New Phytol. (1991), 117, 657–663

Vesicular–arbuscular mycorrhizas respond to corn and soybean cropping history

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(*Received 24 July 1990; accepted 18 December 1990*)

SUMMARY

Communities of vesicular–arbuscular (VA) mycorrhizal fungi were studied in a long-term crop rotation experiment at two locations (Waseca and Lamberton, Minnesota, USA). Spores of mycorrhizal fungi were counted and identified in experimental plots with a cropping history of either corn (*Zea mays* L.) or soybean [*Glycine max* (L.) Merrill]. Mycorrhizal fungal communities were affected by both location and cropping history. At Waseca, *Glomus aggregatum* Schenck & Smith, *G. leptotichum* Schenck & Smith and *G. occultum* Walker spores were more abundant in soil with a corn history than a soybean history, while spores of *G. microcarpum* Tul. & Tul. exhibited the reciprocal pattern. Approximately 90% of the spores recovered at Lamberton were *G. aggregatum* and did not vary with crop history. However, the spores of three other species: *G. albidum* Walker & Rhodes, *G. mosseae* Gerdemann & Trappe, and *G. occultum*, were more abundant in plots with a corn history than a soybean history. Densities of *G. aggretatum* spores were negatively correlated with soil pH at Waseca, but were unrelated to pH at Lamberton were the mean soil pH was lower. Our results indicate that mycorrhizal fungal species are individualistic in their responses to cropping history and edaphic factors.

Key words: VA mycorrhizal fungi, maize, soybean, cropping history, edaphic factors, spore populations.

INTRODUCTION

Most crops form symbiotic associations with vesicular–arbuscular (VA) mycorrhizal fungi (Gerdemann, 1968), and the zygomycetes involved in these associations are often the most abundant fungi in soil (Gerdemann & Nicolson, 1963). VA mycorrhizal fungi are a diverse group of over 144 species (Schenck & Perez, 1990), but little is known about their ecology. Mycorrhizal fungal species, or even isolates of a given species, differ greatly in their effects on plants (e.g. Carling & Brown, 1980; Clarke & Mosse, 1981; Miller, Domoto & Walker, 1985; Modjo & Hendrix, 1986; Bethlenfalvay *et al.*, 1989). Furthermore, the effectiveness of a mycorrhiza in improving plant growth appears to be governed by the interplay between edaphic factors, the host plant, and the fungal isolate (Bethlenfalvay, Ulrich & Brown, 1985; Hall, 1988). Consequently, before VA mycorrhizal fungal isolates can be selected for use in agriculture, it will be necessary to understand how individual fungal species affect plants under local edaphic conditions, and what factors control their populations in agroecosystems (Abbott & Robson, 1982; Menge, 1983; Hall, 1988).

Several studies have examined the affects of cropping sequence on mycorrhizal infection and spore populations (e.g. Black & Tinker, 1979; Sieverding & Leihner, 1984; Dodd *et al.*, 1990) but still relatively little is known about the effects of crop rotation on mycorrhizal fungal communities. The purpose of this study was to compare communities of VA mycorrhizal fungi in field plots with either a corn (*Zea mays* L.) or soybean [*Glycine max* (L.) Merrill] cropping history to better understand how individual species of fungi respond to cropping history and edaphic factors.

MATERIALS AND METHODS

Study sites and experimental design

This study was conducted as part of a long-term

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crop rotation experiment at the University of Minnesota's Agricultural Experiment Stations at Waseca and Lamberton, Minnesota, USA. Soil at the Waseca site was a Nicollet clay loam (fine loamy, mixed, mesic Aquic Hapludoll), while soil at the Lamberton site was a Webster clay loam (fine, loamy, mixed, mesic Typic Haplaquoll). Corn (Pioneer Brand '3737'*) and soybean ('Hodgson 78') were grown continuously, or in rotation, in field plots arranged in a randomized complete block design with four replicates (Crookston *et al.*, 1991). The experimental plots were six rows wide and 18 m long at Waseca and twelve rows wide and 10 m long at Lamberton. Rows were spaced 76 cm apart at both sites. Planting dates were 5 May and 9 May, 1988 at Waseca and Lamberton, respectively. The treatments examined in this study were continuous corn or soybean, first-year corn after a 5 yr soybean history, and first-year soybean after a 5 yr corn history. Throughout the study, fertilizers and pesticides were applied at rates recommended for optimum crop production (Crookston *et al.*, 1991).

Spore populations

Soil samples were collected on 1 June 1988 at Waseca and 2 June 1988 at Lamberton. Composite samples were collected from each plot by taking two soil cores (15 cm deep × 2 cm diameter) *c.* 2·5 cm from the stem of four different seedlings. Thus, each sample was a composite of eight cores. Samples were stored in plastic bags at 4 °C. Soils were air dried and thoroughly mixed before spores were extracted from 25 g aliquots using McKenney & Lindsay's (1987) wet-sieving technique. Spores were placed on a gridded membrane filter and counted with a compound microscope at 40×. Spores were removed from 20% of the total area of the filter paper, mounted on permanent slides and examined using a compound microscope at 400 or 1000×. Spore identification was based on wall structure (Walker, 1983), comparison of spores to holotypes, paratypes, and collections obtained from Oregon State University herbarium, as well as spore reaction in Melzer's reagent, water, and polyvinyl alcohol–lactic acid–glycerine (Schenck & Perez, 1990). Voucher numbers were assigned to representative specimens of each of the species we identified. These slide specimens are available upon request. The percentage of each species in the examined sub-sample was multiplied by the total count of spores on the filter paper to estimate the total abundance of that species in the spore community.

Root colonization

The entire root systems of four corn and soybean seedlings were collected concurrently with the soil samples as an *in situ* infectivity bioassay. It has been shown that root infection is linearly related to soil densities of VA mycorrhizal fungal propagules during the early stages of the colonization process (Carling, Brown & Brown, 1979; Smith & Walker, 1981). We collected corn and soybean seedlings approximately three weeks post emergence. Thus, we assumed that levels of root infection were proportional to soil densities of infective propagules. Seedlings were excavated from each plot using a shovel or a hand trowel. Roots were stored in plastic bags and kept on ice until they were frozen within 6 h of collection. To assess VA mycorrhizal colonization, root systems were washed, cut into 2·5 cm segments, and 0·5 g of randomly selected segments were stained with trypan blue in lactoglycerin (Phillips & Hayman, 1970). Root length containing hyphae, vesicles and/or arbuscules were assessed using a grid-line intersection method (Giovannetti & Mosse, 1980). Percent colonization was calculated as VA mycorrhizal root length/total root length ×100.

Soil analysis

Composite 15 cm deep soil samples were collected from each plot at each location on 15 April 1988 and analysed for pH, extractable phosphorus by the Bray-1 method, and exchangeable potassium by extraction with ammonium acetate.

Statistical analysis

Normality of the data were tested using Kolmogorov–Smirnov one-sample test for goodness-of-fit. Spore count data were normalized using a $\log_e(x+1)$ transformation (St. John & Koske, 1988) and root colonization data were normalized using an arcsine square root transformation (Zar, 1984). Two sample *t* tests were used to compare mean spore densities and root colonization in plots with a corn versus a soybean cropping history. Diversity of the VA mycorrhizal fungal communities was calculated by the Shannon–Wiener index (Krebs, 1985). This index combines two components of diversity: numbers of species and the evenness of allotment of individuals among the species. Two-way analysis of variance was used to evaluate the effects of location and cropping history on soil P, K, and pH, and on the Shannon–Wiener diversity index. Cropping history was defined by a dummy variable (0 for a corn history and 1 for a soybean history). Stepwise multiple regression models were developed to determine if soil variables (P, K, pH) and/or crop history were significant predictors of the spore densities of individual VA mycorrhizal fungal species at Waseca and Lamberton. All statistical tests were performed using Statgraphics (STSC, 1986).

* Mention of a trade name or a proprietary product does not constitute endorsement by the University of Minnesota over products of other manufacturers that may also be suitable.

Table 1. *Occurrence of VA mycorrhizal fungal species at Waseca and Lamberton*

Voucher numbers[a]	Genus, species, authority	Frequency of occurrence[b] at Waseca	Lamberton
WL1	*Acaulospora spinosa*, Walker & Trappe	1	2
WL2	*Entrophospora infrequens*, (Hall) Ames & Schneider	5	3
WL3	*Gigaspora gigantea*, (Nicol. & Gerd.) Gerd. & Trappe	1	16
WL4	*Glomus aggregatum*, Schenck & Smith emend. Koske	16	16
L5	*G. albidum*, Walker & Rhodes	0	11
W6	*G. claroideum*, Schenck & Smith	16	0
WL7	*G. geosporum*, (Nicol. & Gerd.) Walker	11	6
L8	*G. intraradix*, Schenck & Smith	0	1
WL9	*G. leptotichum*, Schenck & Smith	16	7
WL10	*G. macrocarpum*, Tul. & Tul.	10	4
W11	*G. microcarpum*, Tul. & Tul.	16	0
WL12	*G. mosseae*, (Nicol. & Gerd.) Gerd. & Trappe	16	12
WL13	*G. occultum*, Walker	16	10
WL14	spore with peridum (cf. *G. mosseae*)	16	4

[a] Numbers assigned to voucher specimens of each species.
[b] Number of plots, out of 16 possible, in which each species was observed.

Table 2. *Mean spore populations of individual VA mycorrhizal fungal species in experimental plots with a corn or soybean cropping history at Waseca and Lamberton* ($N = 8$)

	Waseca spore populations		Lamberton spore populations	
	Spores (25 g soil)$^{-1}$ CCCCC[a]	SSSSS	Spores (25 g soil)$^{-1}$ CCCCC	SSSSS
A. spinosa	0	0·76[b]	9·1	2·0
E. infrequens	0	4·3[b]	0	6·5[b]
Gi. gigantea	0	0·25[b]	35·6	42·5
G. aggregatum	73·5	41·1*	2188·9	2583·2
G. albidum	—	—	29·6	7·9*
G. claroideum	54·0	76·7	—	—
G. geosporum	8·0	7·4	1·4	9·1
G. intraradix	—	—	2·5	0[b]
G. leptotichum	265·2	105·2***	9·0	7·5
G. macrocarpum	3·7	9·0	6·1	3·6
G. microcarpum	237·3	392·0*	—	—
G. mosseae	161·6	100·1	49·5	17·0*
G. occultum	190·2	38·4***	33·8	14·5*
spore w/peridum	9·1	7·1	1·4	0·6

*, *** Mean pairs are significantly different by t test at $P \leqslant 0{\cdot}05$ and $P \leqslant 0{\cdot}001$ respectively.
[a] CCCCC, 5 yr corn history; SSSSS, 5 yr soybean history.
[b] Mean values were not statistically compared because species did not occur in both cropping histories.

RESULTS

VA mycorrhizal fungal communities

Thirteen species of VA mycorrhizal fungi, plus an unidentified spore type, were observed at the two locations (see Table 1 which includes taxonomic authorities). The composition of the fungal communities differed between the sites. Several species occurred primarily, or solely, at only one of the two locations. For example, *Glomus claroideum* and *G. microcarpum* were observed in every plot at Waseca, but were never observed at Lamberton (Table 1). Conversely, *Gigaspora gigantea* and *G. albidum* were important species at Lamberton, but were rarely, or never observed at Waseca. The Lamberton fungal community was dominated by *G. aggregatum*, which alone accounted for between 89 and 94% of the total spores examined. In contrast, *G. aggregatum* accounted for less than 10% of the spores examined at Waseca.

In temperate ecosystems, sporulation of VA mycorrhizal fungi is triggered late in the growing

Table 3. *Mean Shannon–Wiener diversity index by location and cropping history* (±*SE*, $n = 16$). *The location × crop history interaction was not significant at* $P \leqslant 0{\cdot}05$

	Shannon–Wiener index
Location	
Waseca	1·59±0·03
Lamberton	0·42±0·06
F ratio	338·1***
Cropping history	
CCCCC	1·09±0·15
SSSSS	0·93±0·16
F ratio	5·7*

[a] CCCCC, 5 yr of corn; SSSSS, 5 yr of soybean.
*, *** *F* ratio significant at $P \leqslant 0{\cdot}05$ and $P \leqslant 0{\cdot}001$ respectively.

season after periods of extensive root growth or as the host matures and senesces (Sutton & Barron, 1972; Rich & Schenck, 1981). Our soil samples were collected just 3 wk after the 1988 crop had emerged so we assumed that the spores observed were produced by the crop grown the previous (1987) growing season. Thus, continuous soybean and first-year corn plots had a soybean cropping history and continuous corn and first-year soybean plots had a corn-cropping history. Different fungal communities developed in plots with different cropping histories. At Waseca, *G. aggregatum*, *G. leptotichum* and *G. occultum* spores were more abundant in plots with a corn rather than a soybean history, while spores of *G. microcarpum* exhibited the reciprocal pattern (Table 2). *G. aggregatum* dominated all the plots at Lamberton and did not appear to be influenced by crop history. Nevertheless, *G. albidum*, *G. mosseae* and *G. occultum* spores were more abundant in plots with a corn history than a soybean history (Table 2). At both locations, *Entrophospora infrequens* was observed only in plots with a soybean history (Table 2).

Species diversity, as measured by the Shannon–Wiener diversity index, was affected by both location and cropping history. The VA mycorrhizal fungal community at Waseca was significantly more diverse than the Lamberton community (Table 3). Twelve species were observed at both sites, but the Waseca community had a much greater evenness, and consequently, a higher Shannon–Wiener index. Across both locations the Shannon–Wiener diversity index was higher in plots with a corn history than a soybean history (Table 3).

Total spore count varied significantly with location. Two to three times more spores were recovered from Lamberton than from Waseca. At Waseca, total spore counts tended to be greater in plots with a corn history compared to a soybean history (Table 4). In contrast, total spore counts were not related to crop history at Lamberton (Table 4). Root colonization was generally greater in corn seedlings than soybean seedlings. Colonization of soybean at Waseca was higher in plots with a corn history than a soybean history, but colonization at Lamberton was not related to crop history (Table 4).

Soil analysis

Soil P, K, and pH were significantly higher at Waseca than at Lamberton (Table 5). Soil P was significantly greater in plots with a soybean history than a corn history. The previous crop did not

Table 4. *Mean spore count* (±*SE*, $n = 4$) *and root colonization* (±*SE*, $n = 16$) *of corn and soybean seedlings in experimental plots with a corn or soybean cropping history at Waseca and Lamberton*

Location	Cropping history[a]	Total spore count [spores (25 g soil)$^{-1}$]	VA mycorrhizal colonization (%)
Waseca			
Corn seedlings	CCCCC	1097±98	28·5±1·7
	SSSSS	723±45**	23·2±2·1
Soybean seedlings	CCCCC	908±101	25·1±2·0
	SSSSS	840±110	19·1±1·5*
Lamberton			
Corn seedlings	CCCCC	2521±384	34·0±1·3
	SSSSS	2437±323	36·0±0·7
Soybean seedlings	CCCCC	2211±425	17·0±1·0
	SSSSS	2952±861	17·5±0·7

[a] CCCCC, 5 yr of corn; SSSSS, 5 yr of soybean.
*, ** Mean pairs are significantly different by *t* test at $P \leqslant 0{\cdot}05$ and $P \leqslant 0{\cdot}01$ respectively.

Table 5. *Mean soil, P, K, and pH by location and cropping history (±SE, n = 32). None of the location × cropping history interactions were significant at P ⩽ 0·05*

	Soil P (kg ha^{-1})	Soil K (kg ha^{-1})	pH
Location			
Waseca	75·0±2·1	388·8±8·7	6·36±0·05
Lamberton	60·7±1·7	290·9±9·5	5·79±0·05
F ratio	31·8***	56·6***	70·0***
Cropping history[a]			
CCCCC	64·1±2·0	336·0±12·4	6·11±0·07
SSSSS	71·5±2·4	343·7±12·9	6·05±0·06
F ratio	8·5**	0·3 n.s.	0·75 n.s.

[a] CCCCC, 5 yr of corn; SSSSS, 5 yr of soybean.
, * *F* ratio significant at $P \leqslant 0{\cdot}01$ and $P \leqslant 0{\cdot}001$ respectively.

Table 6. *Coefficients for stepwise multiple regression models to predict spore densities of VA mycorrhizal fungal species at Waseca and Lamberton*

	Explanatory variables				
Species	Crop history[a]	Soil K	Soil P	Soil pH	R^2
Waseca[b]					
E. infrequens	+1·285	—	—	—	0·41
G. microcarpum	+0·553	—	—	—	0·26
G. leptotichum	−0·889	—	—	—	0·53
G. occultum	−1·45	—	—	—	0·62
G. aggregatum	—	—	−0·02	−0·64	0·42
G. geosporum	—	—	—	+2·00	0·18
spore w/peridium	—	−0·0057	—	—	0·34
Lamberton[c]					
E. infrequens	+2·74	—	—	—	0·54
G. albidum	−1·93	—	—	—	0·30
G. mosseae	−1·44	—	—	—	0·23
G. occultum	−1·72	—	—	—	0·18
Gi. gigantea	—	—	—	+1·45	0·22
spore w/peridium	—	—	—	+1·72	0·17

[a] Crop history was defined by a dummy variable: 0, corn history; 1, soybean history. A negative coefficient indicates that species proliferated with a corn history and a positive coefficient indicates proliferation with a soybean history.
[b] None of the explanatory variables were significant predictors for spore densities of *G. mosseae*, *G. claroideum* or *G. macrocarpum* at Waseca. Regression models were not attempted for *Gi. gigantea* and *A. spinosa* because they occurred in only one plot.
[c] None of the explanatory variables were significant predictors for spore densities of *G. aggregatum*, *G. geosporum*, *G. leptotichum* or *G. macrocarpum* at Lamberton. Regression models were not attempted for *G. intraradix* and *A. spinosa* because they occurred in only one or two plots respectively.

influence either the pH or K levels of the soils. There were no significant crop history × location interactions on any of the soil parameters we measured.

Regression models

Significant models could be fitted to the spore densities of seven species at Waseca and six species at Lamberton. Crop history was a significant predictor of *E. infrequens* and *G. occultum* at both locations (Table 6). Furthermore, crop history was a significant predictor of *G. microcarpum* and *G. leptotichum* at Waseca, and *G. albidum* and *G. mosseae* at Lamberton. Dummy variables of 0 and 1 were assigned to plots with a corn or soybean history respectively. Thus, a negative regression coefficient indicates that a species proliferates in corn and a positive coefficient indicates that a species proliferates in soybean. Soil pH was the best predictor of *G. aggregatum* and *G. geosporum* at Waseca, and *Gi. gigantea* and the peridial spore type at Lamberton (Table 6).

DISCUSSION

We observed both site effects and cropping history effects on the composition of the VA mycorrhizal fungal communities. The large difference between the evenness of the communities of Waseca and Lamberton may be attributable to differences in the cultural practices in the two sites. The experimental plots at Waseca contained few weeds during 1987. However, the Lamberton plots were infested with a dense cover of foxtail [*Setaria faberi* Herrm, *S. lutescens* (Weigel) Hubb., and *S. viridis* (L.) Beauv.] during a major portion of the 1987 growing season (James Kurle, personal communication). We suggest that the Lamberton plots may have functioned as monocultures of foxtail rather than monocultures of corn and soybean. Consequently, the cropping history treatment was diluted at Lamberton and there were only subtle differences between the VA mycorrhizal fungal communities in plots with a corn *vs.* a soybean cropping history. This interpretation is supported by the *in situ* bioassay and total spore counts. At Waseca both root colonization and total spore counts were consistently (but not always significantly) greater in plots with a corn history than in plots with a soybean history. This result would be expected if the greater below-ground biomass of corn, compared to soybean, generated higher propagule densities. In contrast, there was no apparent association between root colonization, total spore count and cropping history at Lamberton. Perhaps the high density of weeds throughout the Lamberton plots in 1987 may have largely obliterated any relationship between propagule populations and cropping history.

Differences in the species composition of the fungal communities at Waseca and Lamberton may be attributable to edaphic differences between the sites. Species of VA mycorrhizal fungi are known to differ in their responses to a number of edaphic factors including soil pH (Green, Graham & Schenck, 1976; Abbott & Robson, 1985), soil temperature (Schenck & Smith, 1982), and soil microorganisms (Kitt, Hetrick & Wilson, 1987). Insights into the ecology of these fungi may be gained by comparing the Waseca and Lamberton fungal communities in relation to the edaphic differences of these sites. Porter, Robson & Abbott (1987) found field distributions of VA mycorrhizal fungal species to be sensitive to soil pH. *G. aggregatum* was present at both sites, but at very different densities. This species comprised over 89% of the Lamberton community and none of the measured explanatory variables were significant predictors of its densities. In contrast, *G. aggregatum* never comprised over 10% of the Waseca community, and there its density was negatively correlated with soil pH. Thus, the lower soil pH of Lamberton may have favoured the proliferation of *G. aggregatum* to such an extent that it excluded the proliferation of other species.

Our finding that cropping history influenced the composition of the fungal community corroborates the findings of other workers (Kruckelmann, 1975; Schenck & Kinloch, 1980; McGraw & Hendrix, 1984; Trufem & Bononi, 1985; Schenck, Siqueira & Oliveira, 1989; Dodd *et al.*, 1990). This result is important since VA mycorrhizal fungal species differ in their effects on plant growth, and a shift in the species composition of the fungal community may potentially affect crop growth (Schenck *et al.*, 1989).

VA mycorrhizal fungi are generally believed to lack host specificity because they have been shown to colonize indiscriminately the roots of a vast array of plant species in greenhouse trials (Gerdemann, 1965; Mosse, 1973). However, the survival and spread of these fungi in agroecosystems is not well studied. We suggest that the distinct communities of fungi which we observed in plots with either corn or soybean cropping histories arose because VA mycorrhizal fungal species vary markedly in their ability to spread in, and sporulate around, these crops. The significance of these patterns is strengthened by the fact that some of the species which occurred at both Lamberton and Waseca exhibited similar patterns. For example, at both sites, *G. occultum* and *G. mosseae* spores were more abundant in soils with a corn history than in soils with a soybean history, and *E. infrequens* spores were only recovered from soils with a soybean history.

It is likely that both cropping history and edaphic factors control populations of VA mycorrhizal fungi in agroecosystems. Certainly edaphic effects are not independent of cropping history because crops modify the soil they inhabit. The distinct fungal communities which we observed in soils with different cropping histories probably resulted from differences in the physical, chemical and microbial environments in the rooting zones of corn and soybean.

ACKNOWLEDGEMENTS

We would like to thank J. E. Kurle, M. Bergstedt, and E. Schiefelbein for their technical assistance. Financial support was provided by the James W. Wilkie Fund for Natural History. Published as paper no. 17838 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

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