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Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland

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Summary

1 In laboratory microcosm experiments, co-occurring plant species were found to support very different rates of sporulation of arbuscular mycorrhizal (AM) fungi. These differences were not affected by the time of harvest, suggesting that they reflect host-dependent differences in fungal growth rates, rather than host-dependent timing of sporulation.

2 Spore counts in field soil and estimates from sorghum trap cultures showed that the association of AM fungi with particular host plants in the field was positively correlated with the sporulation rates observed on those hosts in the microcosm experiments.

3 The AM fungal species richness observed at the field site was high relative to estimates made in previous studies. 23 distinct species of AM fungi were found, seven of which have not been previously described.

4 The host-dependence of the relative growth rates of fungal populations may play an important role in the maintenance of fungal species diversity.

Keywords: arbuscular mycorrhizae, community ecology, glomalean fungi, microcosm, population growth rates, species diversity

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Introduction

The majority of vascular plants associate with arbuscular mycorrhizal (AM) fungi (frequently called vesicular arbuscular endomycorrhizal or VAM fungi or glomalean fungi). Despite the importance of AM fungi in the physiology and nutrition of plants, as well as in plant community and ecosystem processes (Miller 1987; Allen & Allen 1990; Allen 1991; Brundrett 1991; Hartnett *et al.* 1993; Miller & Jastrow 1994), factors affecting diversity of AM fungi are poorly understood. By current estimates (Law & Lewis 1983) it appears that, on a global scale, there are vastly fewer species of AM fungi relative to the number of species of mycorrhizal dependent plant hosts. It has been suggested that this low diversity results from the low host-specificity of AM fungi relative to other fungi (Law & Lewis 1983), though other explanations for this pattern have been discussed (Morton 1990; Bever 1992). On a local scale, however, the determinants of AM fungal species diversity are poorly understood (Morton *et al.* 1995).

While most AM fungi can associate with a wide array of hosts, a growing body of work suggests that their performance relative to each other depends upon the host species involved. The sporulation rates of AM fungi have been found to be host-dependent in laboratory systems (Daft & Hogarth 1983; Hetrick & Bloom 1986; Koomen *et al.* 1987; Hung & Sylvia 1988), and such differences in sporulation rates may explain changes in the composition of the AM fungal community in response to the changes in the plant community (Schenck & Kinloch 1980; Johnson *et al.* 1991a, 1992; Sanders & Fitter 1992; Hendrix *et al.* 1995). These host-dependent sporulation rates also may reflect host-dependence in the rate of production of infective propagules (i.e. spores and hyphae), which is a major component of the growth rates of fungal populations.

Host-dependence of fungal population growth rates may be a mechanism for the maintenance of

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fungal species diversity. Enhancement of fungal species diversity is likely when the host-dependence involves changes in the ranking of fungal population growth rates on different plant hosts. With such host-dependence, a diverse plant community would represent a heterogeneous selective environment (*sensu* Brandon 1990) to the AM fungi. Such heterogeneous environments have long been considered to be a primary mechanism for the maintenance of species diversity (MacArthur 1972; Levin 1976; Levins 1979), but the importance of this process in AM fungal communities is poorly understood. Inference from patterns of distribution of AM fungi in the field suggest host species, as well as abiotic factors and competitive interactions, may be important in maintaining fungal species diversity (Koske 1981; Koske 1987; Gemma *et al.* 1989), but only a few studies have used experimental manipulation to evaluate the roles of these different factors (Sanders & Fitter 1992; Johnson *et al.* 1992).

Furthermore, because host-dependent population growth rates of AM fungi may result in the development of distinct fungal communities on plant hosts, these fungal communities may, in turn, affect the performance of plant species relative to each other. Fungal species have indeed been observed to have differential effects on the growth of plant species (Nemec 1978; Powell *et al.* 1982; Ollivier *et al.* 1983; Wilson 1988; Ravnskov & Jakobsen 1995). Given such mutual interdependence of plant and fungal relative growth rates, coexistence of both plants and fungi would be determined by whether their interaction leads to negative or positive feedback (Bever 1992). Negative feedback occurs if a particular plant species promotes the growth of one AM fungal species more than that of others, but this fungal species has a less positive effect on growth of that plant species (but a more positive effect on other plant species) than do other AM fungi. Negative feedback would be expected to promote coexistence of multiple sets of interactants, whereas positive feedback would lead to the predominance of one particular interactant pair (Bever 1992).

In the present study, we test for host-dependence of the growth rate of AM fungi by growing a single fungal community in association with different plant species and monitoring sporulation. We also examine the distribution of fungi in the field with respect to these same plant species and ask whether the plant-fungal associations in the field are similar to the patterns of host-dependence observed experimentally in the glasshouse.

STUDY SYSTEM

The plants and AM fungi used in these experiments were obtained from a mown field on the campus of Duke University, Durham, North Carolina. The field has been maintained by annual mowing for at least 35 years, and consists predominantly of grasses. More than 25 plant species occur frequently, without any attaining clear cut dominance. This plant community is divided temporally into summer and winter assemblages (Fowler & Antonovics 1981). The plants, pathogens and soil of this site have been intensively studied over the last 20 years (e.g. Fowler & Antonovics 1981; Clay 1982; Alexander 1984; Ellstrand & Antonovics 1985; Schmitt & Antonovics 1986; Moloney 1988; Kelley 1989; Broaddus 1991; Ronsheim 1992; Bever 1994). The soil is a sandy loam of the White Store series with a pH of 5.0 and low fertility with extractable phosphorus concentrations of 1.5–6 $\mu g\ g^{-1}$ (Fowler & Antonovics 1981).

We studied four perennial plant species that are abundant at the study site: *Allium vineale* L., *Anthoxanthum odoratum* L., *Panicum sphaerocarpon* Ell., and *Plantago lanceolata* L. Dynamics of these populations have been investigated previously (*Allium*: Ronsheim 1992; Bever 1992; *Anthoxanthum*: Antonovics & Ellstrand 1984; Schmitt & Antonovics 1986; Kelley 1989; Bever 1994; *Panicum*: Bever 1994; *Plantago*: Antonovics & Primack 1982; Alexander 1984), including the role of the soil community in the dynamics of *Anthoxanthum* and *Panicum* (Bever 1994). For each of these species, individual plants were collected from a 5-m × 15-m region of the field and cloned to produce the replicates in our study. In this paper, clones derived from the same field-collected individual will be identified as being of the same 'genotype'.

Methods

GENERAL PROTOCOLS

We tested for host-dependent differences in AM fungal population growth rates using experimental microcosms composed of components which coexisted in the field. Specifically, we started with replicates of a single laboratory mycorrhizal community, grew them with *Allium*, *Anthoxanthum*, *Panicum*, and *Plantago*, and then tested for differentiation of the mycorrhizal fungal communities by monitoring sporulation over successive harvests. We examined the spatial correlations between the distribution of AM fungi and the distribution of the same four plant species in the field using three sampling methods: (1) *Sorghum trap cultures. Sorghum* plants serve as hosts for fungi collected from underneath individuals of each of the four plant species; (2) *Transplant trap cultures.* Field collected individuals of *Anthoxanthum*, *Panicum*, and *Plantago* were transplanted into soil free of AM fungi; and (3) *Field-collected spore counts.* Direct counts were made of field-collected spores from underneath individuals of each of the four plant species. We then compared the patterns observed in the field to the patterns of host-dependent differentiation of the AM fungal communities observed in the microcosm.

In September, 1992, we identified six locations within a 5-m × 15-m study area of the field in which an individual of each of the four plant species was located within 0.5 m of each other (see Fig. 1). These individuals and the soil and roots from underneath them (*c.* 500 mL) were used for the studies described below.

EXPERIMENTAL MICROCOSMS

Portions of the soil and roots from underneath the 6 individuals of each of the four plant species were pooled, diced, and thoroughly mixed with equal parts of sand which had been autoclaved for two hours. Two mycorrhizae-free individuals of each of seven genotypes of each of the four plant species (a total of 56 pots) were planted into 6-cm × 25-cm pots containing this mixture. These genotypes were derived from individuals collected at random from this same site in the previous year. Individuals of *Anthoxanthum* and *Panicum* were cloned by dividing plants into tillers, cutting off the roots, and surface sterilizing the tillers in 10% Clorox for 10 min. (Bever 1994). *Allium* was cloned from asexually produced aerial bulbils (Ronsheim 1992; Bever 1992). Genotypes of *Plantago* were cloned by rooting surface-sterilized leaf cuttings (Wu & Antonovics 1975; Teramura *et al.* 1981).

The plants were grown in a glasshouse in Durham, NC, under natural sunlight and cool conditions (4–21 °C) with one replicate being harvested after 4.5 months and the second replicate being harvested after 6 months. The plants were watered as needed and fertilized during the second and fourth month with a 100 mL of 1/4 strength Hoaglands solution modified to contain a reduced concentration of phosphorus (Millner & Kitt 1992).

DISTRIBUTION OF AM FUNGI IN THE FIELD

Sorghum trap cultures

Soil and roots from underneath each of the six sampled plants of the four species (Fig. 1) were mixed separately with equal parts autoclaved sand. *Sorghum vulgare* was used as a host plant because it grows well in the glasshouse and is a suitable host for a wide variety of fungi (Morton *et al.* 1993). *Sorghum* seeds were planted into 24 6-cm × 25-cm pots (30–50 seeds per pot) containing the soil mixtures and grown in the glasshouse in Durham, NC, under natural sunlight and cool conditions for 5.5 months. They were watered and fertilized as described for the microcosm experiment.

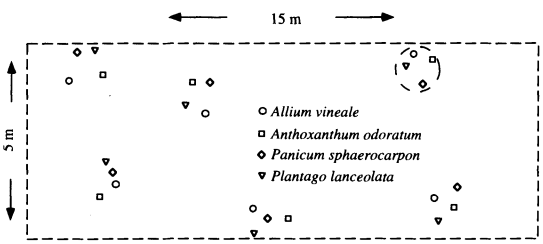

Fig. 1 Sampling design of 5-m × 15-m region of a mown field in North Carolina. Individuals of each of the four species were collected from six locations with individuals at a given location being within 0.5 m of each other.

Transplant trap cultures

Plants were removed from the field as described previously, their roots washed free of soil, and planted into an autoclaved mixture of equal parts soil and sand. *Allium* was not used in this experiment because, as a winter perennial, it did not have roots at the time of collection. Because the root systems of individual plants were only partially sampled and the root architecture differed between plant species, the quantity and nature of roots associated with these transplants could not be held constant between species. The plants were grown for 5 months in the glasshouse in North Carolina and watered and fertilized as described for the microcosm experiment.

Field-collected spore counts

In April 1993, three adjacent soil cores (1.7 cm in diameter and 6 cm deep) were taken from underneath a further seven sets of individuals of each of the four species. All sets had the same clumped arrangement as described above (see Fig. 1) and were taken from the original 5-m × 15-m area. These three cores were pooled so that the soil volume totalled $\approx 50\,cm^3$.

IDENTICATION AND ENUMERATION OF SPORES

All samples were stored in sealed plastic bags at 4 °C for up to 1 month until spores could be counted. Spores were extracted from the soil by blending a solution of a known quantity of soil (either 50 or $100\,cm^3$) in water for two 5-s bursts in a Waring blender, collecting the spores by pouring the slurry through a 45-μm sieve, and separating the spores from sand, soil, and organic debris using sucrose gradient centrifugation (Daniels & Skipper 1982). Spores were examined under a binocular stereomicroscope and Nomarski interference microscope. Species identification was based on spore colour, size, surface ornamentation, and wall structure using reference cultures from the International Collection of Vesicular and Arbuscular Mycorrhizal Fungi, INVAM (Morton *et al.* 1993), and species descriptions (Schenck & Perez 1990). Permanent slide vouchers were made of all fungi, and the majority were subsequently established in single species cultures and deposited in INVAM. A taxonomic treatment of AM fungi at this site will be published separately. Only spores which appeared to be viable (based on colour, shape, surface conditions, and examination of spore contents) were

counted. Spores of all species were counted at 30 × under a binocular stereomicroscope except the small-spored species of *Acaulospora* (*A. trappei*, *A.* D2, *A.* D4) and *Glomus* (*Gl. fasciculatum* and *Gl.* D4). For these species, spores were counted at 42 × within 30 optical squares located randomly across the plate. The average of counts from two separate extractions were used in the analysis of the microcosm experiment.

STATISTICAL ANALYSIS

Species in which only a few spores were found or where counting techniques appeared unreliable (especially the rarer small spored species) were not included in statistical analyses. The spore counts were not normally distributed and the variance of the counts was not homogeneous across treatments, as has been found in other studies of AM fungi (St. John & Koske 1988). Conversion of the spore counts into ranks of counts greatly improved the normality and homogeneity of variances; therefore, analyses were performed on the ranks. These ranks were analysed with a two-way multivariate analysis of variance with profile contrasts (Morrison 1976) using the general linear models procedure of SAS (SAS 1986). The overall effects and the interaction of the effects with the profile were tested using Wilk's Lambda criterion because it is derived from a likelihood-ratio approach (SAS 1986); however, multivariate tests using Pillai's Trace and Hotelling-Lawley Trace gave similar results. The interaction of effects with the profiles (e.g. the dependence of the shape of the profiles on plant species) is of particular interest because it specifically tests whether the relative ranks of spore numbers of the fungal species (i.e. relative abundance) varied with treatments. When the multivariate tests were significant, the ranks of spore numbers of AM fungal species were tested individually using univariate analysis of variance. The similarity of patterns of differentiation observed in the community microcosm experiments to those observed in the field was tested by regressing the mean ranks of spore numbers of different fungal species on different plant hosts between the sorghum traps and the direct field counts against those observed in the community microcosm experiment.

Results

DIFFERENTIATION OF AM FUNGAL COMMUNITIES ON PLANT HOSTS IN THE MICROCOSMS

At the first harvest (4.5 months) the abundance of AM fungal spores present in the pots differed significantly between host species (Table 1, Fig. 2), indicating the original fungal community had strongly differentiated in response to being grown with the different plant species. The sporulation of nine of the 14 most common fungal species depended significantly on host species (Fig. 2, Table 2). Furthermore, the ranks of the spore numbers of the different fungal species were significantly altered when grown with different plant species, as tested by the interaction of rank profiles with the plant species effect (Table 1, Fig. 2). That is, fungal species sporulated differentially on the different species of plant hosts. However, there was no significant effect of plant genotype within species on the composition of the AM fungal species, but the power of the analysis to detect such effects was low because there were only two replicates and the analysis was based on ranks.

The total number of fungal spores and the relative sporulation of the fungal species differed between the two harvests (Table 1). For example, *Gi. gigantea*

Table 1 Multivariate analysis of variance of effects of plant species, genotype within plant species, and harvest on spore counts of AM fungi in the microcosm experiment. Spore counts were made for each pot and these counts were ranked within each fungal species; the analysis was then carried out on the ranks. The heading 'overall differences' presents tests for effects of treatments on total sporulation (considered across all fungal species). The heading 'interaction with the profile' presents tests for the specific hypothesis that the relative rank of sporulation of the different fungal species depends upon the designated treatment effects. The plant species effect was tested over the variation among genotypes within species. Other effects were tested over the residual error

Effect	Num. d.f.	Den. d.f.	Wilk's lambda	*F*	*P*
Overall differences					
plant	48	28	8.0×10^{-4}	5.8	0.0001
genotype within plant	384	176	5.0×10^{-7}	0.9	NS
harvest	16	9	8.0×10^{-2}	5.9	0.005
plant × harvest	48	28	2.0×10^{-2}	1.5	NS
Interaction with profile					
plant	45	31	3.0×10^{-3}	4.1	0.0001
genotype within plant	360	178	1.0×10^{-6}	0.9	NS
harvest	15	10	1.0×10^{-1}	5.8	0.004
plant × harvest	45	31	2.5×10^{-2}	1.7	0.07

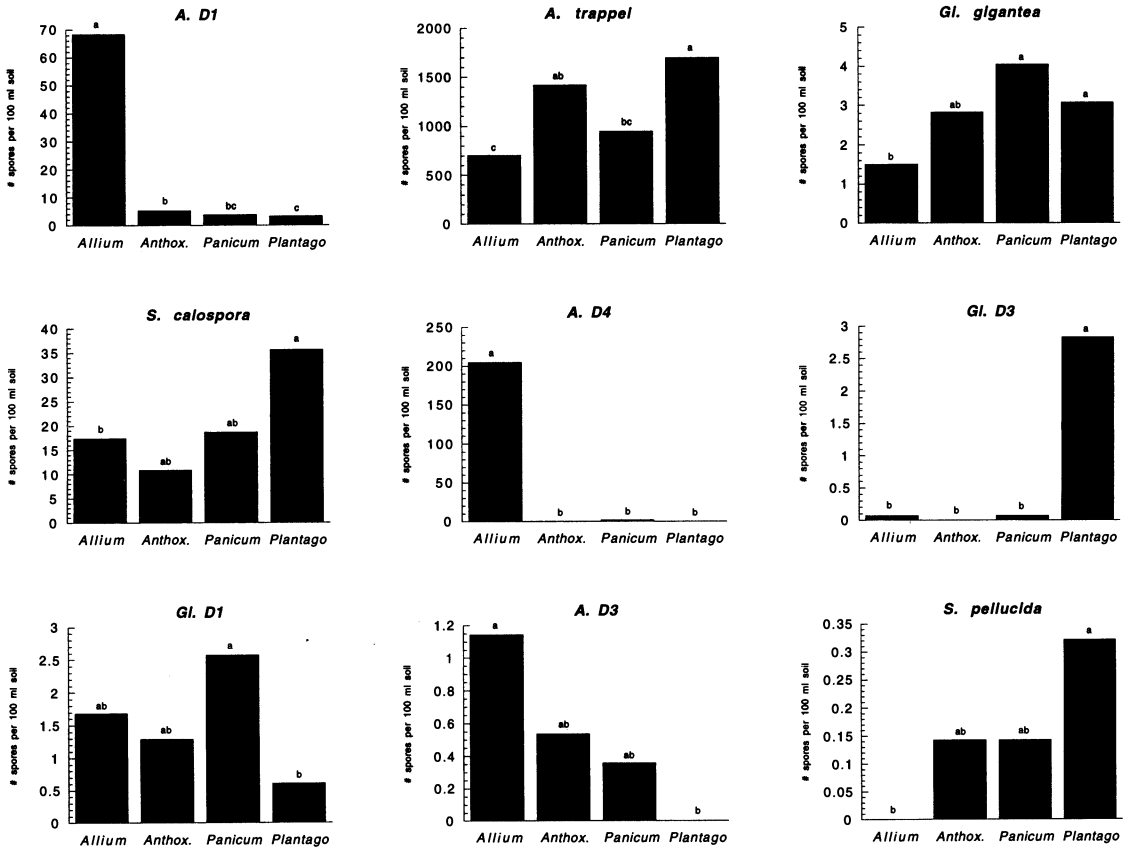

Fig. 2 Sporulation of AM fungi in association with the four plant species in the experimental microcosm. Fungal species with significantly different sporulation between hosts are presented, and are arranged in decreasing order of their average spore volume. Although all statistical tests were performed on the ranks of the spore counts, mean sporulation is depicted for ease of interpretation. Significant differences in sporulation are indicated by different letters.

produced a greater number of spores at the first harvest than the second, while *Acaulospora mellea* showed the opposite trend. However, the effect of the host species on total sporulation did not depend significantly on the harvest (Table 1).

DISTRIBUTION OF AM FUNGI IN THE FIELD

Twenty-three distinct AM fungal species were identified from this site, seven of which have not been described previously (Table 3). No single sampling procedure revealed all of the fungal species, and while there was qualitative agreement in the abundances of the different species recorded by the different sampling methods, there were also some marked discrepancies. For example, *Scutellospora calospora* spores were very abundant in the *Sorghum* trap cultures, but were much less so using the other methods. Also, the transplant trap cultures revealed the lowest diversity and total abundance of fungal spores. The results of the individual sampling methods are described below. The spore abundances in the experimental microcosms were qualitatively similar to those obtained by direct field sampling.

Sorghum trap cultures

Neither overall abundance of spores nor the relative abundance of spores of different species were significantly influenced by the species of plant under which the soil was obtained (Table 4). However, both the total number of spores, and the relative abundance of spores of different species did depend on the location in the field from which the inocula were collected (Table 4), indicating that AM fungal species were not uniformly distributed even in this small area.

Transplant trap cultures

The overall abundance of spores observed was significantly influenced by both the species of plant which was transplanted as well as by the location of that plant in the field (Table 4). The relative abundance of spore types in the transplant traps was significantly affected by plant species but not by location (Table 4), indicating that the composition of the fungal communities differed between plant species.

Field-collected spore counts

In the direct counts of field soil, neither the overall abundance of spores nor the relative abundance of spores of different species depended on the plant species under which the soil was sampled, although the latter effect approached significance ($P < 0.08$, Table 4). Location of the sample in the field influenced both

Table 2 Results from univariate analyses of variance of effects of plant species, genotype within plant species, and harvest on spore counts of AM fungi in microcosm experiment, considering each fungal species separately. Table shows the sums of squares and significance levels of effects for each fungal species. Spore counts were made for each pot and these counts were ranked within each fungal species; the analysis was then carried out on the ranks. The plant species effect was tested over the variation among genotypes within species. Other effects were tested over the residual error

	Plant	Genotype within plant	Plant × harvest	Harvest	Error
d.f.	3	24	1	3	24
A. D1	8628****	2138	944**	354	2513
A. trappei	4116***	4326	1170**	1037	3969
Gi. gigantea	2392*	5124	462	2019*	4425
S. calospora	2295**	3882	3045***	237	5145
Gl. D2	1115	4458	5904****	206	2936
A. D4	1981****	1248	325†	374	2052
S. reticulata	1093	5487	45	286	5532
A. D2	1232	5258	952*	625	4008
Gl. D3	1770***	1512	18	159	2511
Gl. D2	1514*	3802	138	1806	5440
A. mellea	322	2497	3180****	655	3029
A. D3	1221*	3278	208	514	2779
S. pellucida	764**	1306	5	537	2215
Gi. rosea	599	3043	833*	103	4282
S. heterogama	70	3325	71	281	3287
Gl. clarum	144	1922	9	159	2585

†$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

the overall abundance of spores and the relative ranks of spore abundance of the different fungal species (Table 4).

Correlation of field distribution with differentiation in the microcosms

The composition of the AM fungal community associated with the four plant species in the field, as estimated from the sorghum trap cultures and from direct counts of field soil, was significantly similar to the composition of the AM fungal communities observed in the glasshouse microcosms ($P < 0.04$, $R^2 = 0.08$ and $P < 0.05$, $R^2 = 0.08$, for sorghum traps and field soil, respectively). When only the nine fungal species which had significantly different sporulation on plant hosts in the microcosm experiment (Table 3) were included in the analysis, the strength of the regressions increased ($P < 0.03$, $R^2 = 0.17$ and $P < 0.04$, $R^2 = 0.12$ for sorghum traps and field soil, respectively) (Fig. 3). There was no significant correlation between the field distribution of the fungi as estimated from the transplant trap cultures, and their host related abundances in the glasshouse microcosms.

Discussion

The 23 species of AM fungi identified from the 75-m^2 area of the field site in this study probably represents the highest species richness ever recorded for AM fungi within such a small area, and may reflect the intensity of sampling rather than any unique characteristic of the site (Morton *et al.* 1995). Moreover, given that no single sampling procedure revealed all of the fungal species (Table 3), it is unlikely that all of the fungal species present at this site were discovered in this study. In fact, further sampling at this site has revealed several additional species (J. D. Bever, unpublished data).

The glasshouse microcosm experiment provides strong evidence of host-dependent differentiation of AM fungal communities. Not only did overall sporulation of the AM fungi vary significantly on different plant hosts, but, most importantly, fungal species had distinct patterns of differential sporulation such that for a given pair of plant species, one was a better host for one AM fungal species, but a worse host for another. For example, *A.* D1 and *A.* D4 sporulated most prolifically on *Allium* while on this same host *A. trappei* and *Gi. gigantea* had their lowest sporulation (Fig. 2, Table 2). The mechanism of this host-dependent sporulation was not investigated, but it could result directly from host-dependent growth of the fungi, indirectly through host-dependent changes in the mineral soil, or indirectly through host–mediated interactions of individual AM fungal species with each other or with other components of the soil community. Regardless of the mechanism generating the host-dependence, the strength of the host effect suggests that host-dependent processes are important in this system.

Direct evidence of host-dependence in nature was much weaker: of the three methods used to examine association of fungal distribution with plant species, a significant plant species effect was only observed in

Table 3 Abundance of AM fungal species present in a 5 m × 15 m region of a mown grassland in North Carolina, as detected using four methods (see text for detailed description). Data are presented in terms of total spore volume, to give a better indication of overall abundance. For each species and each method, the total spore volume per 100 cm^3 soil is given (in units of 10^6 μm^3). Spore volume was calculated by multiplying the average number of spores observed by the average volume of individual spores, as calculated from their average diameter and the equation of a sphere. The average diameter used in these calculations are reported. Species present but whose counts were unreliable (see text) are indicated by a 'P'

	Total spore volume detected by different methods					
AM fungi[a]	Sorghum cultures	Transplant cultures	Field soil	Experimental microcosm	Spore diam. (μm)	INVAM number
Acaulospora						
bireticulata	0	0	19.7*	0	180	
mellea	71.4	0	7.9	2.4	101	NC 148
trappei	48.0	45.6	13.7	198.2	65	NC 112A
sp. D1[1]	216.2	95.6	217.1	211.6	280	NC 168
sp. D2[2]	2.9	9.2	46.0	5.9	69	
sp. D3[3]	0	0	13.4	2.2	69	
sp. D4[4]	0	0	9.1	10.3	201	NC 174
Gigaspora						
gigantea	102.2	34.2	61.1	64.3	352	NC 120A
rosea	23.7	3.7	P	1.6	260	NC 121A
ramisporophora	0	0	0	P	320	NC 175
Glomus						
clarum	P	0	P	0.1	104	NC 112B
constrictum	P	0	P	P	170	
fasciculatum	0	3.0	0	0	55	
leptotichum	P	0	0	0	160	NC 171
mosseae	3.8	0	P	P	143	
sp. D1[5]	7.1	2.2	0.6	0.9	106	NC 172
sp. D2[6]	16.4	0.9	31.6	12.7	83	
sp. D3[7]	77.5	0	7.1	3.0	160	
sp. D4[8]	P	0	P	P	53	
Scutellospora						
calospora	223.4	4.4	16.3	40.2	154	NC 146
heterogama	1.0	P	1.3	1.0	165	NC 141
pellucida	23.5	0	21.1	3.0	330	NC 155
reticulata	1.6	0	2.4	7.9	237	NC 202

[a]Vouchers available upon request to first author.
*Not included in statistical analysis because of missing data.
[1]Spores are red to orange in colour and 220–360 μm in diameter, with a wall structure resembling that of *A. laevis*
[2]Spores are lightly straw coloured and 50–70 μm in diameter, with a spore wall and inner wall structure resembling that of *A. delicata*.
[3]Spores are copper coloured and 130–230 μm in diameter, with a textured surface and laminae of the spore wall which shatters when squashed.
[4]Spores are straw coloured and 55–80 μm in diameter, with spore wall structure resembling that of *A. scrobiculata*.
[5]Spores are white and 60–120 μm in diameter, with a spore wall structure which most closely resembles that of *Gl. diaphanum*.
[6]Spores are often formed in sporocarps, red to red-orange in colour, and 60–90 μm in diameter, with a wall structure which most closely resembles that of *Gl. etunicatum*.
[7]Spores are tan in colour and 130–180 μm in diameter, having an ephemeral outer wall covering a thick, robust laminated wall.
[8]Spores are formed in sporocarps, yellow to red-brown in colour, and 40–60 μm in diameter. This category is likely composed of several separate sporocarpic species and for that reason was not included in analyses.

the transplant traps. Moreover, this method has the weakness that the quantity of the inoculum is not controlled and is likely to depend on the plant species. The absence of a significant effect of plant species on the distribution of spores in field soil and sorghum traps may not be surprising given our sampling technique. Real differences in the fungal communities in the rhizospheres of the four plant species may have been obscured by the fact that these plants are growing in close proximity and their root systems certainly overlap with each other as well as with other congeners; bulk soil samples taken from under each plant included the roots of other plant species.

The strongest evidence that the host-specific effects seen in the microcosm were relevant to the field situation was the correlation between the patterns of host-dependent differentiation observed in the microcosms and those found in the field (Fig. 3). While this correlation is weak, it does suggest that the process of differential sporulation observed in the laboratory may be active under natural conditions, though this pattern was difficult to detect with our sampling tech-

Table 4 Multivariate analysis of effect of plant species and sample location on spore numbers of AM fungi in field soil samples, as assessed using three methods. Spore counts were made for each sample and these counts were ranked within each fungal species; the analysis was then carried out on the ranks. The heading 'overall differences' presents tests for effects of treatments on total sporulation (considered across all fungal species). The heading 'interaction with the profile' presents tests for the specific hypothesis that the relative rank of sporulation of the different fungal species depends upon the designated treatment effects

Effect	Num. d.f.	Den. d.f.	Wilk's lambda	*F*	*P*
Overall differences					
Sorghum trap cultures					
plant species	42	16	1.0×10^{-1}	1.4	ns
location	70	28	2.5×10^{-5}	3.3	0.0004
Transplant trap cultures					
plant species	18	2	1.4×10^{-8}	92.5	0.001
location	45	8	1.6×10^{-9}	15.5	0.0003
Direct counts of field soil					
plant species	45	10	9.0×10^{-4}	2.1	ns
location	90	24	1.0×10^{-6}	2.7	0.004
Interaction with profile					
Sorghum trap cultures					
plant species	39	18	1.5×10^{-3}	1.5	ns
location	65	32	8.0×10^{-5}	3.1	0.0003
Transplant trap cultures					
plant species	16	4	1.0×10^{-3}	7.6	0.03
location	40	11	2.3×10^{-4}	1.8	ns
Direct counts of field soil					
plant species	42	13	2.0×10^{-3}	2.1	0.08
location	84	29	2.5×10^{-6}	3.1	0.0005

niques in a heterogeneous field. This host-dependence was seen across a wide range of plant and fungal taxa, further suggesting that such host-dependence of sporulation rates are of general importance among co-occurring plants and AM fungi.

To interpret the ecological consequences of the differential sporulation rates of the AM fungi observed in this study, it is important to know if these differences result in corresponding differential rates of population growth. However, measuring the rate of growth of a fungal population is problematical because fungal individuals are usually difficult to distinguish and observe directly (for a discussion of the difficulties for estimating growth rate of fungal genotypes, see Antonovics & Alexander 1989). Most workers in epidemiology use the rate of production of new infections/lesions per infected individual/lesion as a phenomenological measure of fungal spread; and this measure itself is a function of the rate of production of infectious propagules, the time to disease expression, and the duration of the infectious period. However, in AM fungi, the rate of production of infective propagules has been an elusive measurement because new infections can result either from the germination of spores (which are identifiable to species) or from the extension of hyphae from previously infected roots (which cannot currently be distinguished taxonomically). While sporulation has been shown to be correlated with fungal biomass at some level (Gazey *et al.* 1992; Jasper *et al.* 1993; Franke & Morton 1994), the linearity of this correlation within a fungal species and generality of this correlation across fungal and host species is not known. Differences in sporulation, as observed in this study, may therefore reflect differences in the growth rates of fungal populations or differences in the allocation of resources to sporulation relative to hyphal extension. In this study, we tested sporulation at two times of harvest, and while total spore number was significantly different between the two harvests, the strong patterns of differentiation with respect to plant host were not significantly affected by time of harvest. This suggests that the differential sporulation rates are not simply due to timing of allocation of resources to sporulation, and supports the general expectation that these sporulation rates are likely to be correlated with fungal population growth rates.

Host-dependence of fungal population growth rates would play an important role in maintaining AM fungal species diversity whenever there is a reversal of the ranks of population growth rates on distinct hosts (MacArthur 1972; Levin 1976; Levins 1979), as observed in our microcosm experiment. Furthermore, if the relative rate of growth of a plant species is also dependent upon the species of AM fungi with which it associates, as has frequently been observed (Nemec 1978; Powell *et al.* 1982; Ollivier *et al.* 1983; Wilson 1988; Ravnskov & Jakobsen 1995), then the resulting dynamics may be complex, with alternate mechanisms for the coexistence of fungal species (Bever 1992). The power of these mechanisms for maintaining diversity is enhanced by the plant and fungal life histories.

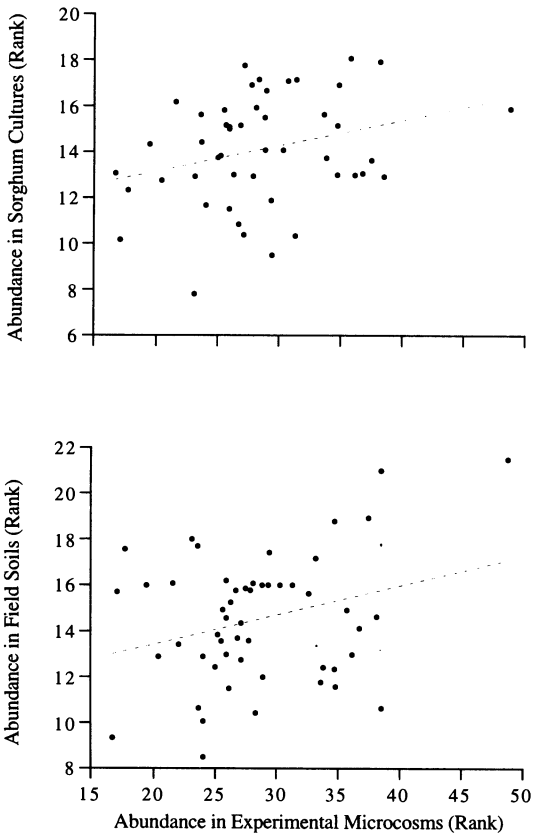

Fig. 3 Regression of ranks of number of spores of AM fungi in association with four plant hosts from (a) sorghum traps and (b) field samples against the corresponding ranks from an experimental microcosm. Each point represents the mean rank of spore numbers of a fungal species in association with a plant species. The lines were derived by regression with $P < 0.03$ and $P < 0.04$ for the sorghum traps and field samples, respectively. Here, we only include the fungal species which were found to have sporulated differentially on host-plant species in the microcosm experiment, although the results are similar if all fungal species are included.

Because of the perennial habit of the plants (quite long relative to the potential rate of turnover of the fungi), the fungi have a high probability of infecting their optimal host plant in successive generations, thereby expanding the range of conditions in which diversity may be maintained. Moreover, with long lived plants and limited fungal dispersal, host-dependence may maintain AM fungal diversity on a local scale.

An expectation of the hypothesis that host-dependence of fungal population growth rates maintains AM fungal species diversity is that species diversity of fungi will correlate with diversity of VA mycorrhizal plant species. It may not be a coincidence that this study site not only has a high AM fungal species diversity, but also has a stable but diverse plant community (Fowler & Antonovics 1981); many of the previous studies of AM fungal diversity have largely focused on samples taken from single plant species or from agricultural settings where plant species diversity is low. It would be interesting to test such a correlation by using systematic and consistent sampling methods over a wide range of plant communities.

Host-dependent fungal sporulation may also depend upon the season. The plant community at this site is temporally divided into distinct warm and cool season associations (Fowler & Antonovics 1981), with the four plant species used in this study being active during the cool season. These experiments were performed in a glasshouse under the seasonally appropriate cool-weather conditions, perhaps facilitating the detection of host-dependent responses. Identification of similar responses in the field soil as in the glasshouse experiments may have been further facilitated by the sampling of the field soil in spring, at which time fungi active during the cool season may be expected to sporulate. Seasonal dependence has been observed in fungal sporulation (Gemma *et al.* 1989) and in the interaction of plants and AM fungi in another grassland site, a prairie in Kansas, with warm and cool season grasses showing their greatest response to fungi at their respective optimal temperatures (Bentivenga & Hetrick 1991; Hetrick *et al.* 1994).

Host-dependent responses in other organisms have been found to vary between geographical isolates and ecological circumstance (Fox & Morrow 1981; Burdon 1987). Geographical isolates of single species of AM fungi have been found to differ in their responses to edaphic factors (Gilden & Tinker 1981; Stahl & Smith 1984; Louis & Lim 1988; Boerner 1990; Stahl *et al.* 1990; Stahl & Christensen 1991), but ecotypic specialization of AM fungi on host species has not been investigated. It is interesting that in contrast to this study, Sanders & Fitter (1992) did not find high sporulation rates of *Scutellospora calospora* on *Plantago lanceolata*, in a seminatural grassland community in England. Distinct patterns in geographically distinct populations and differing ecological circumstances suggests that host-dependence may not be a species-level characteristic in AM fungi.

The sorghum traps, transplant traps, and direct counts of spores in the field all showed evidence of spatial structure in the AM fungal community at the relatively small scale of a few metres. Similar small-scale differentiation was observed in a detailed study of a sand dune by Friese & Koske (1991). Other investigations had previously documented differentiation of the AM fungal community across broader scales (Walker *et al.* 1982; Hetrick & Bloom 1983; Sylvia 1986; Koske & Tews 1987; Koske 1987; Johnson *et al.* 1991b). Such small scale differentiation may be the result of local variation in edaphic factors or in vegetation composition; such effects were not assessed directly in the present study but previous investigations in the same field had shown small scale variation in both these features (Fowler & Antonovics 1981; Antonovics *et al.* 1988; Moloney 1988).

The combined results of the four experiments in this study indicate that caution must be exercised in characterizing AM fungal species diversity. As has been noted previously (Anderson *et al.* 1983; Tews & Koske 1986), spores of fungal species have clumped distributions even within a very small area; therefore repeated sampling from apparently similar locations may yield different compositions of fungal species. Furthermore, the host-dependent sporulation rates indicate that the AM fungal diversity detected using trap cultures may depend upon the species of host plant used in the trap. Finally, a single trapping procedure is unlikely to capture all AM fungal species present in the sample, and a diversity of approaches is necessary for more complete descriptions of AM fungal species diversity at a given location.

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