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DIVERGENT PHENOLOGIES MAY FACILITATE THE COEXISTENCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN A NORTH CAROLINA GRASSLAND[1]

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Interest in the diversity of arbuscular mycorrhizal (AM) fungal communities has been stimulated by recent data that demonstrate that fungal communities influence the competitive hierarchies, productivity, diversity, and successional patterns of plant communities. Although natural communities of AM fungi are diverse, we have a poor understanding of the mechanisms that promote and maintain that diversity. Plants may coexist by inhabiting disparate temporal niches; plants of many grasslands are either warm or cool season specialists. We hypothesized that AM fungi might be similarly seasonal. To test our hypothesis, we tracked the sporulation of individual AM fungal species growing within a North Carolina grassland. Data were collected in 1996 and 1997; in 1997, sampling focused on two common species. We found that AM fungi, especially *Acaulospora colossica* and *Gigaspora gigantea*, maintained different and contrasting seasonalities. *Acaulospora colossica* sporulated more frequently in the warm season, but *Gi. gigantea* sporulated more frequently in the cool season. Moreover, AM fungal species were spatially aggregated at a fine scale. Contrasting seasonal and spatial niches may facilitate the maintenance of a diverse community of AM fungi. Furthermore, these data may illuminate our understanding of the AM fungal influence on plant communities: various fungal species may preferentially associate with different plant species and thereby promote diversity in the plant community.

Key words: coexistence; mutualism; mycorrhizae; niche partitioning; seasonal patterns; spatial patterns; sporulation; VAM (vesicular-arbuscular mycorrhizal) fungi.

Approximately 95% of the world's plant species belong to families that are characteristically mycorrhizal (Smith and Read, 1997); interest in the diversity of fungal communities is increasing because of a growing understanding of the impact of fungal diversity on plant communities. Arbuscular mycorrhizal (AM) fungi inhabit the roots of plants and provide phosphorous, and perhaps other benefits, to plants in exchange for photosynthetically derived carbon compounds. AM fungi are entirely dependent on plant hosts; fungi cannot be cultured axenically. Plant populations and communities are strongly influenced by AM fungi: fungi may increase or decrease plant fitness (Koide, Shumway, and Mabon, 1994; Francis and Read, 1995), regulate inter- or intraspecific competition (Fitter, 1977; Hartnett et al., 1993; Moora and Zobel, 1996), and direct successional patterns (Gange, Brown, and Farmer, 1990; Allen, 1991). A number of studies have demonstrated that species of fungi cannot be substituted for each other within an experiment or habitat (Johnson et al., 1991; Streitwolf-Engel et al., 1997; van der Heijden et al., 1998a); for example, *Glomus macrocarpum* causes tobacco stunt disease (Modjo and Hendrix, 1986), while *Glomus fasciculatum* has no effect on tobacco growth (Modjo and Hendrix, 1986) and *Gigaspora margarita* enhances tobacco growth (Csinos, 1981). Because species of fungi are ecologically distinct, the diversity of an AM fungal community can have large effects on plant population and community dynamics (van der Heijden et al., 1998b), and for this reason, factors that influence the diversity of fungal communities are of interest to plant ecologists. Although AM fungal communities of natural habitats are diverse (e.g., Morton, Bentivenga, and Bever, 1995; Bever et al., 1996; Stutz and Morton, 1996), the specific mechanisms by which coexistence of diverse groups of fungi is facilitated are unknown.

Coexistence might be explained by the partitioning of various biotic or abiotic resources. Although associations between plants and AM fungi appear to be nonspecific, a number of recent studies have demonstrated that the population growth rates of fungal species are dramatically affected by the species of plant with which they are associated (Johnson, Tilman, and Wedin, 1992; Sanders and Fitter, 1992a; Bever et al., 1996; Eom, Hartnett, and Wilson, 2000); this host-specificity in AM fungal response might promote the coexistence of AM fungi. For example, the AM fungal species *Acaulospora colossica* (called A. D1 in Bever et al., 1996) sporulates profusely with the plant *Allium vineale* but not with *Plantago lanceolata*; in contrast, *Scutellospora calospora* sporulates well with *P. lanceolata* but poorly with *A. vineale* (Bever et al., 1996). Although both *Ac. colossica* and *S. calospora* are capable of infecting both *A. vineale* and *P. lanceolata*, the benefits to the fungi are variable; variable benefits might promote the coexistence of AM fungi within a diverse plant community. Coexistence might also be facilitated by the interaction of AM fungi with pathogens. Arbuscular mycorrhizal fungi are commonly infected by other fungi (Daniels and Menge, 1980; Lee and Koske, 1994a; Rousseau et al., 1996) or actinomycetes (Lee and Koske, 1994a) and variability in infection susceptibility might promote coexistence. In addition environmental parameters, for example, soil phosphorus levels (Miranda and Harris, 1994a, b) or pH (Clark, 1997), may provide different

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species of AM fungi with unique niches (see also Johnson, Tilman, and Wedin, 1992; Klironomos et al., 1993; Schultz, 1996).

The coexistence of AM fungal species might also result from fungal partitioning of temporal resources; for example, a fungus that grows in spring will not compete with a fungus that grows in fall. Temporal niche partitioning requires that fungi have distinct seasonalities. The coexistence of plant species is often facilitated by plants' heterogeneous seasonalities; for example, Fowler and Antonovics (1981) documented two seasonal plant guilds within a North Carolina grassland. Species such as *Allium vineale* and *Veronica arvensis* dominate in the cool season, but species such as *Cynodon dactylon* and *Paspalum* spp. dominate in the warm season. AM fungi may demonstrate an analogous seasonality, and disparate seasonal phenologies may contribute to the maintenance of a diverse community of AM fungi.

Moreover, the coexistence of AM fungal species may be facilitated by the fine-scale spatial structure of fungal communities. As the aggregation of species distributions increases, AM fungi are increasingly likely to compete against individuals of the same species, thereby intensifying intraspecific competition relative to interspecific competition. This dynamic may facilitate the coexistence of competing fungal species (Pacala, 1986). In fact, previous studies indicate that AM fungal communities are spatially clumped (Friese and Koske, 1991; Bever et al., 1996).

We investigated the seasonality of AM fungi within the same North Carolina grassland explored by Fowler and Antonovics (1981). The site supports a particularly diverse group of fungi; to date, 37 species have been recorded from approximately 1.5 ha (Bever et al., 1996; Schultz, 1996; J. D. Bever and P. Schultz, Indiana University, unpublished data). Host-specificity in fungal population growth rates may contribute to the maintenance of fungal diversity (Bever et al., 1996); in addition, the high diversity of AM fungi might result from a temporal partitioning of the habitat. In fact, prior sampling suggests that fungi differ in their seasonalities: Schultz, Bever, and Morton (1999) observed that *Acaulospora colossica* sporulates in spring, but other species, particularly *Gigaspora gigantea*, are observed to sporulate in winter (Schultz, 1996). In this study, we aimed to rigorously test for the distinct seasonalities of *Ac. colossica*, *Gi. gigantea*, and other species of AM fungi within the North Carolina grassland. We focused on temporal patterns; however, the same data were used to compare the fine-scale spatial arrangements of collected AM fungi.

MATERIALS AND METHODS

The study site—The habitat is a well-studied (Fowler, 1978; Clay, 1982; Kelley, 1985; Moloney, 1986; Bever, 1992; Schultz, 1996) old field on the campus of Duke University, Durham, North Carolina, USA. Regular mowing over the last 50 yr has halted succession and kept the field as a species-rich grassland. Although certain plant species appear to dominate at specific times of year (e.g., *Anthoxanthum odoratum* in spring), careful observation reveals a mosaic of common plants that belong to a diversity of families (Fowler and Antonovics, 1981). Soil is a sandy loam of the White Store series (Fowler and Antonovics, 1981). Plants are divided into cool and warm season communities; cool season plants are active in fall, winter, and spring, and warm season plants are active in the spring, summer, and fall (Fowler and Antonovics, 1981). The AM fungal community within the field is diverse: a variety of intensive collecting and trapping techniques have revealed 37 morphospecies (Bever et al., 1996; Schultz, 1996; J. D. Bever and P. Schultz, Indiana University, unpublished data), and these fungi are known to be ecologically distinct (Bever et al., 1996; Schultz, 1996; Pringle, 2001).

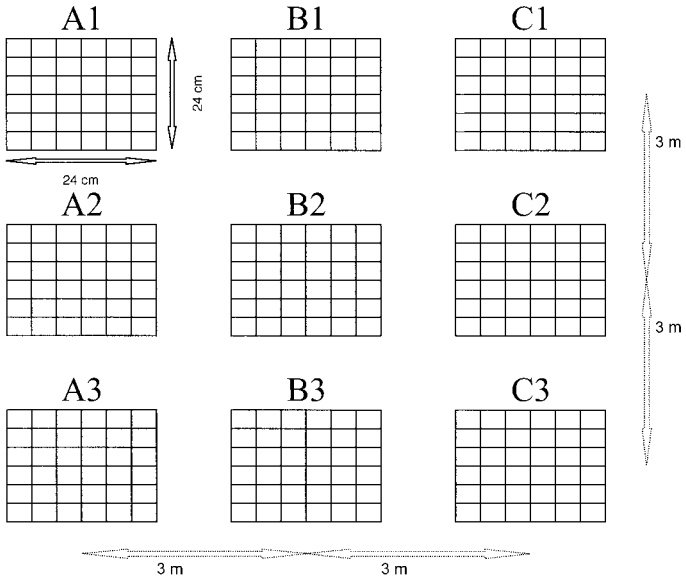

Fig. 1. Sampling design.

Experimental approach and design—Arbuscular mycorrhizal fungi were sampled from an area of the field with a particularly rich fungal community; the site is known to maintain abundant populations of both *Acaulospora colossica* and *Gigaspora gigantea* (Schultz, 1996). Our goal was to evaluate the seasonality of these, and other, fungal species by enumerating spore densities through time.

Numbers of healthy spores are likely to reflect the past physiological activity of an AM fungus. Sporulation can be correlated to the prior growth of a fungus within plant roots (Douds and Schenk, 1990; Gazey, Abbott, and Robson, 1992; see also Abbott and Gazey, 1994), and in agricultural systems, sporulation is associated with plant senescence (see references in Gemma, Koske, and Carreiro, 1989). This evidence suggests that spores form during the final stages of an association between plant and fungus. While this pattern may vary between fungal species, spore counts of *Ac. colossica* and *Gi. gigantea* are likely to reliably track periods of prior physiological activity. *Acaulospora colossica* is closely related to *Ac. laevis* (Schultz, Bever, and Morton, 1999), and *Ac. laevis* has been shown to sporulate following periods of physiological activity (Gazey, Abbott, and Robson, 1992). Moreover, Abbott and Robson (1981) found that mycorrhizae of *Ac. laevis* were ineffective at colonizing new hosts following sporulation. Healthy spores are ephemeral in nature: spores are parasitized or germinated within months of creation. Lee and Koske (1994b) have estimated that spores of *Gi. gigantea* are viable for approximately 5 mo in the field. Therefore, fluctuations in numbers of healthy spores should reflect prior periods of physiological activity, and greatest spore densities should follow peak periods of physiological activity.

Samples were collected from an approximately 36-m^2 site, which represents a relatively small portion of the 1.5-ha field. By minimizing the size of the site, we intended to minimize spatial variability. Plots within the site were arranged in three rows of three plots each (Fig. 1). The nine plots were sampled every other month during the first year. To evaluate the consistencies of *Ac. colossica* and *Gi. gigantea* seasonalities, a subset of the nine sites was monitored in greater detail during the second year.

Sampling in year 1 (1996)—Spores were collected from nine plots within the site on the first day of February, April, June, August, October, and December. Each month, a 24 × 24 cm grid was placed at each plot. The grid was subdivided into 36 4 × 4 cm squares (Fig. 1). Three 2 cm diameter soil cores were taken to a depth of 7 cm from three randomly chosen squares at

each of the nine sites. Soil cores were pooled and stored in sealed plastic bags at 4°C until they could be processed, normally within a month of collection. Immediately prior to spore extraction, soil from a single point was chopped and mechanically homogenized. Spores were extracted from a 50-cm^3 subsample of homogenized soil using a modified sucrose-centrifugation technique (Bever et al., 1996). Spores were identified to species based on spore wall characters using reference cultures from this site (Bever et al., 1996) and from INVAM (International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi; Morton, Bentivenga, and Wheeler, 1993). Essential features were noted for morphotypes that appeared to be distinct from any published species descriptions. Permanent slide vouchers of all species are available upon request from the authors.

Total numbers of healthy spores were recorded for each species. One species creates two kinds of spores, and the different morphologies were tracked individually and are called *Archaeospora leptoticha* and *Glomus leptotichum* (after Morton and Redecker, 2001). A dissecting scope was used to count every spore of the subsample for most species; however, estimates were used to approximate the total number of the small and usually numerous spores of *Acaulospora morrowiae* (referred to as *Ac. mellea* in Bever et al., 1996). We estimated the total number of *Ac. morrowiae* in a subsample by counting the number of spores in 40 randomly located optical squares (2.5 × 2.5 mm). Spores were judged to be healthy if they were completely filled with lipid droplets and devoid of parasitism.

Spores of *Gi. gigantea* were further categorized as either ''new'' or ''old'' on the basis of color (Lee and Koske, 1994b); ''old'' spores are likely to be several months old. ''New'' spores of *Gi. gigantea* are bright yellow-green, but ''old'' spores are dull yellow or yellow-brown. Note that only spores with lipid contents were counted, i.e., dead spores were not counted. Differentiating ''new'' from ''old'' *Gi. gigantea* spores allowed us to more accurately judge the timing of sporulation for this species.

Sampling in year 2 (1997)—Results of sampling in year 1 confirmed that spores of *Gi. gigantea* and *Ac. colossica* were abundant at the study site, were distinctly seasonal, and had contrasting seasonalities. In year 2, sampling focused on *Gi. gigantea* and *Ac. colossica*. However, because of a large and consistent effect of plot identity on spore densities, in the second year sampling was adjusted to more formally evaluate the interaction between spatial and seasonal patterns. Cores were not homogenized; instead, spores were extracted and counted from 25 cm^3 of soil of each core. By evaluating individual cores, we were able to test for the constancy of seasonal patterns within plots. In 1997, only plots A1, A3, B2, C1, and C3 were sampled (Fig. 1). Two 2 cm diameter soil cores were taken to a depth of 7 cm from two randomly chosen squares at each plot. Otherwise, methods were as described above.

Analyses—Only those species that were relatively common (found at every date and in most plots) were included in statistical analyses of spore densities (Table 1). To improve the normality of distributions and homogeneity of variances, spore counts of each species were ranked; further analyses were completed with ranked counts (as in Bever et al., 1996). Multivariate analysis of variance (MANOVA) was used to test for general seasonal and spatial effects (completed with the general linear models procedure; SAS Institute, 1988). Subsequently, a multivariate profile analysis was used to test the variability of fungal species' seasonal and spatial patterns (as developed in Bever et al., 1996). The profile analysis specifically asks whether fungal species display different seasonalities and for this reason, the analysis directly tests the essential question of our study. Finally, significant multivariate effects were further dissected using univariate analyses of variance (ANOVA) on ranked counts of the individual species.

The 1996 data for sites A1, A3, B2, C1, and C3 were combined with the 1997 data to evaluate the similarity of seasonal differences between *Ac. colossica* and *Gi. gigantea* across years. To test whether the seasonalities of the two species contrasted across both years, we used a multivariate profile analysis with the profile × date × year interaction as the error term. The analogous test was used to contrast the spatial patterns in both years. Subsequent to each multivariate analysis, ANOVAs were used to evaluate individual patterns of the two species. ANOVAs allowed us to treat year as a random effect, and therefore, we were able to test the generality of seasonal patterns across both years.

In the analyses described above, counts of new and old *Gi. gigantea* spores were added to give a single value for the total number of spores at each date in each plot. To confirm the seasonal patterns of *Gi. gigantea* that we had inferred from the total spore counts, we tested for seasonal differences in the densities of new and old spores. We predicted that the proportion of new spores would be highest after the fungi sporulate (while spore numbers are increasing) and lowest when fungi do not sporulate (and new spores develop into old spores). We tested our hypothesis by analyzing the effects of date and plot on the proportion of new spores (number of new spores divided by the total number of spores) with MANOVA, as described above, using data from 1996. A subsequent analysis evaluated the similiarity of seasonal patterns in 1996 and 1997 using ANOVA, as described above.

TABLE 1. Sporulation data (1996).

Species	Total number of spores (all dates and sites)	Dates found (of 6)	Sites found (of 9)
Common			
Acaulospora colossica	639	6	9
Scutellospora calospora	579	6	9
Gigaspora gigantea	577	6	9
Archaeospora leptoticha[a]	207	6	4
Acaulospora morrowiae[b]	164	6	7
Glomus clarum	160	6	9
Scutellospora pellucida	98	6	9
Infrequent			
Glomus leptotichum[a]	40	2	3
Acaulospora laevis	16	4	6
Scutellospora reticulata	13	5	1
Scutellospora fulgida	10	2	3
Acaulospora ''copper''[c]	7	3	1
Glomus ''tan''[d]	7	3	2
Scutellospora ''erythropa-like''[c]	5	2	2
Rare			
Acaulospora ''gerdemannii-like''[f]	5	1	1
Acaulospora lacunosa	2	1	1
Scutellospora heterogama	1	1	1
Scutellospora gregaria	1	1	1

[a] Names describe two morphologies of the same species (Morton and Redecker 2001).
[b] Identified as *Acaulospora mellea* in Bever et al. (1996).
[c] Undescribed species: see description for *Acaulospora* sp. D3 in Bever et al. (1996).
[d] Undescribed species: see description for *Glomus* sp. D3 in Bever et al. (1996).
[e] Appeared to be *Scutellospora erythropa*, however, the near-opaque color of the spore wall hindered identification and characterization of the three flexible inner walls.
[f] Undescribed species: spores are white and approximately 120 μm in diameter; a reticulate spore wall appears to slough with age.

RESULTS

Numbers of AM fungal species (1996)—Spores of 17 species were collected from our site within the old field (Table 1). Three of the species are undescribed (Table 1). The majority had been previously observed within the site by Bever et al. (1996) and Schultz (1996). Only seven of the species occurred frequently enough to include in statistical analyses (Table 1).

Seasonal patterns of the common species (1996)—Sporulation was distinctly seasonal, and seasonal differences between species were significant. Spore counts varied signifi-

TABLE 2. Effects of sampling date and site (1996 data), multivariate analysis (MANOVA).

Effect of	Numerator df	Denominator df	Wilks' lambda	*F*
Sampling date	35	141	0.115	2.72***
Site	56	183	2.17×10^{-3}	6.93***

*** $P \leq 0.0005$.

cantly with date, as indicated by the multivariate analysis of variance (Table 2). Seasonality is the result of differences in sporulation patterns between fungal species, as tested by the seasonal term within the multivariate profile analysis (interaction of species' profiles with sampling date: Table 3; Fig. 2). The latter analysis demonstrates that AM fungal species displayed contrasting seasonal patterns.

For example, spores of *Ac. colossica* were significantly more common in the warm season (Table 4); four times as many spores were recovered in June than in either February or December (Fig. 2A). In contrast, spores of *Gi. gigantea* were significantly more common in the cool season (Table 4); half as many spores were found in June vs. either February or December (Fig. 2A). While spore densities of *S. calospora* varied significantly with date (Table 4), the fungus was not an exclusively warm or cool season sporulator. Rather, sporulation of *S. calospora* peaked at both the end of spring and the end of summer (Fig. 2A). However, spores of *S. calospora* appeared to be most common in the warm season (Fig. 2A). Spore densities of *Archaeospora leptoticha*, *Acaulospora morrowiae*, and *S. pellucida* appeared to rise and fall in concert (Fig. 2C); these spores were common in February and declined in number through August, but were common again in October and December. However, seasonal trends were significant for *S. pellucida* only (Table 4). Densities of *Glomus clarum* spores increased throughout the year (Fig. 2C); seasonal trends were significant (Table 4).

Seasonal patterns of Ac. colossica and Gi. gigantea (1996 and 1997)—Contrasting and seasonal sporulation patterns were recorded in both 1996 and 1997. The United States' National Climatic Data Center (www.ncdc.noaa.gov) recorded 142.21 cm of rain in Durham, North Carolina in 1996, and 103.71 cm of rain in 1997. Differences in precipitation were concentrated in 1 mo; in September 1996, Hurricane Fran brought 42.27 cm of rain to Durham. In September 1997, only 7.8 cm of rain were recorded in Durham. Despite the very different rainfall patterns in 1996 and 1997, seasonal sporulation patterns of *Ac. colossica* and *Gi. gigantea* were consistent in 1996 and 1997. Patterns were first evaluated by testing for a seasonal profile effect (profile × date) using the interaction between the seasonal profile effect and year (profile × date × year) as the error term (as described in MATERIALS AND METHODS; Wilks' lambda $F_{5,5} = 9.39$ and $P < 0.014$);

TABLE 3. Effects of sampling date and site (1996 data), multivariate analysis (Multivariate Profile Analysis).

Interaction of species' profiles	Numerator df	Denominator df	Wilks' lambda	*F*
With sampling date	30	138	0.179	2.47***
With site	48	171	5.95×10^{-3}	6.54***

*** $P \leq 0.0005$.

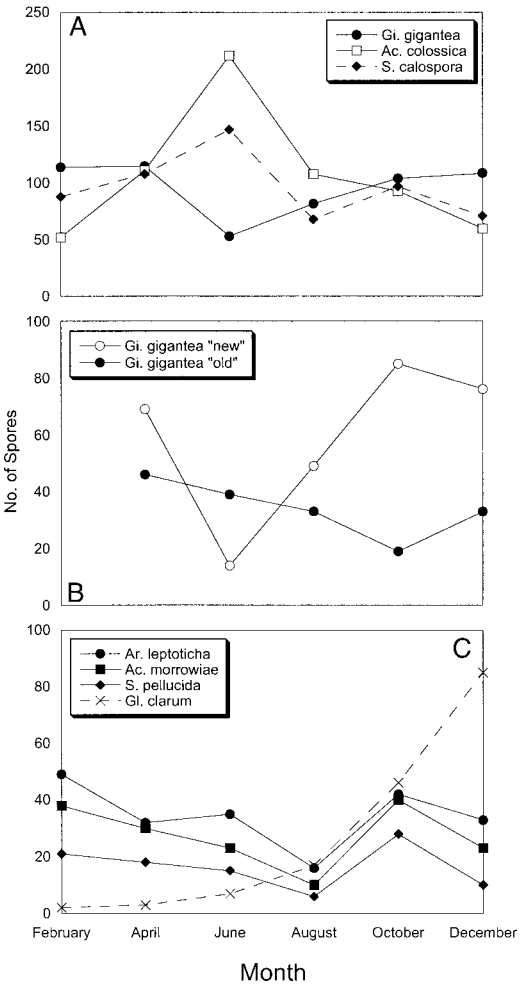

Fig. 2. Seasonal patterns of arbuscular mycorrhizal fungal sporulation, 1996. Number of spores is the total number of spores counted in a given month from nine sites (450 cm^3 of soil). (A) Seasonality of *Gigaspora gigantea*, *Acaulospora colossica*, and *Scutellospora calospora*. (B) Seasonality of *Gigaspora gigantea* "new" and "old" spores. (C) Seasonality of *Archaeospora leptoticha*, *Acaulospora morrowiae*, *Scutellospora pellucida*, and *Glomus clarum*.

the analysis demonstrated that *Ac. colossica* and *Gi. gigantea* maintained contrasting seasonalities across the two years. In individual ANOVAs, date proved to be a strong and significant effect for each species (*Ac. colossica*, $F_{5,44} = 4.89$, $P < 0.0012$; *Gi. gigantea*, $F_{5,44} = 2.45$, $P < 0.049$), demonstrating

TABLE 4. Effects of sampling date and site (1996 data), individual analyses (ANOVA).

AM fungal species	Sampling date, *F*†	Site, *F*††
Acaulospora colossica	3.13*	3.05*
Acaulospora morrowiae	1.78	7.01***
Archaeospora leptoticha	1.81	21.36***
Glomus clarum	4.29**	1.51
Gigaspora gigantea	2.82*	11.15***
Scutellospora calospora	2.42*	13.95***
Scutellospora pellucida	3.45*	6.24***

* $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.0005$.
† df = 5, error df = 39.
†† df = 8, error df = 39.

that seasonal patterns were strong across these two years. In order to test whether seasonal trends are predictable, we treated year as a random effect and tested the seasonal patterns by using the interaction of date and year as an error term. In this more conservative analysis, date remained a significant predictor of *Ac. colossica* sporulation ($F_{5,5} = 5.81$, $P < 0.038$), but not of *Gi. gigantea* sporulation ($F_{5,5} = 2.26$, $P < 0.20$). For both species, the numbers of spores were not significantly different in the same month of different years (*Ac. colossica*: interaction of date and year, $F_{5,21} = 0.84$, $P < 0.53$; *Gi. gigantea*: interaction of date and year, $F_{5,21} = 1.08$, $P < 0.38$).

Seasonal patterns of new and old Gi. gigantea spores (1996 and 1997)—By comparing the seasonalities of new and old *Gi. gigantea* spores, we were able to confirm that *Gi. gigantea* sporulates in the cool season. In 1996, new spores were common in the cool months, but were rare in June (Fig. 2b). Older spores were consistently found in each month (Fig. 2b). Numbers of new *Gi. gigantea* spores varied significantly with date ($F_4 = 5.80$, $P < 0.0013$), but numbers of old *Gi. gigantea* spores did not ($F_4 = 0.92$, $P < 0.46$). Furthermore, ratios of new to old *Gi. gigantea* spores differed significantly with date ($F_4 = 4.15$, $P < 0.0083$) and peaked in the cool months (Fig. 2b). By combining data for 1996 and 1997, we tested the consistency of the seasonal patterns across both years, again treating year as a random effect and using the interaction of date and year as an error term (as described above). In contrast to the results for total numbers of *Gi. gigantea* spores, date was a significant predictor of the ratio of new to old spores across years ($F_{4,4} = 7.90$, $P < 0.034$), even under this conservative test. Furthermore, ratios of new to old spores were not significantly different in the same month of different years, as tested by ANOVA (interaction of date and year, $F_{4,33} = 0.32$, $P < 0.87$).

Spatial patterns (1996 and 1997)—The seven common species displayed contrasting spatial distributions in 1996. The overall spatial effect on spore counts was highly significant in the multivariate analysis of variance (Wilks' lambda $F_{56,183} = 6.93$, $P < 0.0001$), with much of this effect being due to the consistent differences in spatial patterns between the fungal species as tested by profile analysis (interaction of species' profiles with site: Wilks' lambda $F_{48,171} = 6.54$, $P < 0.0001$). Spores of *Ac. colossica*, *Gi. gigantea*, *S. calospora*, and *S. pellucida* were extracted from every site (Fig. 3); however, analyses of the individual data sets indicated that spores were significantly more common in some sites versus others (Table 4). Both *Ac. colossica* and *Gi. gigantea* were particularly abundant at sites A3 and C3 (Fig. 3). *Archaeospora leptoticha* and *Acaulospora morrowiae* displayed significant spatial patterns (Table 4). Spores of *Ar. leptoticha* were generally restricted to sites C3 and C2 (Fig. 3) (as were spores of *Glomus leptotichum*). *Acaulospora morrowiae* spores were scattered throughout B and C sites, but were particularly common at site B2 (Fig. 3). *Glomus clarum* demonstrated no spatial pattern (Table 4); although a disproportionate number of spores appear to have been found at site A3 (Fig. 3), the data are biased by the exceptional number of spores found from this site in one month (44 of 160 total spores, December 1996). As the spatial pattern was not consistent through time, it was not significant.

Spores of infrequent and rare species were found in low numbers and the data were not amenable to statistical analyses,

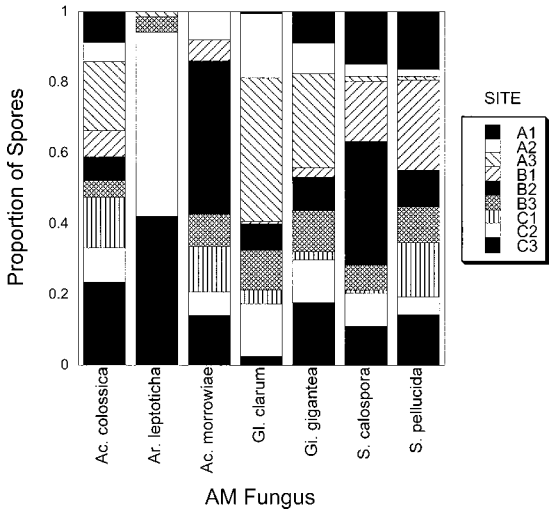

Fig. 3. Spatial patterns of arbuscular mycorrhizal (AM) fungal sporulation, 1996. The patterns of each bar illustrate the proportion of species' spores collected from each site. Proportions can be understood as follows, e.g., of every 100 *Acaulospora colossica* spores approximately 22 were found at site C3.

however, species were spatially structured. For example, spores of *S. reticulata* were found in five months and in each month the spores were extracted from a single site (Table 1). Similarly, spores of the undescribed species *Acaulospora* ''copper'' were restricted to a single site (Table 1).

Although spores of *Gi. gigantea* were found at the same sites in 1996 and 1997, spores of *Ac. colossica* were not. The test for a spatial profile effect (profile × site) using the interaction between the spatial profile effect and year (profile × site × year) as the error term (as described above) was not significant (Wilks' lambda $F_{4,4} = 2.46$, $P < 0.20$); *Ac. colossica* and *Gi. gigantea* did not maintain contrasting spatial distributions across the two years. This is probably a result of the shifting spatial patterns displayed by *Ac. colossica*: when year was treated as a random effect (as described above) spatial patterns proved to be predictable for *Gi. gigantea* ($F_{4,4} = 13.09$, $P < 0.014$) but not for *Ac. colossica* ($F_{4,4} = 3.97$, $P < 0.11$).

DISCUSSION

Seasonal patterns—Arbuscular mycorrhizal fungal species were found to have contrasting and seasonal phenologies (Tables 2 and 3). The contrast is especially obvious when the sporulation of *Ac. colossica* is compared to the sporulation of *Gi. gigantea*; *Ac. colossica* spores were most abundant in the warm months of summer, but *Gi. gigantea* spores were common in the cool months of winter (Fig. 2). Seasonalities were consistent across the two years of this study, despite the very different patterns of precipitation in 1996 versus 1997. Differences between *Ac. colossica* and *Gi. gigantea* were also consistent with seasonal patterns recorded in previous years at this site (Schultz, 1996; Schultz, Bever, and Morton, 1999). The seasonal differences in spore densities probably reflect seasonal differences in spore formation. As detailed in MATERIALS AND METHODS (Gemma, Koske, and Carreiro, 1989; Douds and Schenk, 1990; Gazey, Abbott, and Robson, 1992), sporulation follows periods of physiological activity. Therefore, our data suggest that *Ac. colossica* is physiologi-

cally active in the cool season, while *Gi. gigantea* is physiologically active in the warm season.

Our data are supported by several additional lines of evidence. *Gigaspora gigantea* is easy to culture in a warm season greenhouse (Schultz, 1996; personal observation), but *Ac. colossica* is very difficult to culture in a warm season greenhouse (Schultz, Bever, and Morton, 1999). In contrast, *Ac. colossica* sporulates profusely in a cool season greenhouse (Bever et al., 1996). Furthermore, when grown in a cool season greenhouse, *Ac. colossica* sporulates abundantly with a markedly cool season host (*Allium vineale*). *Gigaspora gigantea* sporulated poorly with this cool season host (Bever et al., 1996); however, *Gi. gigantea* is easily cultured with the tropical grass *Sorghum sudanense*. Finally, developmental evidence suggests that *Ac. colossica* sporulates in late spring. *Acaulospora colossica* sporulates by developing a thin-walled saccule on the terminal branch of a fertile hypha; each saccule forms a spore. After spores are formed, the saccule degrades or breaks off (Schultz, Bever, and Morton, 1999); in this study, saccules were only found in April (personal observation). Our data strongly imply that *Ac. colossica* is physiologically active in the cool season and lasts through the summer as a dormant spore. Conversely, *Gi. gigantea* is likely to be physiologically active in the warm season and over-winter as a dormant spore.

Populations of *Gi. gigantea* in coastal sand dune communities of the eastern United States also sporulate in late fall and winter. Gemma and Koske (1988) recorded peak numbers of *Gi. gigantea* spores in late fall (November and December); Lee and Koske (1994b) recorded peak numbers of healthy *Gi. gigantea* spores in winter (January and February). To our knowledge, the sand dunes of Rhode Island and grasslands of North Carolina have no species of plant in common. The climate of the two habitats is also very different. The concordance of data across different communities suggests that the seasonality of *Gi. gigantea* is a species-level property.

While our study was designed to document seasonal differences between *Ac. collosica* and *Gi. gigantea*, we found that most of the common fungi were significantly seasonal (Table 4). Many of these fungi seemed to sporulate in the fall (e.g., *S. pellucida*). However, *S. calospora* appeared to sporulate in the spring as well as the fall, and spores of *Gl. clarum* were most common in winter.

Aseasonal species may be cryptically seasonal. The data are observational and a conservative measure of AM fungal seasonality. For example, AM fungal species may experience differential parasitism or predation in nature: the spores of a palatable species will be ephemeral and the species' seasonality obvious; the spores of a resistant species will persist in the soil. The second species may appear aseasonal.

The unusual exponential pattern of *Gl. clarum* sporulation may have been caused by a hurricane that swept through our field site on 6 September 1996. The sporulation of *Gl. clarum* increased dramatically in October and December (Fig. 2). In February and April, *Gl. clarum* had been found in a single plot, but in October and December, *Gl. clarum* was recovered from each of the nine plots (data not shown). *Glomus clarum* may have responded to the extraordinary amounts of rain associated with the hurricane by invasively sporulating through the site, and perhaps the population of *Gl. clarum* at this site sporulates in association with exceptional amounts of precipitation.

The seasonality of AM fungal sporulation in natural habitats has been documented in only a few studies. In contrast, a number of classic papers explore temporal patterns of fungal sporulation in agricultural systems (Hayman, 1970; Sutton and Barron, 1972; Saif, 1977; Rich and Schenck, 1981); a majority of these studies record general increases in the sporulation of all species as crops mature and are harvested. Although work by Merryweather and Fitter (1998) indicated that spores of AM fungi within sycamore- or oak-dominated woodlands are aseasonal, studies by Sylvia (1986), Gemma, Koske, and Carreiro (1992), and Stürmer and Bellei (1994) in maritime dune systems of Florida, Massachusetts, and Santa Catarina Island (Brazil) suggested distinct seasonalities for different species of fungi within a single habitat. A study by Sanders and Fitter (1992b) of root infection also demonstrated that mycorrhizal colonization of plants might be temporally variable, although in this study, fungi were not identified to species. Distinct seasonal guilds of AM fungi might be a typical feature of fungal communities.

The distinct seasonalities of AM fungal species suggest a temporal partitioning of plant resources; by specializing on cool or warm season plants, fungi would minimize interspecific competition for roots. In fact, our data suggest that fungi derive variable benefits from their associations with plants. For example, if *Ac. colossica* is a specialist of *Allium vineale* or other cool season plants, it will derive a limited benefit from associations with warm season plants; conversely, *Gi. gigantea* may derive its greatest benefit from associations with warm season plants. The divergent seasonalities of *Ac. colossica*, *Gi. gigantea*, and other fungi may facilitate the coexistence of a diverse group of AM fungal species within the North Carolina grassland. Our data also provide additional evidence for the distinct ecologies of fungi that coexist within a single site (e.g., Bever et al., 1996; Van der Heijden, 1998a).

Matching seasonalities of plants and AM fungi might reflect a level of specificity between plants and fungi. In fact, three lines of correlational evidence are suggestive of specificity between *Ac. colossica* and the cool season plant *Allium vineale*. First, *Ac. colossica* and *A. vineale* are both physiologically active during the cool season and dormant over the warm season. The peak sporulation period of *Ac. colossica* coincides with the period in which *A. vineale* dies back into its bulb (Fowler and Antonovics, 1981). Second, as mentioned above, *Ac. colossica* sporulates preferentially with *A. vineale* when growth of *Ac. colossica* is compared in *A. vineale* and other cool season hosts (Bever et al., 1996). Finally, the distributions of *Ac. colossica* and *A. vineale* are positively correlated in the field (Schultz, 1996). Specificity between *Ac. colossica* and *A. vineale* could generate a positive feedback dynamic in which a high initial abundance of *A. vineale* would increase the abundance of *Ac. colossica*, thereby increasing the relative success of *A. vineale* (Bever, Westover, and Antonovics, 1997; Bever, 1999). However, a demonstration of this dynamic will require further experimentation.

Spatial patterns—Fine-scale spatial patterns were recorded for both common (Tables 2 and 3) and infrequent species. For example, the common fungus *Archaeospora leptoticha* was generally restricted to two plots (Table 4; Fig. 3); although the rare species *S. reticulata* sporulated in 5 mo, spores were only recovered from a single plot (Table 1). The spatial arrangements of *Ac. leptoticha* and its hypothesized synanamorph, *Glomus leptotichum*, provide further evidence of their formation from a common mycelium (Morton, Bever, and Pfleger, 1997; Morton and Redecker, 2001); although spores of *Gl.*

leptotichum were rare, they were generally collected within the distribution of the more common *Ac. leptoticha*. The spatial structure of plant communities is obvious to ecologists, and this work demonstrates that fungal communities can also be spatially patterned. Fungal communities may be highly variable at a local scale; comparisons of the individual plots of this study show that the community of one site can be very different from the community of a second and nearby site (e.g., compare plots A3 and B3, Fig. 3).

Implications for plant ecology—The coexistence of disparately seasonal and spatial AM fungi illuminates our understanding of the interactions between AM fungi and plants. It suggests a mechanism through which the diversity of an AM fungal community might drive the diversity of a plant community, as has been demonstrated in experimental grasslands of North America and Europe (Van der Heijden et al., 1998b). Our observations suggest that the success of cool and warm season guilds of plants is dependent upon plants' associations with cool and warm season guilds of fungi. The maintenance of a functionally diverse plant community may depend on the coexistence of guilds within the fungal community. Furthermore, spatial patterns within the fungal community might also have direct impacts on plant community diversity and structure. A local community of fungi may control the local composition and diversity of plants (van der Heijden et al., 1998b). Therefore, the fine-scale heterogeneity of the fungal community may increase the regional diversity of the plant community. Small and diverse patches of AM fungi may structure the plant community as a mosaic of plant types, especially if some plants are restricted within the boundaries of a particularly effective symbiont. Clearly, the seasonal and spatial heterogeneity of the fungal community might have strong impacts on plant community processes.

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