of the population densities of the individual species.

Colonization of roots present in the soil samples from the field plots was determined by picking roots from soil samples and storing and staining as above. Colonization was determined by the gridline intersect method of Giovannetti and Mosse (1980).

Community analysis

Four indices, species richness, the Simpson dominance index, the Shannon-Weiner index of species diversity, and species equitability, were computed for community analysis. Species richness (S) is the mean number of species detected in a plot. The Simpson index (C) was computed according to the formula:

$$C = \Sigma(X_i/X_o)^2$$

where Xi = the population density for an individual species and X_o = the total population densities of the plot. The Shannon-Weiner index of species diversity (H′) was calculated according to the formula:

$$H' = -\Sigma(X_i/X_o)\ln(X_i/X_o)$$

The species equitability index (J) was computed according to the formula:

$$J = H'/\ln S$$

All statistical analyses were done using SAS (SAS Institute 1985a). In the text, means are considered to be different if $P < 0.05$.

Table 1. Populations of mycorrhizal fungal propagules in continuous soybean or rotated plots on 1 March 1988, fumigated or not fumigated in previous years. *NF*, Soil not fumigated; *F*, soil fumigated 3 April 1986 for all crops and 6 May 1987 for soybean. Means of 4 replications ± standard error. Means followed by same letter on a line are not different statistically (LSD, $P=0.05$

Species	Soybean[a]		Corn		Milo		Fescue	
	NF	F	NF	F	NF	F	NF	F
Glomus spp.								
G. aggregatum Schenck & Smith emend Koske	0 a	7± 3 a	21± 13 a	44± 16 a	44± 44 a	29± 12 a	14± 14 a	7± 7 a
G. ambisporum Smith & Schenck	13± 8 b	10± 3 b	69± 26 a	0 b	106± 33 a	92± 52 a	88± 37 a	29± 17 ab
Unidentified brown spp.[b]	115± 78 ab	22±15 b	78± 34 ab	48± 30 b	160± 40 a	90± 3 ab	70± 26 ab	14± 8 b
G. caledonium (Nicol. & Gerd.) Trappe & Gerd.	14± 8 ab	0 b	0 b	0 b	26± 26 ab	0 b	48± 29 a	0 b
G. canadense (Thaxter) Trappe & Gerd.	0 d	21±11 cd	128± 76 ab	75± 10 bcd	109± 13 abc	29± 12 cd	169± 40 a	79± 32 bcd
G. claroideum Schenck & Smith	0 a	0 a	14± 14 a	0 a	0 a	0 a	0 a	0 a
G. clarum Nicolson & Schenck	7± 7 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
G. etunicatum Becker & Gerdemann	0 a	0 a	7± 7 a	0 a	0 a	0 a	0 a	0 a
G. fecundisporum Schenck & Smith	44± 20 b	51±35 b	261±115 ab	393±107 ab	411±200 ab	190± 67 b	602±256 a	333± 64 ab
G. intraradices Schenck & Smith	49± 49 ce	26±16 c	112± 30 abc	195± 38 a	179± 41 ab	113± 41 abc	211± 77 a	136± 55 abc
G. leptotichum Schenck & Smith	36± 19 b	50±17 ab	85± 43 ab	32± 32 b	104± 59 ab	152± 55 a	120± 21 ab	20± 7 b
G. macrocarpum Tul. & Tul.	110± 42 d	219±60 cd	532±120 bc	797±195 ab	903±106 ab	499± 35 bcd	1071±247 a	621±183 bc
G. maculosum Miller & Walker	21± 21 c	36±15 bc	106± 45 abc	95± 4 abc	208± 95 a	190± 54 ab	214± 78 a	117± 40 abc
G. manihotis Howeler, Sieverding & Schenck	0 b	0 b	0 b	0 b	0 b	0 b	29± 17 a	0 b
G. microcarpum Tul. & Tul.	21± 7 d	35± 8 d	242± 84 bc	170± 55 cd	352± 84 ab	110± 31 cd	406± 48 a	90± 37 cd
G. monosporum Gerdemann & Trappe	0 a	9± 6 a	39± 30 a	7± 7 a	78± 78 a	65± 56 a	75± 38 a	0 a
G. mosseae (Nicol. & Gerd.) Gerd. & Trappe	7± 7 b	0 b	7± 7 b	0 b	51± 22 a	22± 22 ab	28± 12 ab	0 b
Gigaspora spp.								
G. gigantea (Nicol. & Gerd.) Gerd. & Trappe	85± 25 a	7± 4 b	7± 7 b	0 b	14± 8 b	0 b	7± 7 b	7± 7 b
G. margarita Becker & Hall	66± 22 a	2± 2 c	22± 22 bc	0 c	0 c	22± 22 bc	51± 8 ab	7± 7 c
Scutellospora spp.								
S. savannicola (Herr. & Ferr.) Walker & Sanders	0 a	0 a	0 a	0 a	0 a	0 a	0 a	0 a
Total	586±177 de	500±98 e	1729±255 bc	1855±352 bc	2770±488 ab	1602±245 cd	3222±660 a	1460±297 cde

[a] Crop planted in the previous years
[b] Spore diameter 95–125 μm, wall thickness 5–10 μm, brown color

Results

The mycorrhizal fungal community

Twenty mycorrhizal fungal species in three genera were identified by bioassay (Table 1). Species of the genus *Glomus* predominated both in number of species and population densities. Predominant species on 1 March, based on population densities for all treatments, were *G. macrocarpum* Tul. & Tul., *G. fecundisporum* Schenck & Smith, *G. microcarpum* Tul. & Tul., *G. intraradices* Schenck & Smith, *G. maculosum* Miller & Walker, *G. canadense* (Thaxter) Trappe & Gerd., *G. leptotichum* Schenck & Smith, and an unidentified brown-spored species. A few propagules of *Scutellospora savannicola* (Herr. & Ferr.) Walker & Sanders were found in the September sampling in fumigated plots of each of the crop rotation treatments.

Population densities of species

Propagule population densities differed among rotation treatments in nonfumigated plots at the start of the season. The lowest total densities on 1 March were in continuous soybean plots (Table 1). The highest densities at the beginning of the season were in fescue and milo plots. Overall, densities in rotation plots were at least three-fold higher than those in continuous soybean plots. The higher densities associated with crop rotation were due primarily to higher densities of species of *Glomus,* notably *G. macrocarpum, G. maculosum, G. microcarpum,* and *G. canadense.* The two species of *Gigaspora, G. gigantea* and *G. margarita,* were particularly prominent in continuous, nonfumigated soybean plots (Table 1). Although their densities are low relative to those of many species of *Glomus,* they are significant because of the large mass of *Gigaspora* spores. Rotation with any crop severely depressed densities of *G. gigantea,* and rotation with corn and milo reduced densities of *G. margarita.*

In rotated plots, population densities of most species of *Glomus* decreased during the season (Figs. 1, 2). However, the densities of *G. mosseae* (except for the milo plots) (data not shown), *G. aggregatum* Schenck & Smith emend Koske (Fig. 2), and *G. monosporum* (Fig. 2) showed no changes with time. Densities of *Gigaspora gigantea* did not change in rotated plots, but densities in continuous soybean plots decreased during the course of the season to the levels present in the rotated plots (Fig. 1). Densities of *G. margarita* were unchanged in soybean and corn plots, increased in milo plots, and decreased in fescue plots (Fig. 1). Densities of *G. margarita* in nonfumigated continuous soybean plots were higher than in the rotated plots by the end of the season.

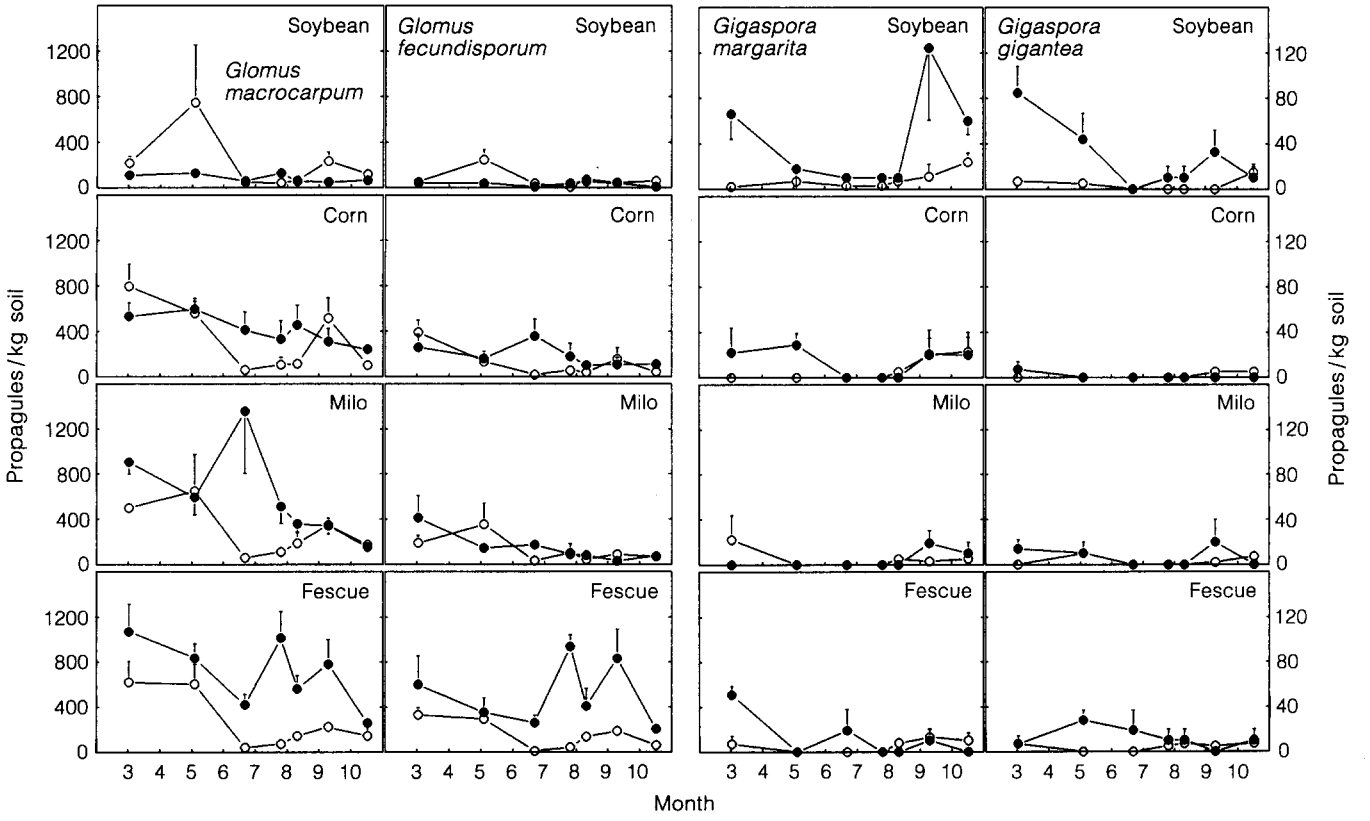

Fig. 1. Population densities of *Glomus macrocarpum, Glomus fecundisporum, Gigaspora gigantea,* and *Gigaspora margarita* during a soybean production season as influenced by soil fumigation and crop rotation in the 2 previous years. Fumigation was done on 3 May. *Bars* are standard errors of the means; some very low standard errors are not readly visible. O—O, Fumigated; ●—●, nonfumigated

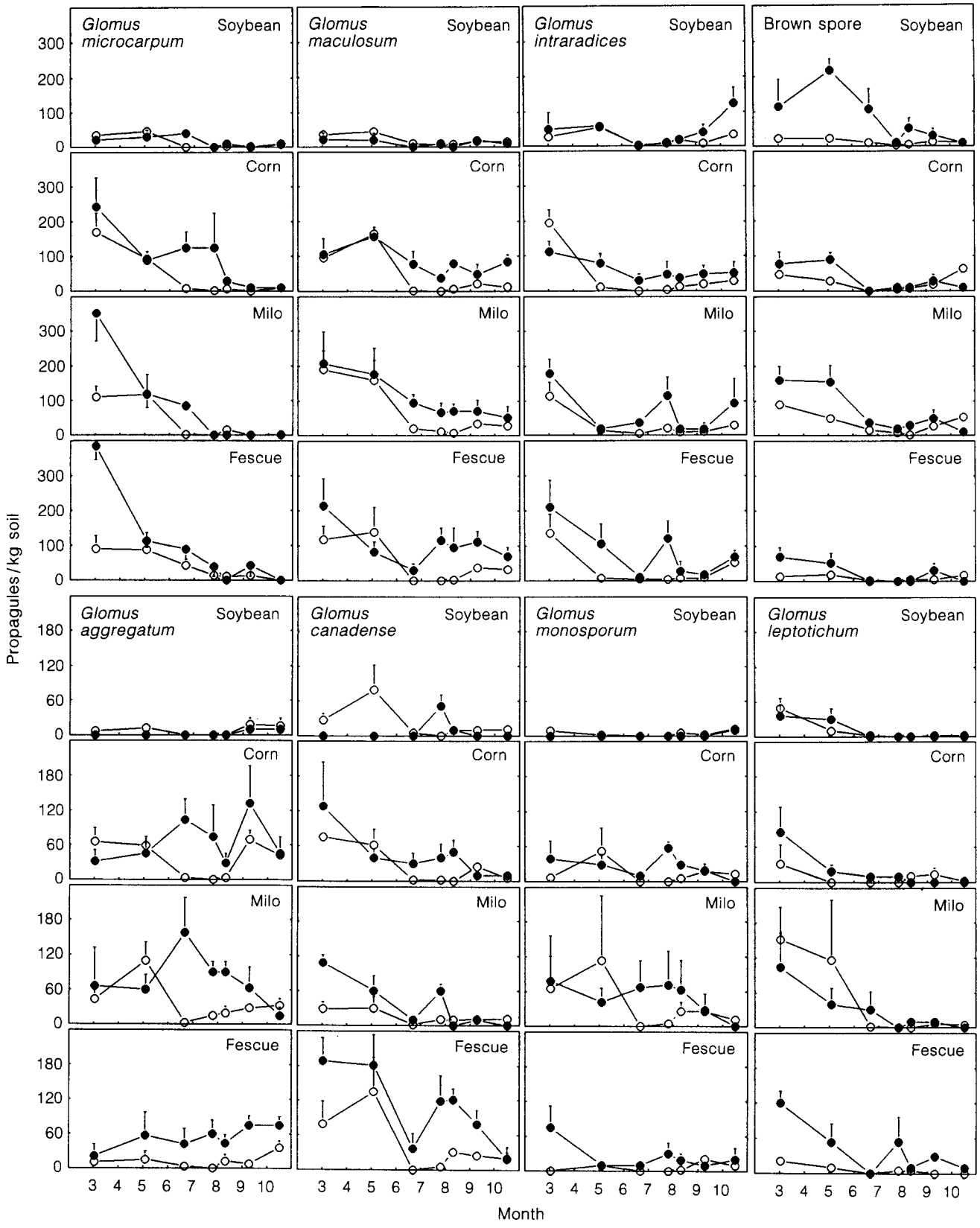

Fig. 2. Population densities of eight species of *Glomus* during a soybean production season as influenced by soil fumigation and crop rotation in the 2 previous years. Fumigation was done on 3 May. ○—○, Fumigated; ●—●, nonfumigated

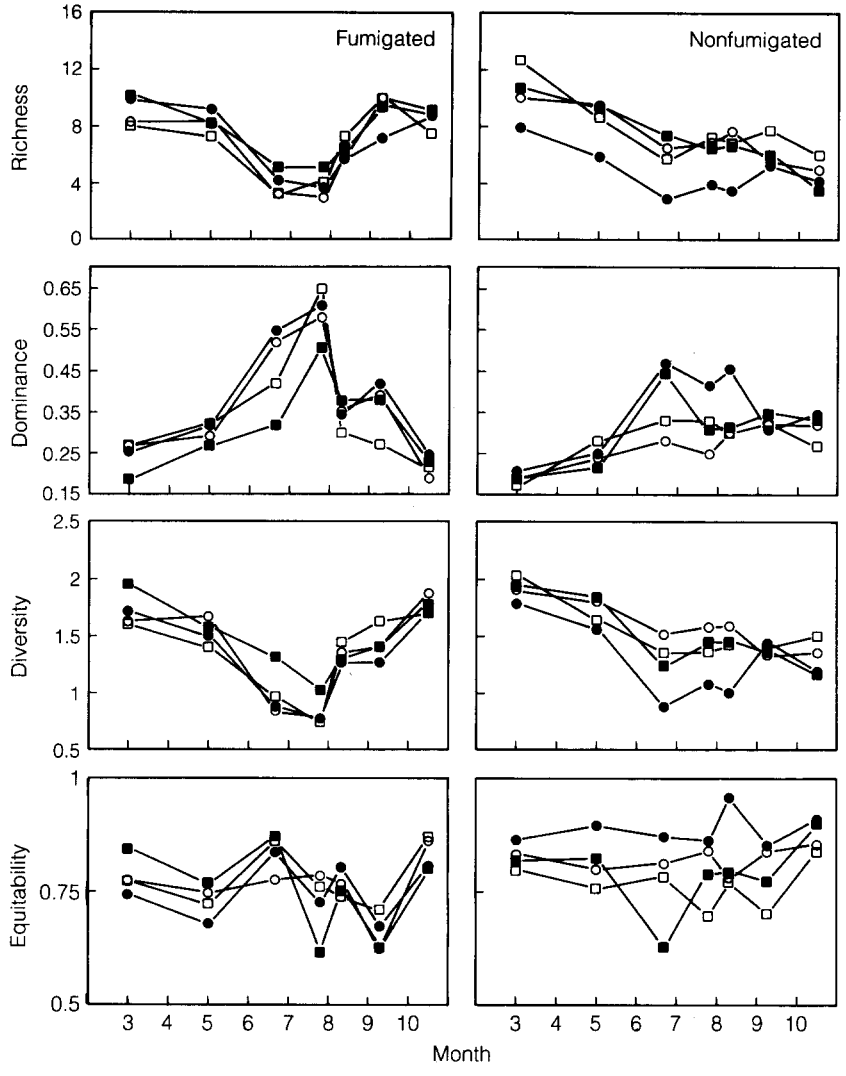

Fig. 3. Indices for species richness, dominance, diversity, and equitability for the mycorrhizal fungal community during a soybean production season as influenced by soil fumigation and crop rotation in the 2 previous years. Fumigation was done on 3 May. ●, Soybean; ■, milo; ○, corn; □, fescue

Total population densities in fescue and milo plots fumigated 2 years previously were lower than those in nonfumigated plots (Table 1). Nearly all species were affected, especially in the fescue plots. Although the fumigated soybean plots were fumigated in 1987 as well as 1986, densities were not appreciably less than in nonfumigated soybean plots, except for *Gigaspora gigantea* and *G. margarita.*

Fumigation in the final year drastically reduced but did not eliminate populations of propagules, as indicated in the June population densities (Figs. 1, 2). On 21 June, 47 days after fumigation and 30 days after planting, total densities ranged from 93 to 154 propagules/kg soil. As the soybean crop grew and reached maturity, densities increased steadily, reaching parity with nonfumigated plots in all crop rotation treatments except fescue. Most species followed this trend.

Densities of *Gigaspora* species were generally lower in plots fumigated 1 or 2 years earlier than in nonfumigated plots (Fig. 1, March and May sampling dates). In contrast to most species of *Glomus,* densities of *Gigaspora* did not recover in the third year of the experiment (Fig. 1).

Community dynamics

Species richness was affected by crop rotation, fumigation, and time ($P = 0.0001$ for each), and all interactions between these factors were significant (each at $P = 0.0001$). Species dominance was affected by fumigation ($P = 0.008$) and time ($P = 0.0001$). Species diversity was affected by rotation ($P = 0.013$), fumigation ($P = 0.023$), and time ($P = 0.0001$). Species equitability was affected by rotation ($P = 0.0014$), fumigation, and time ($P = 0.0001$ each).

In nonfumigated continuous soybean plots, species richness was lower than that in rotated plots before and during most of the growing season (Fig. 3). During the early part of the growing season, species dominance and equitability was higher and diversity lower in nonfumigated continuous soybean plots than in rotated plots (Fig. 3). By October, nonfumigated continuous soybean plots did not differ from rotated plots in any of these indices.

Fumigation drastically reduced species richness and diversity and increased dominance (Fig. 3). The effect of fumigation on equitability was primarily to elimi-

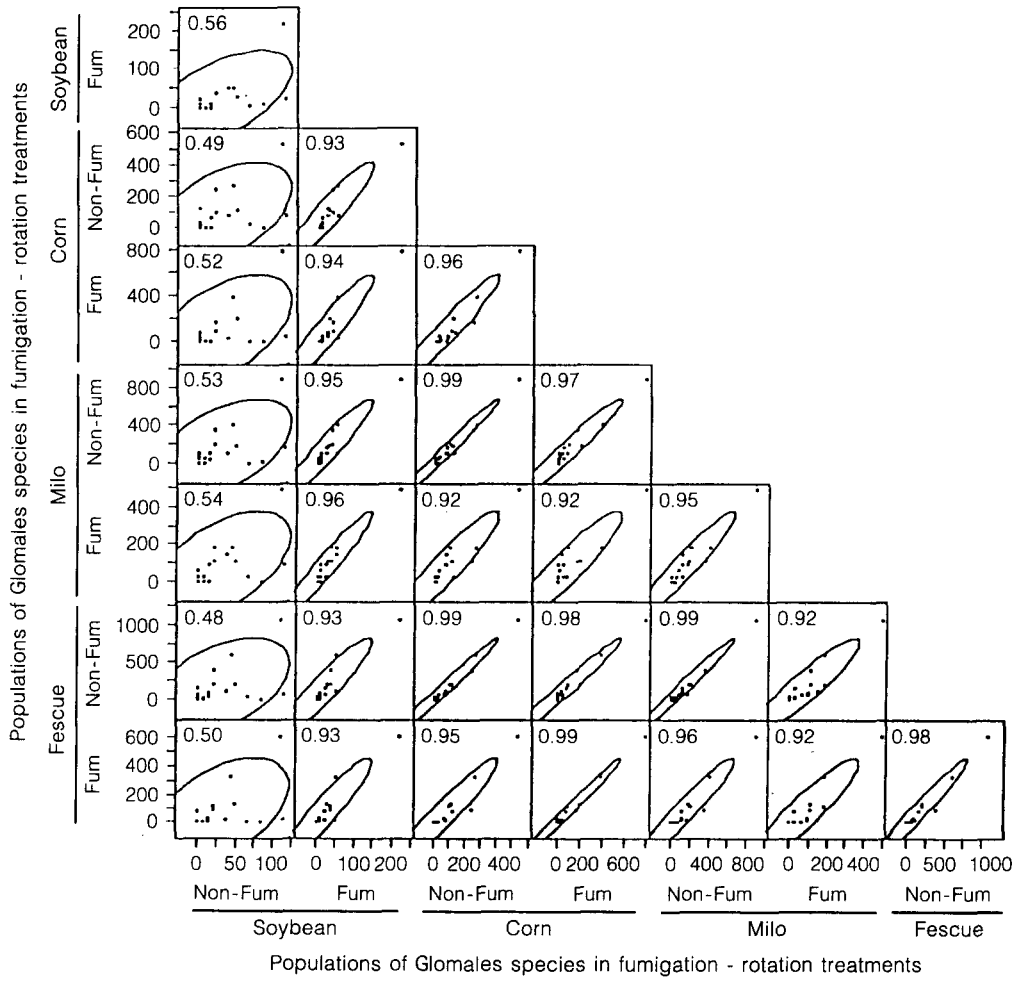

Fig. 4. Relationships between propagule population densities of individual species for pairs of rotation and fumigation treatments for the 1 March sampling, with Pearson correlation coefficients. Oval lines indicate 95% confidence limits. *Fum*, Fumigated; *Non-Fum*, nonfumigated

nate the high equitability associated with nonfumigated continuous soybeans (Fig. 3). However, these effects, evident in the June sampling, were largely reversed by the end of the season. For the October sampling, species richness and diversity were higher and dominance lower in fumigated than in nonfumigated plots (Fig. 3).

Relative population densities of the individual species among the rotation and fumigation treatments on 1 March were closely correlated except for nonfumigated soybean (Fig. 4). Pearson correlation coefficients were about 0.5 for the relationships of densities of species in nonfumigated soybean plots with those in any other treatment and above 0.9 for any pairs of treatments not involving nonfumigated soybean. The correlation matrices for species between fumigation-crop rotation treatments were summarized using principal components analysis. Most of the variance was accounted for by the first component, since the second eigenvalue for each month was less than one. The disparity of the relationships between nonfumigated soybean and any other rotation or fumigation treatment that was apparent on 1 March (Fig. 4) persisted until late July (Fig. 5), when the soybean plants were in a period of vigorous vegetative growth. In July and to a lesser extent early August, when plants were entering the reproductive stage, population densities of species

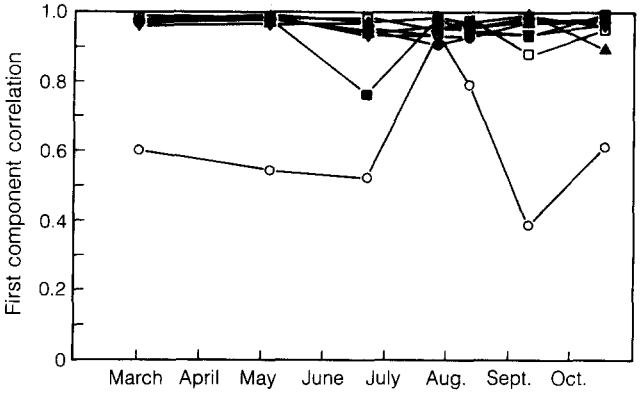

Fig. 5. Principal components factor analysis of Pearson correlation coefficients for population densities of propagules. ○—○, Soybean, nonfumigated; ●—●, soybean, fumigated; △—△, corn, nonfumigated; ▲—▲, corn, fumigated; □—□, fescue, nonfumigated; ■—■, fescue, fumigated; ▽—▽, milo, nonfumigated; ▼—▼, milo, fumigated

in nonfumigated soybean plots resembled those in the other fumigation and rotation plots. In September and October, when plants were in the final reproductive and senescence stages, densities in nonfumigated soybean plots again differed from those in the other plots (Fig. 5).

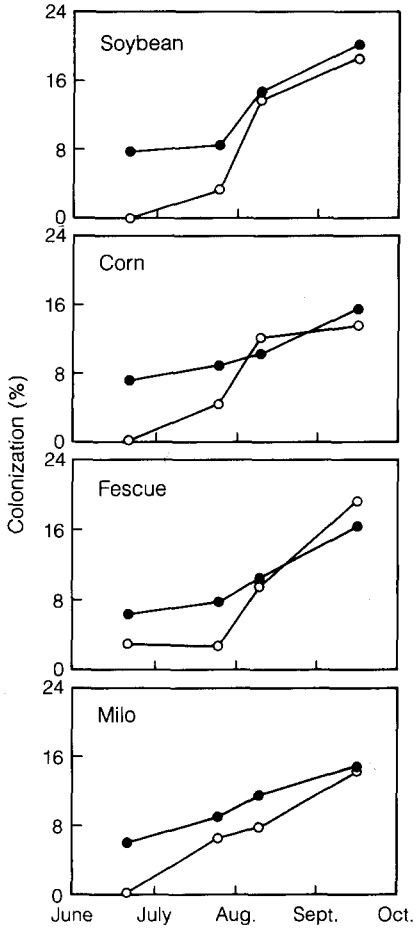

Fig. 6. Mycorrhizal colonization of soybean roots in 1988 as influenced by crop rotation and soil fumigation. Fumigation was 3 May; the planting date was 21 May. O, Fumigated; ●, nonfumigated

Mycorrhizal colonization, growth, and yield of soybean

Colonization by mycorrhizal fungi, low in nonfumigated plots and virtually nil in fumigated plots during vegetative growth, increased rapidly after reproduction began (Fig. 6). Crop rotation had no effect on colonization at any time in nonfumigated plots. For the August and September sampling dates, fumigated and nonfumigated plots did not differ in colonization.

Colonization patterns differed in nonfumigated soybean plots in 1987 and 1988. In 1987, colonization was ca. 1% in nonfumigated plots until 10 August, when the level rose to ca. 3%. On 1 September 1987, colonization was ca. 33% in nonfumigated plots and 23% in fumigated plots, the difference being significant. Thus in 1988, colonization was higher early in the season but lower late in the season than in 1987.

Early in the season, soybean plants in continuous soybean plots were significantly taller in fumigated than in nonfumigated plots, although the differences were not large (Table 2). However, the reverse was true in rotated plots. A similar effect of fumigation on plant height was also apparent in the soybean plots in 1986 and 1987. By 10 August 1988, plants in nonfumigated continuous soybean plots were slightly taller than those in fumigated plots (Table 2). For both 1988 sampling dates, rotation crops and fumigation effects were significant ($P = 0.0001$).

Soybean plants in fumigated fescue plots were stunted (Table 2). There was also a significant rotation crop × fumigation interaction because of the effect of fumigation in fescue rotated plots. If fescue rotation plots were not considered, the interaction terms were insignificant for both dates.

Fumigation, but not rotation, significantly increased yields (Table 2). Fumigation significantly increased yields in soybean and milo plots. While fumigation reduced growth in fescue plots, yields were not reduced. There was no relationship between yield and plant height.

Discussion

Disruption of the mycorrhizal fungal community by crop rotation and fumigation

The data obtained suggest that these two dissimilar production practices disrupt a stable mycorrhizal fungal community associated with continuous production

Table 2. Effect of crop rotation and soil fumigation on growth and yield of soybean. The yield was measured at 11.7% moisture. Crops were planted in 1986, 1987, and 1988. Soybeans were planted on 21 May 1988. *S*, Soybean; *M*, milo; *C*, corn; *F*, fescue; *NF*, soil not fumigated; *F*, soil fumigated. The data for height are the means of four replications with 30 plants measured per replication. Means for soybean height followed by the same letter for a date are not different (LSD, $P=0.05$). The data for yield are the means of four replications. Yield means followed by the same letter are not different (LSD, $P=0.05$)

Cropping sequence	Height (cm)				Yield (kg/ha)	
	25 July		10 August			
	NF	F	NF	F	NF	F
S-S-S	51.9 c	58.9 d	103.8 c	97.7 e	2581 c	2904 ab
C-C-S	70.2 a	64.7 b	107.3 b	101.3 d	2780 bc	3055 ab
M-M-S	68.9 a	65.5 b	110.0 a	102.9 cd	2856 bc	3170 a
F-F-S	70.9 a	53.8 e	107.6 ab	83.5 f	2575 c	2942 ab

of soybean. In land rotated to other crops for 2 years, the community at the beginning of the season exhibited higher species richness and diversity and lower equitability than land planted continuously to soybean. Barbour et al. (1987) pointed out that maintaining high diversity in plant communities may require episodic disturbances. Rotation effects were evident during most of the growing season. By the end of the season, however, continuous soybean and rotated plots did not differ in these indices. Thus, growing a single crop of soybeans on rotated land caused a convergence of community indices. As a test of this hypothesis, it would be of great interest to obtain data for the first 2 years of such an experiment or earlier, to see whether a divergence due to rotation followed by convergence after a return to soybean also occurs.

Fumigation negated the differences between rotated and nonrotated plots. The shape of the indices curves during the season was also changed. During vegetative growth, fumigation resulted in reduced species richness and diversity and much higher dominance, while these effects were reversed at the end of the season.

Changes in the community during the growing season

During the late July-early August period, the soybean crop was in transition from vegetative to reproductive phases. These were the sampling dates when the nonfumigated soybean plots did not differ from others with regard to relationships among population densities of species (Fig. 5) but marked changes in community indices occurred in fumigated plots (Fig. 3) between the July and August sampling dates. Such changes in community indices did not occur in nonfumigated plots. Associated with the extreme changes in community indices in fumigated plots during this period was an equalization of mycorrhizal colonization of roots in fumigated and nonfumigated plots.

It may be useful in future work to reduce experimental variables so as to allow more frequent sampling in relation to crop development. The relationships between root mass and length densities, population densities of spores, population densities of propagules, and plant development would be of interest.

Crop rotation effects on mycorrhizal communities

Species richness and population densities of fungi which colonize soybean were increased by rotation. Since the densities of most species were increased by rotation, the apparent increase in number of species may be due in part simply to increase in densities in continuous soybean plots above the detection limit. Since the assay host for all plots was soybean, the higher densities in rotated plots cannot readily be explained by host specificity.

Crop rotation also changed quantitatively the composition of the mycorrhizal fungal community in nonfumigated plots. Population densities of most species were increased, particularly *Glomus canadense* and *Glomus monosporum*. In contrast, densities of the unidentified brown-spored species and *Gigaspora* species were clearly favored by continuous soybeans. In rotated plots, the densities of these species were usually only a fraction of those in continuous soybean plots, and their occurrence was sporadic (Table 1).

To a large extent, a single crop of soybeans nullified the effects of crop rotation and returned densities and community structure in general to those in continuous soybean plots. A similar effect was observed when barley was rotated with kale, a nonmycorrhizal crop, or fallow (no crop) (Black and Tinker 1979). When the previous crop was barley, colonization in barley roots and densities of mycorrhizal spores were twofold higher than when the land was previously in kale or was fallow. Our data and those of Balck and Tinker indicate that densities of propagules of mycorrhizal fungi in general or of a single species might be managed by careful design of cropping systems. However, implementation of such management will require much more knowledge of the effects of the different fungal species on plants and of different plants on individual species of Glomales.

Soil fumigation effects

Soil fumigation as expected virtually eliminated propagules in the surface soil (Menge 1982; McGraw and Hendrix 1986) but by the end of the growing season, population densities had recovered to the levels in nonfumigated plots. Fumigation had little effect below the level of soil disturbance by tillage equipment, i.e. ca. 20 cm (An et al. 1990a). Ross (1971) grew soybeans in fiberglass bins of soil with or without mycorrhizal fungi and fumigated with chloropicrin injected at a depth of 90 cm and with methyl bromide released on the soil surface. At the end of the season, population densities of spores in noninoculated plots were only about 10% of those in inoculated plots. This result suggests that extraordinarily deep fumigation retards recolonization more effectively than the surface-soil fumigation that we applied. However, Ross also included a physical barrier which prevented spread of inoculum laterally from the roots of adjacent plants. However, mycorrhizal fungi are not known to move laterally over the distance necessary to reach the portions of the plots from which we obtained soil samples. Scheltema et al. (1985) recorded spread of *G. fasciculatum* in subterranean clover and ryegrass of less than 20 cm after 2 months, and movement of *Gigaspora calospora* was even less (Scheltema et al. 1987). In competition with indigenous fungi, *Glomus caledonium* (Nicol. & Gerd.) Trappe & Gerd. moved 7.5 cm after 13 weeks through soil planted to hosts (Mosse et al. 1982). Thus, the inoculum for recolonization of the soil may have come primarily from below the depth of effective fumigation, or may have been transported from adjacent nonfumigated soil by insects, rodents, tillage equipment, surface water, or wind (Warner et al. 1987).

The mycorrhizal fungal community associated with soybean

The mycorrhizal fungal community colonizing soybean in the studied field consisted of at least 20 species, with total population densities of less than 600 propagules/kg soil in the nonfumigated continuous soybean plots. An estimate of total propagules present cannot be made without taking into consideration other plants inhabiting the land. Weeds were not a significant factor in this field due to intensive management using herbicides and tillage. However, since this land has a history of rotation with corn, the number of species and their densities might have been higher than those we found if corn had also been included as an assay host. About half of the species occurred patchily, as indicated by standard errors which equal or are a high proportion of the means (Table 1); these species may be inconsequential to soybean production on this land. The other half occurred consistently and usually at relatively high densities; these species may be important in soybean production on this land.

About half of the species we found were also found at one of two sites in Minnesota with a history of soybean or corn production (Johnson et al. 1991). However, the preponderant species differed considerably from those we found.

In terms of population density, *Glomus macrocarpum* was dominant in all rotations, even continuous soybean plots. This is perhaps the most prevalent species worldwide; it was the first species to be described by the Tulasnes in 1845 and has been associated with many hosts and a variety of soil conditions around the world (An 1991). Because of changing taxonomic criteria such as color and the number of wall layers of spores, it is probable that *Glomus fecundisporum* and *Glomus fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske have been identified as *Glomus macrocarpum* in some cases. As a group, however, these fungi are adaptable and prevalent.

Crop rotation and fumigation disrupted the mycorrhizal fungal community associated with continuous soybeans, favoring *Glomus macrocarpum* and other species of *Glomus,* and reducing populations of species of *Gigaspora.*

One explanation for the prominence of species of *Gigaspora* in continuous nonfumigated soybean plots is that these species have affinity for soybean due to host specificity or some other cause. Among a wide variety of mycorrhizal species, *Gigaspora margarita* (according to Schenck and Smith 1982) was the most common species associated with soybean (Schenck and Hinson 1971). However, Johnson et al. (1991) found a high density of *Gigaspora gigantea* at one of two Minnesota locations with a 5-year history of soybean, but no *Gigaspora margarita* at either location. Schenck and Schroder (1974) found this species to have an affinity for high temperatures and suggested it competes better on summer crops than crops grown in other seasons. In a study in which soybean plants were inoculated with six species of mycorrhizal fungi in single culture, most species exhibited higher spore germination, penetration, and colonization of soybean roots than *Gigaspora margarita* (Schenck and Smith 1982).

Mycorrhizal fungi in relation to crop development

Fitter (1990) suggested that most plants are mutualistic with fungi only at times when plant demand for P is much greater than the supply capacity of their root systems, specifically mentioning seedling establishment and flowering. The present experiment suggests that mutualism did not occur during seedling establishment or subsequent vegetative growth. In most fumigated plots, colonization was not detected until late in vegetative development. In nonfumigated plots, colonization was quite low during early growth, especially in 1987, and seemed to plateau at this level until the onset of reproduction of soybean. Fumigation virtually eliminated mycorrhizal propagules in the surface soil, and appreciable propagule populations were not observed until the reproductive stage. The extensive growth of plants in fumigated plots late in the vegetative stages in the absence of appreciable mycorrhizal colonization or propagules was much greater than can be accounted for by seed nutrient supply to the plants pending development of mycorrhizae.

The slight growth reductions due to fumigation measured in rotated plots suggest mutualism. Even in the continuous soybean plots, in which fumigation had increased growth by the time of the earlier measurement, a stunting effect of pathogens such as *Macrophomina phaseolina, Heterodera glycines,* and the *Fusarium solani* strain which causes "sudden death syndrome" (Roy et al. 1989, Rupe 1989) may have been observed; these pathogens are controlled extensively by fumigation and, to some extent, by rotation. The plants in the fumigated plots did not appear stunted but were consistently vigorous and appeared greener than those in nonfumigated plots.

While our data do not provide evidence that mutualism occurred during the reproductive stages of soybean development, they are not inconsistent with this possibility. During pod development, mycorrhizal colonization escalated rapidly in both fumigated and nonfumigated plots, and propagule populations in fumigated plots increased rapidly to levels not different from those in nonfumigated plots. Predominant in fumigated plots at the end of the season were species of *Glomus,* notably *Glomus macrocarpum, Glomus fecundisporum, Glomus maculosum,* and *Glomus intraradix* (An 1991), whereas *Gigaspora margarita* and *Gigaspora gigantea* were prominent in nonfumigated continuous soybean plots. If the *Gigaspora* species are only commensal or even pathogenic, their suppression by fumigation and rotation may permit the greater expression of potentially mutualistic *Glomus* species.

There is little evidence for either pathogenicity or mutualism between mycorrhizal fungi and soybean in the field. The question of whether mutualism occurs late in the development of soybean in the field is tech-

nically difficult to approach. Mycorrhizal fungi reinvade the surface soil during the period of soybean reproduction and their exclusion may be impossible, especially since the propagules for reinvasion may come from soil below the zone of effective fumigation (An et al. 1990a). It is difficult to grow plants in the greenhouse which are indistinguishable from field-grown plants at maturity.

The absence of mutualism may be due to the high P level in this soil. In Kentucky, P fertilization is not recommended if soil P is higher than 67 kg/ha, a level far less than that occurring in this field. Mutualism has been observed for soybean in P-deficient soil (Ross 1971; Fredeen and Terry 1988; Carling et al. 1989). However, pathogenicity has also been observed. Plants grown in a perlite-sand mixture were stunted by *Glomus fasciculatum* throughout a 15-week period, and shoot and root weights of inoculated plants never reached the maximum measured in noninoculated plants (Bethlenfalvay et al. 1982). P content of plants was also reduced by the fungus for the first 2 months. Fredeen and Terry (1988) observed stunting of soybean roots and shoots by *Glomus fasciculatum.* In barley (Black and Tinker 1979) and tobacco (Hendrix et al. 1992), a negative relationship between colonization and yield has been observed. Other instances of plant stunting by mycorrhizal fungi have been observed (Tinker 1978; Modjo and Hendrix 1986). With soybean, pathogenicity by some species has been reported, with only *Glomus mosseae* consistently being mutualistic (Schenck and Smith 1982; Van Nuffelen and Schenk 1984).

The timing and extent of root colonization appears to differ from one season to another. In 1987, root colonization was less than 5% until about mid-August, then increased sharply to about 35% for the nonfumigated treatment and 23% for the fumigated treatment in less than 3 weeks. In 1988, colonization was higher in the nonfumigated than the fumigated treatment early, remained at this level through July, then increased sharply late in the growing season, peaking at ca. 20%. Similarly, Black and Tinker (1979) observed seasonal differences in the plateau level of colonization in plots planted to barley for 3 consecutive years, ca. 20% one year and 40% another.

The pattern of low or undetectable colonization during vegetative growth, followed by a rapid increase in colonization during host reproduction, has been described in wheat (Hetrick and Bloom 1983) and barley (Black and Tinker 1979). In soybean, the appearance of a low level of colonization followed by a lag period before a rapid increase in colonization may be related to the relatively high content of soil P. Fredeen and Terry (1988) observed such a lag in colonization of soybean in a low-P soil fertilized with a high rate of P fertilizer but not a low rate. However, Black and Tinker (1979) found no relationship between colonization and soil P.

Whether mycorrhizal fungi are pathogenic, mutualistic, or commensal with respect to their hosts is important for decisions on strategies to suppress, encourage, or ignore these fungi. Since farmers seldom attempt soybean production in deficient soils, it is important to determine whether and under what conditions mutualism occurs, e.g., in soils which strongly sorb P fertilizer or during pod development. Information is also needed on mutualistic or pathogenic effects of individual mycorrhizal species and the role of the composition of the mycorrhizal community in the expression of mutualism. This information will be useful because propagule density of the mycorrhizal fungal community and of individual species can be altered by crop rotation.

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