Oecologia (2009) 160:807–816
DOI 10.1007/s00442-009-1345-6

COMMUNITY ECOLOGY - ORIGINAL PAPER

Trade-offs between arbuscular mycorrhizal fungal competitive ability and host growth promotion in *Plantago lanceolata*

Alison Elizabeth Bennett · James D. Bever

Received: 29 January 2008 / Accepted: 26 March 2009 / Published online: 18 April 2009

Abstract In this study we tested for trade-offs between the benefit arbuscular mycorrhizal (AM) fungi provide for hosts and their competitive ability in host roots, and whether this potential trade-off shifts in the presence of a plant stress (herbivory). We used three species of AM fungi previously determined to vary in host growth promotion and spore production in association with host plants. We found that these AM fungal species competed for root space, and the best competitor, *Scutellospora calospora*, was the worst mutualist. In addition, the worst competitor, *Glomus* white, was the best mutualist. Competition proved to have stronger effects on fungal infection patterns than herbivory, and competitive dominance was not altered by herbivory. We found a similar pattern in a previous test of competition among AM fungi, and we discuss the implications of these results for the persistence of the mutualism and feedbacks between AM fungi and their plant hosts.

Keywords *Scutellospora calospora* · Feedback · Host growth promotion · *Archaeospora trappei* · *Glomus*

Communicated by Jeremy Burdon.

A. E. Bennett (✉) · J. D. Bever
Indiana University, 1001 E. 3rd Street, Bloomington, IN 47405, USA
e-mail: alibenne@bio.indiana.edu

J. D. Bever
e-mail: jbever@bio.indiana.edu

Present Address:
A. E. Bennett
University of Wisconsin, 237 Russell Labs, 1630 Linden Drive, Madison, WI 53706-1598, USA

Introduction

Both empirical research and theory in ecology and evolution have long suggested that all living organisms pay costs for their activities, and these costs determine how they allocate their resources. Early research focused on trade-offs between growth or survival and reproduction (reviewed in Stearns 1989), but in the last few decades trade-offs have been described between a wide variety of other activities including plant defense and growth (Herms and Mattson 1992), plant defense and tolerance to herbivory (Fineblum and Rausher 1995), and pathogen virulence and spore production (Thrall and Burdon 2003). Trade-offs determine how organisms allocate their resources, and, when resources are scarce, determine the evolutionary trajectories along which organisms may move. Thus, exploring trade-offs allows us to predict evolutionary changes that will occur in organisms given knowledge of the selective forces acting on those organisms.

While trade-offs in plants have received great attention, little attention has been paid to their common mutualists, such as arbuscular mycorrhizal (AM) fungi. When associated with plant roots, AM fungi access sources of phosphorous and other trace minerals unavailable to plants, and in return plants shuttle carbon to the fungi (Smith and Read 1997). Just like plants, AM fungi allocate their resources to many different activities. In order to produce new spores, AM fungi must compete with other fungi for host resources, search for nutrients for hosts and themselves, and defend themselves or tolerate attack by fungivores. There are many species of AM fungi, and their coexistence is likely mediated by variation in ecological attributes (Bever et al. 2001), including resource allocation patterns. In fact, previous research has demonstrated that some fungal species excel at providing host benefits, as measured by

plant growth, while other AM fungal species fail to provide any benefit at all (Johnson et al. 1997). Moreover, previous research has suggested trade-offs in life history characteristics such as extra-radical versus internal hyphal proliferation and colonization strategies (Hart and Reader 2002, 2005).

Do AM fungal species that fail to provide host benefits allocate their resources to other activities, such as competition? Previous studies have reported that competition occurs among AM fungal species (Cano and Bago 2005; Hepper et al. 1988; Pearson et al. 1993, 1994; Sen et al. 1990; Wilson 1984; Wilson and Trinick 1983), and, in some cases, competition between two or more fungi can be strong enough to result in the exclusion of an AM fungal species from host roots (Hepper et al. 1988; Sen et al. 1990; Wilson and Trinick 1983). This competition is most commonly documented as changes in root infection or occupied space within root systems (Cano and Bago 2005; Wilson 1984; Wilson and Trinick 1983). While the relationship between competitive success and plant growth promotion was not tested in these studies, observations that the fungal species that proliferates on *Plantago lanceolata* delivered the least benefit to *P. lanceolata* (Bever 2002a) suggest a negative relationship between competitive success and growth promotion. However, whether fungal proliferation results from a difference in competitive ability of the fungi is unknown. The relationship between mutualist benefit and competitive ability has been most frequently explored in ant–plant mutualisms where competition occurs between ant species that provide different levels of benefit to host plants. In most of these systems the better competitor provided the greatest mutualistic benefit (e.g., Miller 2007; Yu and Pierce 1998). In another microbial system, among three ectomycorrhizal fungal species, the better fungal competitors may have also been the better symbionts, although host benefit varied between competitive and non-competitive settings (Kennedy et al. 2007).

AM fungi, however, are likely to suffer not only from competition, but stresses in their host's environment as well. Both abiotic and biotic stresses are likely to limit plant carbon availability, and limited carbon availability may limit plant allocation to large carbon sinks such as AM fungi. Biotic stresses, like herbivory, have also been shown to influence mycorrhizal infection in the absence of fungal competition (reviewed in Gange 2007; Gehring and Whitham 1994, 2002). For example, insect herbivory has been shown to both reduce (Gange et al. 2002; Wamberg et al. 2003) and increase (Currie et al. 2006; Kula et al. 2005; Mueller et al. 2005; Wamberg et al. 2003) AM fungal colonization. If stresses, like herbivory, result in similar changes in mycorrhizal fungal colonization across species, then we would not expect changes in competitive relationships among fungal species. However, if host stress does not equally impact fungal species, then competitive hierarchies could change, particularly if competitive dominants are more negatively or positively impacted by stress than less competitive species then they may benefit from host stress. Alternatively, if competition among fungal species is the dominant factor determining host colonization by individual fungi, effects of herbivory may be minimal. An examination of plants infected with multiple fungal partners found no change in overall mycorrhizal colonization following herbivory (Gange et al. 2005) suggesting that perhaps fungal competition has stronger effects on mycorrhizal fungal colonization than plant stress on fungal growth. However, this study did not examine changes in infection by individual fungi, thus we can not determine which mechanism is acting. A study in pollinator systems found that a host stress such as herbivory can increase the effectiveness of the pollinator mutualism (Strauss et al. 2001).

The purpose of this study was two-fold: first, to determine whether there is a trade-off between the benefit AM fungi provide for their hosts and their competitive ability in host roots, and second, whether this trade-off shifts in the presence of a plant stress such as herbivory. We chose to focus on herbivory, because plant abiotic stresses vary with environment, but almost every plant in nature encounters herbivory. In addition, the amount, frequency, and severity of herbivory can easily be controlled, and thus it is an excellent way to address how biotic stresses might alter the plant–mycorrhizal mutualism. This study directly quantifies competition among AM fungal species in host roots, and consequently evaluates the relationship between competition and plant growth promotion. We tested these questions using the interaction between *P. lanceolata* and three species of mycorrhizal fungi which have previously been shown to vary in their effect on, and response to, *P. lanceolata* (Bever 2002a), and we subjected half of each treatment to herbivory by *Junonia coenia* (Lepidoptera, Nymphalidae) (common buckeye butterfly) larvae.

Materials and methods

Study system

To answer the proposed questions we worked with the butterfly *Junonia coenia*, its plant host *Plantago lanceolata* (Plantaginaceae), and three AM fungal symbionts of *P. lanceolata*. These species co-occur in an old field on the Duke University campus in Durham, North Carolina (Bever et al. 2001). *J. coenia*, a native butterfly, feeds on members of the Plantaginaceae including *P. lanceolata*. *P. lanceolata* is widely distributed across the United States in old fields, mowed lawns, and disturbed sites. This species is well

defended, containing carbon-based secondary compounds derived from iridoid glucosides (commonly considered to be defensive chemicals) (Bobbitt and Segebarth 1969; Duff et al. 1965). *J. coenia* larvae are specialists on *P. lanceolata* and sequester the iridoid glucosides found in plantain leaf tissues (Bowers and Collinge 1992; Bowers and Puttick 1986).

P. lanceolata, like many plants, associates with AM fungi. Although over 37 species of AM fungi have been isolated from the field at Duke University (Bever et al. 2001), we chose three species [an unidentified species of *Glomus* we call *Glomus* white (Bennett and Bever 2007), *Archaespora trappei*, and *Scutellospora calospora*] based on prior evidence of specificity in growth promotion of *P. lanceolata* and fungal response of these species (Bennett and Bever 2007; Bever 2002a). Association with *Glomus* white and *A. trappei* produces large plants, but *S. calospora* does not promote host growth and may even reduce plant growth (Bennett and Bever 2007). Plants simultaneously associated with all three fungi produce the same amount of biomass as plants associated with only *Glomus* white (Bennett and Bever 2007). However, *S. calospora* experiences a higher population growth rate than the other fungi when grown with *P. lanceolata* (Bever 2002a, b).

Experimental design

The experiment consisted of a factorial design with two levels of herbivory (yes or no) and five different fungal treatments (plants grown with *Glomus* white, *A. trappei, S. calospora* individually, in combination, and with a sterilized combination of spores). Inoculum was obtained from cultures containing spores of *Glomus* white, *A. trappei,* and *S. calospora* (from a mowed old field near Duke University in North Carolina) maintained in the greenhouses on the Indiana University campus. Soil collected from the same North Carolina old field was mixed 1:1 with sand to promote drainage in pots, and sterilized with steam for 2 h at 123°C, allowed to cool for 24 h, and re-sterilized with steam for 2 h at 123°C. Inocula consisted of one-sixth of the volume of the pot, and, given our sole interest in mycorrhizal infection, no microbial wash was added. In order to control for natural variation in mycorrhizal affinity we used seven independent full-sib genotypes of *P. lanceolata* created by mating plants collected from the same North Carolina old field, and four seedlings of each genotype were grown in each treatment producing 28 plants per soil treatment. Plants were arranged in two blocks, and randomized. *J. coenia* larvae were obtained from a colony maintained by Fred Nijhout at Duke University.

One *P. lanceolata* seedling from the seven crosses described above were transplanted into 6-inch pots containing one of the five soil treatments described above. Half the plants in each soil treatment were subjected to three rounds (at weeks 5, 6, and 7) of 20% defoliation (determined by measuring total leaf length of the plant) by *J. coenia* larvae. During herbivory events, larvae were contained within clip cages (petri dish lids held together by hair clips to form cages that easily open and close) throughout the duration of each herbivory event to insure that only 20% of the total leaf area was eaten at each time point. Empty clip cages were placed on plants that did not receive herbivory to control for clip cage effects.

Half the plants were harvested at week 8 in order to examine root colonization. During the first harvest, roots were harvested by washing them over sieves, and stained with Trypan Blue. The number of arbuscules, vesicles, coils, and hyphae were counted to assay the percentage of AM fungal infection. Each of the fungal species belongs to different families which produce morphologically distinct characteristics (for keys to these characteristics see Abbott 1982; Merryweather and Fitter 1998). The hyphae of *Glomus* white are thick, bend frequently, and produce knobs at the bends, while the hyphae of *A. trappei* are much finer and generally stain lightly (Fig. 1). *S. calospora* forms two types of hyphae: a thick hyphae similar to that of *Glomus* (without frequent bends and knobs) and a very thick bulbous hyphae (Fig. 1). Arbuscules of these three genera are also easily distinguishable as *Glomus* produces arbuscules that fill the cell, *A. trappei* produces finer arbuscules that do not typically fill the cell, and *S. calospora* forms arbuscules in which thick hyphae circle the cell as well as filling the cell with finer hyphae. As a result, these mycorrhizal species are easily distinguishable within the root. In the combination treatment plants always hosted all three species in their root system, and occasions when infection by multiple species (coinfection) occurred in the same root section were also recorded. Given that molecular methods could only have told us the identity and, depending on the technique, the amount of fungi located in each root sample, but not where the fungal structures were located, what type of fungal structures were present, or whether there were differences in co-infection at individual sites within roots, we found that morphological methods were a more appropriate tool for this study than molecular methods.

Following the initial harvest, the remaining plants were fertilized with a no-phosphorous fertilizer biweekly beginning in week 13. After 20 weeks of growth the remaining plants were harvested for total, above- and belowground biomass, reproductive biomass (defined as the stem and flower spike), and stem biomass (data reported in Bennett and Bever 2007). Cores from these pots were taken and AM fungal spores were extracted from these cores using sucrose centrifugation. Spores were then counted to assay differences in AM fungal spore density between treatments. Only one species of fungus, *Glomus* white, sporulated.

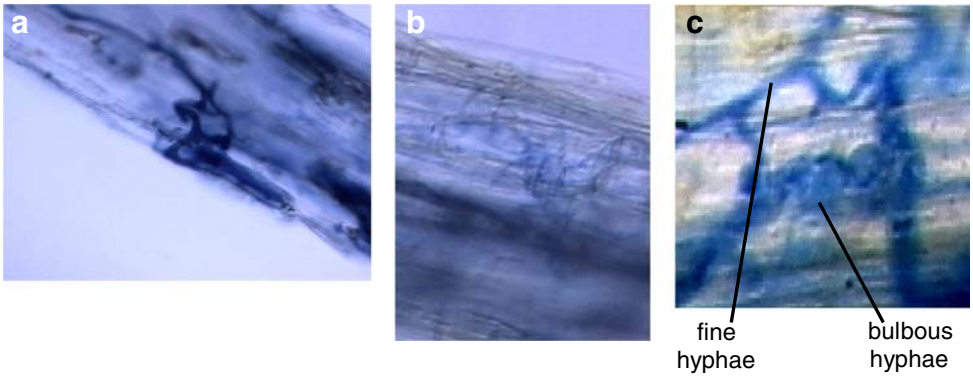

Fig. 1 Micrographs of **a** *Glomus* white, **b** *Archaespora trappei* hyphae, and **c** *Scutellospora calospora* bulbous and fine hyphae

Test of mycorrhizal inoculum potential

We examined each mycorrhizal inoculum potential using sorghum plants grown with each fungal inocula (*Glomus* white, *A. trappei*, *S. calospora*, a mixture of all three fungal species, and a sterilized mixture of all three species) at three different inocula concentrations: half the inocula concentration, equal inocula concentration, and double the inocula concentration present in experimental pots. An analysis of percent root length colonized revealed that mycorrhizal infection levels did not vary based on inocula type ($F_{48,3} = 1.52$, $P = 0.2209$) or the interaction between inocula type and inocula concentration ($F_{48,6} = 0.39$, $P = 0.8812$).

Competitive ability

In order to contrast fungal competitive ability and plant growth promotion we constructed a metric of competitive ability by dividing the percent root infection by a single fungal species in competition with other fungal species by the percent root infection by that same fungal species in the absence of competition. We calculated competitive ability in this manner for both percent root length colonized and percent root length containing resource exchange sites. Growth promotion was calculated for each fungal species as growth with that fungal species minus growth with sterile spores divided by growth with sterile spores.

Statistical analysis and interpretation

Fungal colonization

We analyzed the colonization data in three complementary ways. In the first analysis, we addressed whether herbivory affected AM fungi in the absence of competition (i.e., only between plants associated with one fungal species). This analysis excluded plants associated with all three fungal species. We tested differences in fungal infection due to fungal species, herbivory, and their interaction and block using a mixed model ANOVA with genotype and its interactions as random effects. In this analysis, as well as those that followed, the response variables percent root length colonized by mycorrhizal fungi and percent arbuscules and coils (resource exchange sites) in the total plant root length were arcsin square root transformed to satisfy the assumption of normality. All analyses were conducted in the general linear models procedure of SAS 2000.

In the second analysis, we addressed whether herbivory affected AM fungi in the presence of competition (i.e., between plants only associated with all three fungal species). Given the multivariate nature of the measures of the three species, we first analyzed plants inoculated with all three fungi using mixed model multivariate ANOVAs (MANOVAs) on the overall infection of the three species and the exchange sites of the three species. We followed the MANOVA with corresponding ANOVAs. Again, genotype and genotype×herbivory were identified as random effects.

In the third analysis we tested whether competition with other AM fungal species affected colonization by individual fungal species. This analysis included plants inoculated with both a single species of fungus and that fungal species in mixture with the other two fungal species. To examine this question we performed a mixed model ANOVA for each individual fungal species with genotype and its interactions as random effects, and herbivory, block, and inocula type (sterilized spores, single inocula, or combined inocula) as fixed effects.

Frequency of co-infection

On plants associated with all three species of fungi, we tested whether infection by multiple fungal species (the frequency of coinfection) occurred more or less frequently than expected if infection events were completely independent. In order to answer this question we constructed a metric of the difference in observed rates of coinfection within a plant and the rates of coinfection expected from the products of the respective individual rates of coinfection in that

plant. We tested whether this difference was affected by herbivory using ANOVA. We then use the least squared means and corresponding SEs from the ANOVA to construct *t*-tests to determine if the observed frequencies of multiple infections were significantly greater than expected.

Fungal sporulation

We examined whether herbivory or competition impacted spore production by the fungus *Glomus* white, by performing an ANOVA on the log transformed number of spores per pot with the independent fixed variables block, fungal treatment (mixture of fungi or *Glomus* white alone), and herbivory, and random independent variable plant genotype.

Results

Fungal colonization: species effects

Percent *P. lanceolata* root length colonized varied by mycorrhizal species (Table 1). When fungi were grown alone with *P. lanceolata*, *Glomus* white and *A. trappei* experienced greater levels of root length colonization than *S. calospora* (Fig. 2a). When all three fungi were grown together each fungus experienced a decrease in individual overall colonization relative to when they were alone (Table 3; Fig. 2a).

Fungal colonization: herbivore effects

In plants inoculated with individual fungal species, herbivory decreased the overall colonization and percentage of arbuscules and coils of *A. trappei* and *S. calospora*, but did not affect infection or the percentage of arbuscules and coils of *Glomus* white (Fig. 2b). These results differed from an examination of only roots from plants associated with all three fungal species (Table 2). The MANOVA showed that herbivory influenced overall root infection (Wilks' λ $F_{3,10} = 29.79$, $P < 0.0001$), but not resource exchange sites (Wilks' λ $F_{3,10} = 0.11$, $P = 0.9495$) in plants associated with all three fungal species (Fig. 2). Herbivory did not lead to a decrease in overall colonization of the mixture of all three fungi, or to a decrease in colonization by any fungi in particular. In fact, the combination of herbivory and competition led to an increase in colonization by *A. trappei* (Fig. 2a).

Fungal colonization: competitive effects

There was a significant effect of competition experienced by all three fungal species, and expressed in both percent root length colonized and percent resource exchange sites (Table 3). Repeating the original analysis while excluding herbivory treatments revealed that in the absence of herbivory, both *A. trappei* ($F_{1,18} = 40.46$, $P < 0.0001$) and *Glomus* white ($F_{1,17} = 144.11$, $P < 0.0001$) experienced a decrease in the percentage of arbuscules in competition relative to

Table 1 ANOVA results for percent of root length colonization (*RLC*; arcsin transformed) by mycorrhizae and percentage of arbuscules and coils in roots by individual fungal species infecting *Plantago lanceolata*

	df	RLC (%)		Arbuscules/coils (%)	
		SS	*P*	SS	*P*
Block	1	0.000147	0.9241	0.019155	0.1885
Genotype[a]	6	0.293840	0.3393	0.086009	0.8567
Fungal inocula[b]	4	0.968936	<0.0001	0.051924	0.0797
Herbivory[c]	1	0.022531	0.1831	0.094079	0.1691
Genotype × Herbivory	6	0.059784	0.7120	0.232292	0.0048
Genotype × Fungal inocula	12	0.127447	0.7781	0.099266	0.6802
Herbivory × Fungal inocula	2	0.031386	0.3826	0.021505	0.3760
Herbivory by Species of inocula	2	0.031386	0.3826	0.021505	0.3760
Archaeospora trappei vs. *Glomus* white	1	0.030490	0.1739	0.014825	0.2464
A. trappei vs. *Scutellospora calospora*	1	0.003575	0.6388	0.000128	0.9138
Glomus white vs. *S. calospora*	1	0.013497	0.3631	0.017698	0.2059
Error	51	0.817461		0.549943	

SS sum of squares

[a] Genotype was considered a random effect, so terms were tested across genotype interaction terms. Genotype was tested across Genotype × Fungal inocula and Genotype × Herbivory error

[b] Fungal inocula was tested across Genotype × Fungal inocula error

[c] Herbivory was tested across Genotype × Herbivory error

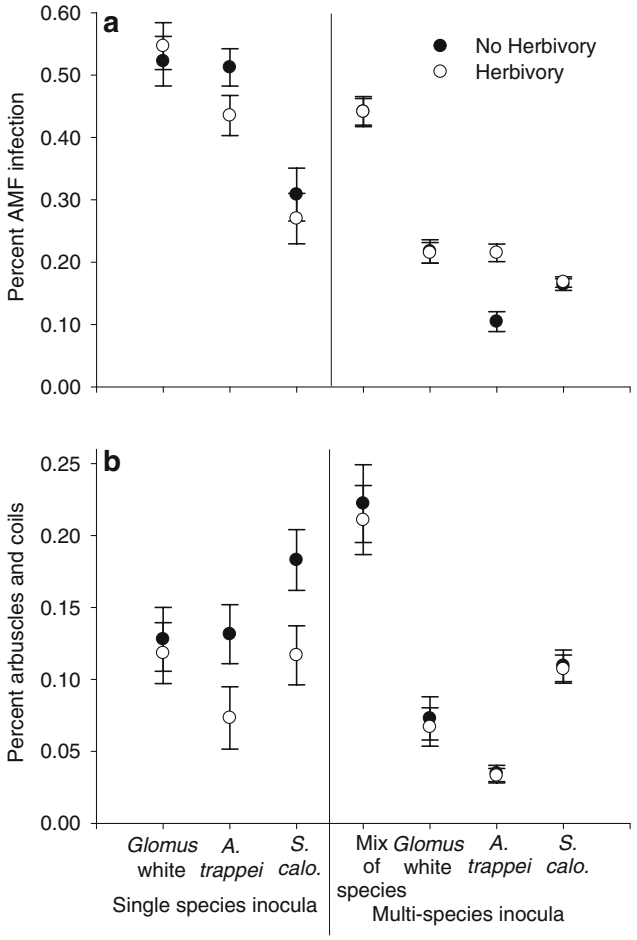

Fig. 2 Percent of *Plantago lanceolata* **a** root length colonized and **b** resource exchange sites (arbuscules and coils) by four different inocula types. *Points to the left of the line* are for treatments when fungi were grown alone, *points to the right of the line* were for all three fungi (*Mix of species*) and each fungal species in this treatment. Values are for mean ± SE. *S. calo. S. calospora, A. trappei Archaeospora trappei, AMF* arbuscular mycorrhizal fungi

when they were grown alone, whereas *S. calospora* ($F_{1,18} = 4.14$, $P = 0.0568$) did not (Fig. 2b). Only plants associated with *A. trappei* experienced a significant interaction between the effects of competition and herbivory due to an increase in *A. trappei* infection following herbivory in competition, but a decline in *A. trappei* infection following herbivory in the absence of competition (Table 3; Fig. 2a).

Frequency of co-infection

The analysis of co-infection revealed that the presence of fungal structures produced by any two fungal species or all three fungal species within the root was significantly less than expected [*Glomus* white vs. *A. trappei* ($t_{25} = -4.622$, $P < 0.0001$), *Glomus* white vs. *S. calospora* ($t_{25} = -6.536$, $P < 0.0001$), *A. trappei* vs. *S. calospora* ($t_{25} = -5.781$, $P < 0.0001$), all three species together ($t_{25} = -3.801$, $P = 0.0008$)]. The difference between the observed and expected value of co-infection of *Glomus* white and *S. calospora* was almost double that of any other combination. The difference between the observed and expected co-infection was not dependent upon herbivory, block, or genotype (Fig. 3).

Table 2 ANOVA results for percent of RLC (arcsin transformed) by individual fungal species and percentage of arbuscules and coils in roots when grown in combination in the roots of *P. lanceolata*

Mixture of species[a]	*df*	RLC (%)		Arbuscules/coils (%)	
		SS	*P*	SS	*P*
Block	1	0.009586	0.2473	0.000182	0.9067
Genotype[b]	6	0.074272	0.1163	0.027791	0.4854
Herbivory[c]	1	0.000007894	0.9677	0.000781	0.6992
Genotype × Herbivory	6	0.026337	0.6710	0.026941	0.8947
Error	12	0.077757		0.152571	
Glomus white					
Block	1	0.002565	0.5072	0.012682	0.2938
Genotype[b]	6	0.117493	0.0278	0.079348	0.2368
Herbivory[c]	1	0.000307	0.7790	0.003012	0.5416
Genotype × Herbivory	6	0.021130	0.6963	0.042922	0.6694
Error	12	0.065876		0.126281	
A. trappei					
Block	1	0.000781	0.6962	0.005554	0.1745
Genotype[b]	6	0.071492	0.5900	0.005712	0.5388
Herbivory[c]	1	0.142551	0.0182	0.000051402	0.8350
Genotype × Herbivory	6	0.086771	0.0517	0.006205	0.8729
Error	12	0.058580		0.031992	
S. calospora					
Block	1	0.005095	0.1184	0.001850	0.5323
Genotype[b]	6	0.007092	0.7911	0.018671	0.5999
Herbivory[c]	1	0.000117	0.8306	0.000034454	0.9278
Genotype × Herbivory	6	0.014226	0.3217	0.023164	0.5480
Error	12	0.021609		0.053675	

Results are presented for all three fungal species combined and by individual species

[a] Genotype × Fungal species mix was never significant so it was not included in the model

[b] Genotype was considered a random effect, so terms were tested across genotype interaction terms. Genotype was tested across Genotype × Herbivory error

[c] Herbivory was tested across Genotype × Herbivory error

Relationship between growth promotion and competitive ability

In order to contrast fungal competitive ability and plant growth promotion we graphed the metric of competitive abil-

Table 3 ANOVA results for three analyses of percent of RLC (arcsin transformed) by mycorrhizae and percentage of arbuscules and coils in roots of individual fungal species when grown singly or in combination (*Fungal inocula*) in the roots of *P. lanceolata*

	df	RLC (%)		Arbuscules/coils (%)	
		SS	*P*	SS	*P*
Glomus white					
Block	1	0.001692	0.4978	0.004622	0.3430
Genotype[a]	6	0.060633	0.0281	0.035927	0.2447
Fungal inocula[b]	1	3.099402	<0.0001	0.883333	<0.0001
Herbivory[c]	1	0.000277	<0.0001	0.003128	0.3687
Genotype×Herbivory	6	0.010954	0.8002	0.019867	0.6817
Herbivory× Fungal inocula	1	0.000277	0.7835	0.003128	0.4345
Error	38	0.137207		0.190499	
A. trappei					
Block	1	0.000308	0.8482	0.000115	0.8675
Genotype[a]	6	0.106303	0.2676	0.010364	0.8873
Fungal inocula[b]	1	1.667434	<0.0001	0.285729	<0.0001
Herbivory[c]	1	0.013338	0.3001	0.016954	0.1129
Genotype×Herbivory	6	0.062535	0.3003	0.029730	0.3222
Herbivory× Fungal inocula	1	0.177017	<0.0001	0.028454	0.0121
Error	37	0.306587		0.151292	
S. calospora					
Block	1	0.002179	0.1320	0.000211	0.7457
Genotype[a]	6	0.003357	0.8136	0.007370	0.6309
Fungal inocula[b]	1	2.342252	<0.0001	1.434630	<0.0001
Herbivory[c]	1	0.000199	0.6980	0.000469	0.6114
Genotype×Herbivory	6	0.007230	0.2761	0.009798	0.5558
Herbivory× Fungal inocula	1	0.000199	0.6441	0.000469	0.6288
Error	38	0.034936		0.074971	

Results are presented for each fungal species

[a] Genotype was considered a random effect, so terms were tested across genotype interaction terms. Genotype was tested across Genotype×Herbivory error

[b] Genotype×Fungal species mix was never significant so it was not included in the model

[c] Herbivory was tested across Genotype×Herbivory error

ity for both percent root length colonized (Fig. 4a) and percent root length containing resource exchange sites (Fig. 4b) from this study as well as percent root length colonized from Pearson et al. (1994) (Fig. 4c). Graphing this metric allows us to determine that across both studies and types of measurements, *S. calospora*, the less beneficial fungus, is competitively superior to both *Glomus* white and *A. trappei* (Fig. 4).

Fungal sporulation

Herbivory did not affect the number of spores produced by *Glomus* white ($F_{1,33} = 0.85$, $P = 0.3897$), but competition did ($F_{1,33} = 10.9$, $P < 0.04$). When *Glomus* white was included in combination with *A. trappei* and *S. calospora* the average number of spores produced per pot was 7,718 (SE = 1,558) which was significantly less than 12,360 (SE = 1,558), the average number of spores per pot produced when *Glomus* white was grown alone.

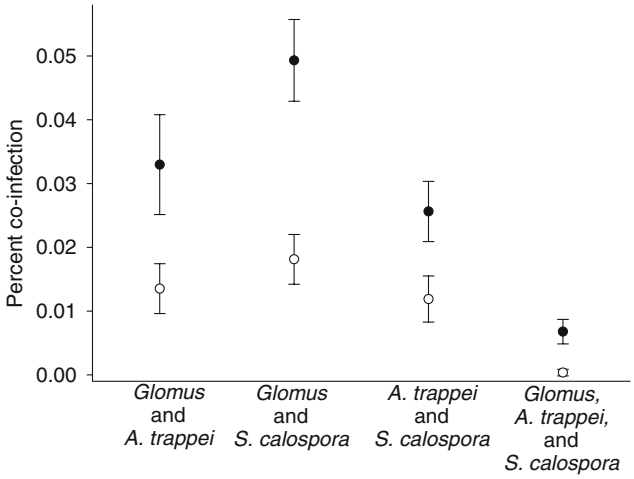

Fig. 3 Expected (*filled circles*) and observed (*open circles*) values of co-infection of *P. lanceolata* roots by more than one fungus graphed against all possible combinations of the fungal species. Values are mean ± SE

Discussion

Our results are consistent with a trade-off between host growth promotion and competitive ability, unlike ant–plant systems which lack this trade-off (e.g., Miller 2007; Yu and Pierce 1998). *S. calospora*, the poorest growth promoter (Bennett and Bever 2007), suffers the least from competition with other fungi (Fig. 4), and produces the greatest number of arbuscules or coils when co-infecting host plants (Fig. 2). In fact, it appears that *S. calospora* competitively suppresses the better growth promoter *Glomus* white (Bennett and Bever 2007): *Glomus* white was very unlikely to co-occur in a root segment with *S. calospora* (Fig. 3). In addition, our estimate of *S. calospora* competitive ability, using only percent mycorrhizal colonization, is fairly conservative as alternative measurements, like percent root length colonized, would take into consideration the significantly smaller size of plants associated with *S. calospora* (Bennett and Bever 2007) and demonstrate that *S. calospora* would have a proportionally greater amount of fungi than any of the other fungal species. A second study comparing another *Glomus* sp. and *S. calospora* revealed that *S. calospora* also promoted the growth of subterranean clover less than *Glomus* sp. (as measured by aboveground dry weight), but had the greater competitive ability (as measured by percent root infection) (Pearson et al. 1994).

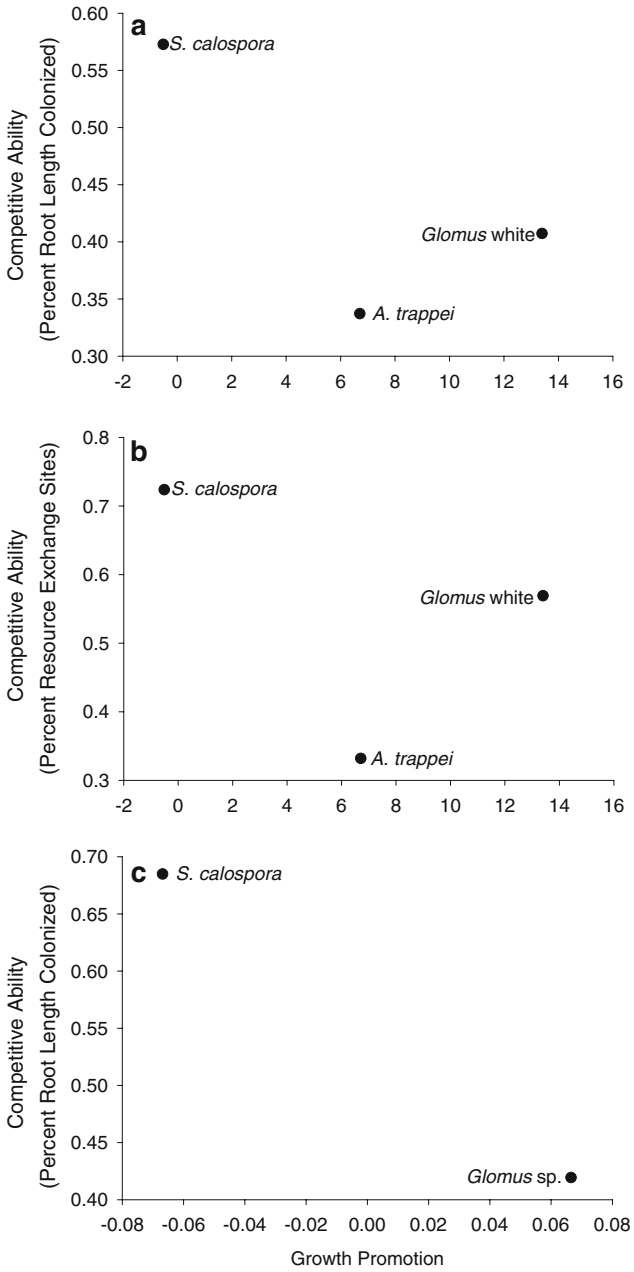

Fig. 4 Competitive ability of each fungal type from **a**, **b** this study and **c** Pearson et al. (1994) graphed against growth promotion. Competitive ability is calculated as the ratio of percent of **a**, **c** *P. lanceolata* percent root length colonized or **b** resource exchange sites of that fungus when grown in mixture divided by **a**, **c** percent root length colonized or **b** resource exchange sites of that fungus when grown alone

In addition, other studies have documented competition for space between *Glomus* sp. and *Scutellospora* or *Gigaspora* species (Cano and Bago 2005; Wilson 1984; Wilson and Trinick 1983). Taken all together, these studies suggest that poor mutualists like *S. calospora* tend to be better competitors for root space, and would be expected to dominate in root systems thereby decreasing the mutualism. In fact, *S. calospora* has been previously observed to dominate on *P. lanceolata* root systems and thereby contribute to a decline in fungal community benefits for *P. lanceolata* (Bever 2002a, b). Although colonization and external hyphae in the soil matrix were not measured in this experiment, previous experiments examining these characteristics in fungi colonizing *P. lanceolata* found faster colonization rates among *Glomus* species, but a greater proportion of external than internal hyphae within pots inoculated with *S. calospora* (Hart and Reader 2002). Despite the fact that *S. calospora* is likely allocating the majority of its resources outside host roots, it is still competitively superior inside host roots. Therefore, again, our measurement of fungal competitive ability is conservative. Thus, this and other studies support the notion that there is a trade-off between fungal competitive ability and host growth promotion.

All three fungal species in this study experienced a reduction in overall root infection when grown in combination. Moreover, these fungi were significantly less likely to be found together within the same section of root than expected by chance (Fig. 3). In fact, when multiple fungal species were observed within a single section of root, the structures of the different species were usually in different sides or layers of the root (A. E. Bennett, personal observation). This spatial separation of the fungi is consistent with expectations of local-scale interference competition. Local-scale allelochemical interference is common in saprophytic fungi (Gloer 1995 and references therein), but has not yet been demonstrated in AM fungi.

The pattern of fungal dominance observed in this study is not consistent with the expectations of partner choice of fungal symbionts by plants, which has been suggested as a mechanism by which hosts can avoid cheaters in mutualisms (Bronstein et al. 2003; Hoeksema and Kummel 2003; Kiers and van der Heijden 2006; Knowlton and Rohwer 2003; Palmer et al. 2003). If partner choice explained the interaction between host and fungus, changes in mycorrhizal colonization should reflect the host benefit received by a mutualist. However, *P. lanceolata* appears unable to choose to associate with better mutualists even when offered a choice.

Just as in previous studies of herbivore effects on single AM fungal species associated with hosts, herbivory tended to reduce fungal infection in host roots (Fig. 2) (reviewed in Gehring and Whitham 1994). However, the effect of herbivory on all three fungal species disappeared in the presence of competition, suggesting that antagonistic interactions, like herbivory, are unlikely to destabilize mutualistic interactions (Strauss et al. 2001). By contrast, the competition among fungal species did not negate the influence of AM fungi over host plant responses to herbivory. All fungal species in this study but *A. trappei*, associated with plant roots singly and in combination suppressed chemical responses to herbivory that appeared in plants subjected to

herbivory but not associated with AM fungi (Bennett et al. 2009). Even though herbivory is less of a factor for AM fungal colonization than competition, AM fungi still strongly impact aboveground plant interactions.

Implications

Our observation of high competitive ability in the least beneficial fungus is consistent with expectations of a fitness cost of mutualism in AM fungi. Given that this fitness cost is expressed in competition, the abundances of beneficial fungi are predicted to decline in mixture despite these fungi thriving in isolation. Such a decline in beneficial fungi in mixture was observed by Bever (2002a) where *S. calospora* proliferated when associated with *P. lanceolata*, causing a decline in the relative growth of *P. lanceolata* in the following generation. This dynamic would likely lead to the breakdown of the mutualism. Thus we can ask: what forces can counteract the cost of mutualism thereby allowing a beneficial fungus to persist in the population? One possibility is that the competitive advantage of the non-beneficial *S. calospora* is host specific, as observed by Bever (2002a, b). Therefore, while *S. calospora* proliferates on *P. lanceolata*, it decreases in abundance when associated with a second co-occurring plant species: *Panicum sphaerocarpon*. The net dynamic prevents *S. calospora* from excluding fungi that benefit *P. lanceolata* from the system. *P. lanceolata* will benefit from AM fungi when establishing in locations in the field with low relative abundance of *S. calospora*. In this study we found that biotic stresses, like herbivory and competition, would not be expected to alter the nature of the negative feedback between *P. lanceolata* and its AM fungal community. This suggests that, instead of the traditional view of extreme beneficial specialization by two mutualist species, one is more likely to encounter a suite of mutualists whose "benefits" will range from negative to positive depending on the exact pairing (Bronstein et al. 2003; Stanton 2003). The populations of both mutualists are thus kept in check by negative feedback loops, and the frequency with which they encounter beneficial and cheater partners.

This study is the first study, to our knowledge, to examine how individual mycorrhizal species within a community respond to a stress such as herbivory, and the first to provide evidence of a trade-off between competition and growth promotion between mycorrhizal fungal species. These results provide greater insight into the nature of the plant–mycorrhizal association and competition between mycorrhizal species.

Acknowledgements We would like to thank David McNutt, Julie Gummow, Dacia Montemayor, Alex Nguyen, Tommy Zajac, and Deane Bowers for assistance with data collection, protocols, and equipment; and Fred Nijhout, Laura Grunert, and Deane Bowers for providing *J. coenia* butterflies, and help rearing them. We would like to thank Keith Clay, Heather Reynolds, Curt Lively, Peggy Schultz, Scott Mangan, Tom Platt, Jennifer Rudgers, Jon Haloin, Michael Dawson, Mirka Macel, Pat McIntyre, Elizabeth Seifer, and the Bever/Schultz lab group for taking the time to review and discuss this paper with us. We would like to thank Jason Hoeksema, Peter Kennedy, and two anonymous reviewers for their comments on the manuscript. We acknowledge the support of Sigma Xi Grant-In-Aid of Research, Indiana University McCormick Grant, and NSF grants DEB-0407816 to Alison Bennett and DEB-0049080 to Jim Bever. All experiments comply with the current laws of the United States.

References

Abbott LK (1982) Comparative anatomy of vesicular arbuscular mycorrhizas formed on subterranean clover. Aust J Bot 30:485–499

Bennett AE, Bever JD (2007) Mycorrhizal species differentially alter plant growth and response to herbivory. Ecology 88:210–218

Bennett AE, Bever JD, Bowers MD (2009) Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. Oecologia (in press)

Bever JD (2002a) Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. Proc R Soc Lond B Biol Sci 269:2595–2601

Bever JD (2002b) Host-specificity of AM fungal population growth rates can generate feedback on plant growth. Plant Soil 244:281–290

Bever JD, Schultz PA, Pringle A, Morton JB (2001) Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. Bioscience 51:923–931

Bobbitt JM, Segebarth KP (1969) Iridoid glycosides and similar substances. In: Taylor WI, Battersby AR (eds) Cyclopentanoid terpene derivatives. Dekker, New York, pp 1–145

Bowers MD, Collinge SK (1992) Fate of iridoid glycosides in different life stages of the buckeye, *Junonia coenia* (Lepidoptera, Nymphalidae). J Chem Ecol 18:817–831

Bowers MD, Puttick GM (1986) Fate of ingested iridoid glycosides in Lepidopteran herbivores. J Chem Ecol 12:169–178

Bronstein JL, Wilson WG, Morris WE (2003) Ecological dynamics of mutualist/antagonist communities. Am Nat 162:S24–S39

Cano C, Bago A (2005) Competition and substrate colonization strategies of three polyxenically grown arbuscular mycorrhizal fungi. Mycologia 97:1201–1214

Currie AF, Murray PJ, Gange AC (2006) Root herbivory by *Tipula paludosa* larvae increases colonization of *Agrostis capillaris* by arbuscular mycorrhizal fungi. Soil Biol Biochem 38:1994–1997

Duff RB, Bacon JSD, Mundie CM, Farmer VC, Russell JD, Forrester AR (1965) Catalpol and methylcatalpol—naturally occurring glycosides in *Plantago* and *Buddleia* species. Biochem J 96:1–7

Fineblum WL, Rausher MD (1995) Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature 377:517–520

Gange AC (2007) Insect–mycorrhizal interactions: patterns, processes, and consequences. In: Ohgushi T, Craig TP, Price PW (eds) Ecological communities: plant mediation in indirect interaction webs. Cambridge University Press, London, pp 124–143

Gange AC, Bower E, Brown VK (2002) Differential effects of insect herbivory on arbuscular mycorrhizal colonization. Oecologia 131:103–112

Gange AC, Brown VK, Aplin DM (2005) Ecological specificity of arbuscular mycorrhzae: evidence from foliar- and seed-feeding insects. Ecology 86:603–611

Gehring CA, Whitham TG (1994) Interactions between aboveground herbivores and the mycorrhizal mutualists of plants. Trends Ecol Evol 9:251–255

Gehring CA, Whitham TG (2002) Mycorrhizae–herbivore interactions: population and community consequences. In: van der Heijden MGA, Sanders IR (eds) Mycorrhizal ecology, vol 157. Springer, Berlin, pp 295–320

Gloer JB (1995) The chemistry of fungal antagonism and defense. Can J Bot 73:S1265–S1274

Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol 153:335–344

Hart MM, Reader RJ (2005) The role of the external mycelium in early colonization for three arbuscular mycorrhizal fungal species with different colonization strategies. Pedobiologia 49:269–279

Hepper CM, Azconaguilar C, Rosendahl S, Sen R (1988) Competition between 3 species of *Glomus* used as spatially separated introduced and indigenous mycorrhizal inocula for leek (*Allium porrum* L). New Phytol 110:207–215

Herms DA, Mattson WJ (1992) The Dilemma of plants—to grow or defend. Q Rev Biol 67:283–335

Hoeksema JD, Kummel M (2003) Ecological persistence of the plant–mycorrhizal mutualism: a hypothesis from species coexistence theory. Am Nat 162:S40–S50

Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytol 135:575–586

Kennedy PG, Hortal S, Bergemann SE, Bruns TD (2007) Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. J Ecol 95:1338–1345

Kiers ET, van der Heijden MGA (2006) Mutualistic stability in the arbuscular mycorrhizal symbiosis: exploring hypotheses of evolutionary cooperation. Ecology 87:1627–1636

Knowlton N, Rohwer F (2003) Multispecies microbial mutualisms on coral reefs: the host as a habitat. Am Nat 162:S51–S62

Kula AAR, Hartnett DC, Wilson GWT (2005) Effects of mycorrhizal symbiosis on tallgrass prairie plant–herbivore interactions. Ecol Lett 8:61–69

Merryweather J, Fitter A (1998) The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*. I. Diversity of fungal taxa. New Phytol 138:117–129

Miller TEX (2007) Does having multiple partners weaken the benefits of facultative mutualism? A test with cacti and cactus-tending ants. Oikos 116:500–512

Mueller RC, Sthultz CM, Martinez T, Gehring CA, Whitham TG (2005) The relationship between stem-galling wasps and mycorrhizal colonization of *Quercus turbinella*. Can J Bot 83:1349–1353

Palmer TM, Stanton ML, Young TP (2003) Competition and coexistence: exploring mechanisms that restrict and maintain diversity within mutualist guilds. Am Nat 162:S63–S79

Pearson JN, Abbott LK, Jasper DA (1993) Mediation of competition between 2 colonizing va mycorrhizal fungi by the host plant. New Phytol 123:93–98

Pearson JN, Abbott LK, Jasper DA (1994) Phosphorus, soluble carbohydrates and the competition between 2 arbuscular mycorrhizal fungi colonizing subterranean clover. New Phytol 127:101–106

Sen R, Hepper CM, Azconaguilar C, Rosendahl S (1990) Competition between introduced and indigenous mycorrhizal fungi (Glomus spp) for root colonization of leek. Agric Ecosyst Environ 29:355–359

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic Press, London

Stanton ML (2003) Interacting guilds: moving beyond the pairwise perspective on mutualisms. Am Nat 162:S10–S23

Stearns SC (1989) Trade-offs in life-history evolution. Funct Ecol 3:259–268

Strauss SY, Conner JK, Lehtila KP (2001) Effects of foliar herbivory by insects on the fitness of *Raphanus raphanistrum*: damage can increase male fitness. Am Nat 158:496–504

Thrall PH, Burdon JJ (2003) Evolution of virulence in a plant host–pathogen metapopulation. Science 299:1735–1737

Wamberg C, Christensen S, Jakobsen I (2003) Interaction between foliar-feeding insects, mycorrhizal fungi, and rhizosphere Protozoa on pea plants. Pedobiologia 47:281–287

Wilson JM (1984) Competition for infection between vesicular arbuscular mycorrhizal fungi. New Phytol 97:427–435

Wilson JM, Trinick MJ (1983) Infection development and interactions between vesicular–arbuscular mycorrhizal fungi. New Phytol 93:543–553

Yu DW, Pierce NE (1998) A castration parasite of an ant–plant mutualism. Proc R Soc Lond B Biol Sci 265:375–382