New Phytologist

Analogous effects of arbuscular mycorrhizal fungi in the laboratory and a North Carolina field

Anne Pringle[1] and James D. Bever[2]

[1]Department of Organismic and Evolutionary Biology, 16 Divinity Avenue, Harvard University, Cambridge, MA 02138, USA; [2]Department of Biology, Indiana University, Bloomington, IN 47405, USA

Author for correspondence:
Anne Pringle
Tel: +1 617 4969707
Fax: +1 617 4958848
Email: pringle@oeb.harvard.edu

Received: *5 March 2008*
Accepted: *5 May 2008*

Summary

• Although arbuscular mycorrhizal (AM) fungi are ubiquitous symbionts of plants, the mutualism has rarely been tested in nature.

• In experiments designed to explore the ecological relevance of associations between different fungal and plant species in a natural environment, plant species were infected with different species of fungi and grown in separate trials in the laboratory and a North Carolina (USA) field.

• The benefits to plants varied dramatically as plant species were grown with different species of AM fungi. Effects of mycorrhizal fungi in nature were generally correlated to effects in the growth chamber, suggesting that laboratory data do reflect dynamics between plants and AM fungi in the field. Initial size at transplant and experimental block were also significant predictors of plant growth in the field. Correlation statistics between laboratory and field data were weaker when analyses involved plant species less responsive to infection by any AM fungus, suggesting that the response of a species to inoculation is a good predictor of its sensitivity to specific AM fungi in the field.

• AM fungal identity appears to influence the growth and reproduction of plants in the field.

Key words: cooperation, feedback, fitness, mutualism, mycorrhizal benefit in the field or wild, organic agriculture, specificity, symbiosis.

New Phytologist (2008) **180**: 162–175

doi: 10.1111/j.1469-8137.2008.02537.x

Introduction

Laboratory plants associated with arbuscular mycorrhizal (AM) fungi may grow larger or smaller (Hayman & Mosse, 1971; Mosse & Hayman, 1971; Francis & Read, 1995), or shift allocation patterns (Miller *et al.*, 1987; Cook *et al.*, 1988; Streitwolf-Engel *et al.*, 1997), or vary in reproductive success (Koide *et al.*, 1994; Francis & Read, 1995), as compared with plants grown without AM fungi. AM fungi inhabit the roots of plants and provide phosphorus (P), and perhaps other benefits, to plants in exchange for photosynthetically derived carbon compounds. A majority of the world's plant species belong to families that are characteristically mycorrhizal (Smith & Read, 1997). In the laboratory, a single plant species may derive different benefits from its associations with different species of fungi (Streitwolf-Engel *et al.*, 1997; Van der Heijden *et al.*, 1998a). This variability is thought to be ecologically important, as it underlies the interdependence of plant and AM fungal community dynamics (Bever, 1999, 2002; Hart *et al.*, 2003), as well as explanations for observations of plant diversity response to manipulations of fungal diversity (van der Heijden *et al.*, 1998b; Vogelsang *et al.*, 2006). However, the costs and benefits ascribed to the association in the laboratory have been difficult to confirm in nature. Field experiments have focused on plants in agricultural habitats (e.g. Hetrick *et al.*, 1984), or plants grown with a single species of AM fungus (e.g. *Glomus intraradices*; Koide *et al.*, 1994; Sanders & Koide, 1994; Pendleton, 2000).

Data on the function of diverse mycorrhizal associations in nature are rare (Fitter, 1985, 1990; Francis & Read, 1995; Read, 2002).

In this study we sought to understand how patterns described from the laboratory translate to the field by growing a group of plants with the same species of AM fungi in growth chambers, and then in nature. Plant species were chosen to represent possible mutualism, neutralism, or antagonism, according to Francis & Read's (1995) hypothesis that certain plant families are more or less likely to experience mutualism or parasitism in association with AM fungi. Both plant seeds and AM fungal cultures were isolated from the same North Carolina grassland and this site was also used for our field experiment. The latter experiment used plants infected at germination in the laboratory, and isolated from other AM fungi for a minimum of 7 wk before transplantation. By establishing a priority of infection for AM fungi in the laboratory, we aimed to minimize secondary infections in the field. Other data suggest that AM fungi can exclude other species of fungi from roots (Mosse & Hayman, 1971). Newsham *et al.* (1995) capitalized on this logic to inoculate *Vulpia ciliata* with a *Glomus* sp. in the laboratory, and subsequently grew the plants in nature, successfully demonstrating that plants infected with the *Glomus* sp. avoided infection by a pathogenic fungus. The same technique was used as early as 1975 to inoculate glasshouse seedlings of fourwing saltbush plants (*Atriplex canescens*) with *Glomus mosseae* for later use at a coal mine spoil (Aldon, 1975). In our experiments we asked: is this group of AM fungi beneficial to plants in the laboratory; what is the relative benefit to each plant species of association with different AM fungal species; and finally, are the patterns observed in the laboratory equivalent to the patterns observed in the field?

Materials and Methods

Plant and fungal species

Plant seeds and AM fungal cultures were isolated from a well-studied (Fowler, 1978; Clay, 1982; Kelley, 1985; Moloney, 1986; Bever, 1992; Schultz, 1996) semi-natural grassland on the campus of Duke University, Durham, NC, USA. In this field certain plant species appear to form a majority at specific times of year (e.g. *Anthoxanthum odoratum* in spring), but careful observation reveals a mosaic of co-dominants from a diversity of families (Fowler & Antonovics, 1981). At least 37 species of AM fungi grow within the grassland, and a third of the species are or were undescribed (Bever *et al.*, 2001). The fungi are patchily distributed and sporulation is seasonal (Schultz, 1996; Pringle & Bever, 2001). Surface soil is a nutrient-poor sandy loam with an average pH of 5.0 and P concentrations of 1.5–6 $\mu g\ g^{-1}$ of soil (Fowler & Antonovics, 1981). A clay hardpan is found underneath the surface soil at a depth of *c.* 15 cm.

Experimental plant species were chosen to represent a spectrum of interactions, according to Francis & Read's (1995) hypothesis that certain plant families are more or less likely to experience mutualism or parasitism in association with AM fungi. Species included *Allium vineale* L. (Liliaceae; mutualism), *Anthoxanthum odoratum* L. (Graminae; neutralism), *Cerastium glomeratum* Thuillier (Caryophyllaceae; neutralism), *Plantago lanceolata* L. (Plantaginaceae; mutualism), *Rumex acetosella* L. (Polygonaceae; parasitism), and *Veronica arvensis* L. (Scrophulariaceae; neutralism). From this point forward we will refer to the plant species by using generic names. Species are either winter annuals (*Cerastium* and *Veronica*) or perennials (all others). In the field plants germinate in fall or winter, actively grow in spring and die or, if perennial, experience high mortality in summer. Seeds for these experiments were collected from different areas of the grassland, and mixed.

Experimental AM fungi are representative of the fungal diversity of the grassland, and are easily trapped in culture: spores of *Acaulospora morrowiae*, *Archaeospora trappei*, *Gigaspora gigantea*, and *Scutellospora pellucida* are abundant in the field (Schultz, 1996), but *Glomus* 'white' spores are relatively rare. (For more information on *A. morrowiae*, *A. trappei* and *Glomus* 'white', see Bever *et al.*, 1996; note that *Glomus* 'white' = *Glomus* sp. D1 and *Acaulospora morrowiae* = *Acaulospora mellea*. For *S. pellucida*, see Bever & Morton, 1999. In Pringle, 2001, *A. morrowiae* was discussed as *A. mellea*, and *Archaeospora trappei* as *Acaulospora trappei*.) Cultures were established from single spores and were maintained on *Sorghum vulgare* Persoon. Each inoculum was homogenized before its use in experiments. All fungal species have been deposited in the International Culture Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi, as described in Bever *et al.* (1996).

The growth chamber experiment

Germinating seeds To minimize microbial contamination, seeds of all species except *Veronica* were surface-sterilized in 10% bleach; *Veronica* seeds are very small and were judged too fragile. Seeds were germinated in a 3 : 1 mixture of sterilized sand:seedling mix (seedling mix is Metro 200 growth medium; Scotts-Sierra Horticultural Products Co., Marysville, OH, USA). Germinating seeds were kept in a growth chamber at 25°C day : 10°C night with 10-h day lengths. Seedlings were misted five or more times each day.

Inoculation Seedlings and AM fungi were planted in a factorial design when seedlings were *c.* 2.5 (*Allium* and *Plantago*) or 4 (*Anthoxanthum*, *Cerastium*, *Rumex* and *Veronica*) wk old. Ten replicates of each combination were established, and 10 replicates of each plant species were planted without any fungus as controls. Sterilized 7.5 cm diameter × 13.3 cm deep pots were filled with a bottom layer of *c.* 50 ml of sterilized gravel and a middle layer of *c.* 250 ml of sterilized 1 : 1 sand:field soil mix. The pots were inoculated with 15 cm^3 of

the appropriate fungus (or 15 cm^3 of sterilized sand:field soil mix for controls). Seedlings were rinsed free of potting mixture and planted. To minimize pot-to-pot contamination, pots were topped off with *c*. 25 cm^3 of sterile sand, and nine randomly chosen pots of different plant species inoculated with the same fungus were placed in a single pot rack. Racks were placed randomly in a growth chamber and re-randomized within the chamber every 3 wk. The chamber was kept at 22°C day : 10°C night with 12-h day lengths. Plants were watered three times a day for the first 5 wk and once a day subsequently. Plants that died in the first 3 wk of the experiment were replaced. Plants were watered with *c*. 33 ml of low-P fertilizer at 14 wk (0.45 mg KH_2PO_4 per 33 ml dose).

Estimating the benefit to plants of association with AM fungi

All species except *Cerastium* Pots were harvested after 19 or 20 wk. Shoots were cut and bagged. Root biomass and fungal sporulation were estimated for each pot as follows: roots and soil of *Anthoxanthum* and *Plantago* pots were divided vertically and each half weighed. Roots of one half were washed free of soil and bagged. Roots and soil of the second half were stored in a 4°C cold room and used in assays of fungal sporulation, which were performed to confirm the purity and effectiveness of inoculations. Because *Rumex* grows only a few large roots per plant, unevenly distributed in pots, roots of half of the *Rumex* pots were washed free of soil and dried while roots and soil of the second half of the *Rumex* pots were preserved in their entirety for measurement of sporulation. Bagged shoots and roots of all species were dried for several weeks at 66°C, and weighed. Total root mass was estimated by extrapolating from the measures of per cent fresh weight of the divided roots and soil.

Cerastium *Cerastium* began to flower at 8 wk after planting. Capsules were counted and harvested as they matured. *Cerastium* plants finished flowering and senesced over a period of 31 wk. The roots and soil of each pot were preserved at 4°C for assay of fungal fitness. To estimate plant fitness, the total number of capsules from each plant was tallied, and 20 capsules of each plant were dissected, and seeds counted. The 20 capsules were chosen in proportion to the number of capsules collected at each collection date; for example, if 50% of the capsules were collected on 4 July, 10 capsules from this date were randomly chosen and dissected for seed counts.

The field experiment

Germination and inoculation The field experiment included all AM fungal species used in the growth chamber experiment, but excluded the plant species *Cerastium*. *Cerastium* was excluded because of its very different phenology and because both *Anthoxanthum* and *Veronica* also represent possible neutral interactions with AM fungi (Francis & Read, 1995). Excluding *Cerastium* also allowed us to increase the replicates of other species.

Associations between plants and AM fungi were established within cell packs of seedling flats. Field soil was sieved, mixed 1 : 1 with sand (to facilitate subsequent transplanting) and sterilized in an autoclave for 4 h. Cell packs were half-filled with the sterile soil and sand mix, and 5 cm^3 of a fungal inoculum was added before cell packs were filled with the soil and sand mix. Generous numbers of seeds were loosely scattered across each pot. Species of plants and AM fungi were combined factorially; the experiment included 25 combinations. Plant species differed in their germination requirements (specifically, time between planting and germination) and sowing was staggered so that different species would emerge at approximately the same time. Pots were placed in a growth chamber at 25°C day : 10°C night with 9-h day lengths, and misted five or more times each day.

Plants were grouped according to fungal infection to limit contamination. Data on the size of plants as they were taken to the field were used to statistically control for the early influence of blocking by fungal species (see 'Analyses'). In late October 1999, seedlings were taken from growth chambers and left to harden on mist benches within a cold room of the glasshouse. In a few cases, seedlings from cells with multiple plants were transplanted to cells where development of a plant stand failed.

Transplanting plants to the field

Seedlings were transplanted to the North Carolina grassland when individuals were large enough to withstand transplant shock (*Allium* and *Anthoxanthum*: 7 wk old; *Plantago*: 8 wk old; *Rumex*: 12 wk old, and *Veronica*: 18 wk old). Each plant species was transplanted on a single day. All species except *Veronica* were transplanted in mid-November. Because *Veronica* seedlings grew poorly, they were transplanted in late January. To distinguish early growth effects from AM fungal effects in the field, the initial size of each plant was recorded: for *Allium*, *Anthoxanthum* and *Plantago*, the number of leaves and height of each plant; for *Rumex*, the number of leaves and rosette width; for *Veronica*, the number of leaves.

At planting, an auger was used to dig holes and remove surface vegetation. The destruction of plant roots and shoots around each focal plant minimized the competition experienced by each seedling in its first weeks in the field. Each hole was *c*. 10 cm deep and 5 cm wide; although surface vegetation was discarded, the soil from each hole was crumbled and used at planting. Seedlings were transplanted with the soil and fungus of the germination pot and mulched with a mix of composted pine bark, peat moss and sand. The blocking design is illustrated in Fig. 1: a single representative of each plant and fungal combination was transplanted to each block; a total of 500 plants (20 of each combination) were grown in the field.

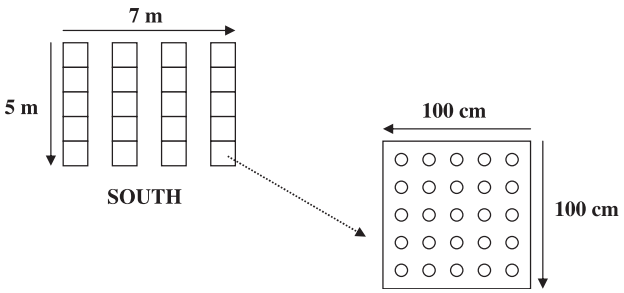

Fig. 1 The blocking design of the field experiment.

In the first 2 wk after transplanting, seedlings were watered occasionally and covered with plastic on especially cold nights. To protect against grasshoppers, clear plastic cups with the bottoms cut off were turned upside down and placed around *Plantago* seedlings, and secured to the ground with toothpicks. These were removed after several weeks of cold weather decimated the grasshopper population.

Estimating the benefit to plants via reproductive data

Anthoxanthum plants were flowering by early March. From that point, this species was censused every 3–4 d. The length of each fully mature inflorescence was measured and stems of measured inflorescences were marked. Several dozen mature inflorescences were sampled and the seeds of these inflorescences were counted; data were used to regress seed number on inflorescence length using the general linear models procedure of SAS version 6.12 (SAS Institute Inc., 1996). In fact, *Anthoxanthum* inflorescence length was a significant predictor of seed number ($r^2 = 0.7$; $F = 60.91$, d.f. = 1, $P < 0.0001$) and therefore inflorescence length is a reasonable estimate of *Anthoxanthum* fitness.

Reproductive data were also recorded for *Veronica* and *Rumex*, but neither *Allium* nor *Plantago* flowered. *Veronica* plants flowered in early April. The number of capsules on each plant was counted at harvest. Very few *Rumex* flowered, and because *Rumex* is dioecious seeds were not counted.

Estimating the benefit to plants via biomass data

As plants died (*Veronica*) or grew quiescent in the summer heat (*Anthoxanthum*, *Plantago* and *Rumex*), shoots were harvested, dried and weighed. *Allium* bulbs of even blocks were dug from the ground, dried and weighed (*Allium* bulbs of odd blocks were inadvertently destroyed).

Analyses

Early mortality of plants in the laboratory and field, and *Rumex* reproduction in the field χ^2 tests were used to evaluate the early mortality of laboratory *Plantago* and *Rumex* seedlings when grown without fungi, or when grown with different fungal species. Separate χ^2 tests were used to evaluate the early mortality and later reproduction of *Rumex* plants in the field.

Laboratory biomass data (all plant species except *Cerastium*) Treatment effects were analyzed with an analysis of variance (ANOVA). To improve normality, biomass data were log-transformed. The treatment and interaction sums of squares were decomposed into orthogonal tests of (1) the control treatment versus the average effects of AM fungal species and (2) AM fungal species versus each other. This technique allowed us to distinguish the impact of the AM fungi in general from the impact of individual fungal species. Because treatment and interaction terms were significant, subsequent analyses focused on individual plant species.

Treatment effects for each plant species were analyzed with individual analyses of variance (ANOVA). To improve normality, *Allium* shoot and root data were log-transformed, and *Rumex* root data were ranked. As already described, the treatment and interaction sums of squares were decomposed into orthogonal tests of; the control treatment versus the average effects of AM fungal species, and AM fungal species versus each other. Tukey's multiple contrast test was used to test for significant differences between fungal treatments.

Fitness of *Cerastium* from the laboratory Treatment effects on *Cerastium* capsule and seed number were analyzed using ANOVA. Tukey's multiple contrast test was used to test for significant differences between fungal treatments.

Reproductive and biomass data of field plants Reproductive and biomass data were evaluated in a single ANOVA executed with the general linear models procedure of SAS version 6.12 (SAS Institute Inc., 1996). The following measures of initial size at transplanting, and performance at harvest, were used: for *Allium* data were initial heights and harvest bulb weights; for *Anthoxanthum* data were initial number of leaves and harvest inflorescence lengths (the total length of all inflorescences for each plant); for *Plantago* data were initial heights and harvest shoot weights; *Rumex* data were initial number of leaves and harvest shoot weights; and *Veronica* data were initial number of leaves and harvest capsule number. The analysis assessed effects of plant species, initial size at transplanting, experimental block and AM fungal species, and interactions, on harvest data. As these data were distributed irregularly, measures for each species were ranked and the ANOVA completed with ranked counts. Because data were ranked, the analysis is considered nonparametric. Type III sums of squares are reported. Because the interaction of plant and fungal species was significant, subsequent analyses examined each species of plant individually.

Individual species' harvest data were analyzed with ANOVA (SAS Institute Inc., 1996); the analyses were used to test a hypothesis of 'no effect of fungal species' on reproductive or biomass measures. Initial size at transplanting was used as a

co-variate to control for early growth chamber effects; because the interaction of initial size and AM fungal species was not a significant predictor of size at harvest for any plant species we do not discuss it further (but see Supporting Information Fig. S1 for data on initial sizes). Because sample sizes were unequal, a Studentized maximum modulus (GT2) multiple contrast test was used to evaluate differences in individual species' growth with different AM fungal species.

Linking field performance to laboratory measures To explore the relationship between field and growth chamber data, the mean performance of each plant species with each fungal species in the field was regressed against the mean performance of that plant species with the fungal species in the laboratory (using general linear models procedures; SAS Institute Inc., 1996). We took mean biomass as a single measure of plant performance in the laboratory, and either mean biomass or mean reproduction as a measure of plant performance in the field. Least-squares means were used for field data, allowing us to remove the effect of initial size. The predictive power of the growth chamber means for field performance differed for the different species of plants (as reflected by a significant interaction between growth chamber biomass and plant species when the analysis included every species of the experiments, and plant species was included as a predictive variable: $F_{4,15} = 3.12$, $P < 0.05$), and so the dependence of field performance on laboratory performance was tested for each plant species separately. We then tested whether the strength of the individual relationships was influenced by plant responsiveness to inoculation with any AM fungus by calculating the nonparametric correlation between the r^2 of the field-growth chamber regressions and *F*-statistics of the contrasts between controls and plants infected with any AM fungus. To calculate these contrasts, we used total plant biomass data from the laboratory.

Results

Early mortality of plants in the laboratory and field, and *Rumex* reproduction in the field

Infection with diverse AM fungal species influenced the survival of two plant species in the first 3 wk of the growth chamber experiment. Three of 10 *Plantago* plants grown without any fungus died, as compared with 0 deaths of the 50 *Plantago* plants grown with AM fungi ($\chi^2 = 12.83$, d.f. = 5, $P < 0.025$). Three of 10 *Rumex* plants grown with *S. pellucida* died, as compared with 0 deaths of the 50 *Rumex* plants grown without AM fungi or with any other species of AM fungi ($\chi^2 = 12.83$, d.f. = 5, $P < 0.025$).

Eighteen (of 100) *Rumex* plants died in the course of the field experiment. The majority were infected by *S. pellucida* (seven plants), *G. gigantea* (six plants) or *Glomus* 'white' (four plants). None was infected by *A. trappei*. The differences between fungal treatments were significant ($\chi^2 = 10.33$, d.f. = 4, $P < 0.05$). Nine plants flowered in the course of the experiment, but the differences between fungal treatments were not significant ($\chi^2 = 1.56$, d.f. = 4, $P > 0.05$).

Laboratory biomass data (all species except *Cerastium*)

Plant species were variably affected by infection with AM fungi. Fresh spores were collected from every combination of fungus and plant, confirming that each fungal species was able to infect every plant species (data not shown). Spores were not found in the soil of nonmycorrhizal controls. In a comparison of uninfected plants versus plants infected by any species of AM fungus, plants were found to be differently influenced by the presence of any AM fungus (significant plant species × control vs AM fungi interaction; Table 1). In

Table 1 Effects of diverse fungal species on plant biomass in the laboratory experiment (ANOVA for combined data for *Allium*, *Anthoxanthum*, *Plantago*, *Rumex* and *Veronica*)

		Shoot weights		Root weights	
Source of variation	d.f.	MS	*p*	MS	*p*
Plant species	4	9.19	0.0001	26.41	0.0001
Treatment	5	0.53	0.0001	0.13	NS
AMF vs sterile[1]	1	0.10	0.001	0.025	NS
Among AMF[2]	4	0.43	0.0001	0.10	NS
Plant species × treatment[3]	20	0.67	0.0001	0.96	0.0001
Plant species × AMF vs sterile	4	0.15	0.005	0.72	0.0001
Plant species × among AMF	16	0.52	0.0001	0.24	NS

[1]Comparison is between uninfected plants and plants infected with any species of arbuscular mycorrhizal fungus (AMF).
[2]Comparison is among plants infected with *Acaulospora morrowiae*, plants infected with *Archaeospora trappei*, plants infected with *Gigaspora gigantea*, plants infected with *Glomus* 'white', and plants infected with *Scutellospora pellucida*.
[3]The same contrasts as for 'treatment', but parsed according to individual plant species. This tests for the different growth of plant species in association with any AMF, or different species of AMF.
NS, not significant.

Table 2 Effects of diverse fungal species on plant biomass in the laboratory experiment (ANOVA for individual species)

Plant		d.f.[2]	Shoot weights		Root weights	
			F	*p*	*F*	*p*
Allium	Treatment[1]	5, 54	24.6	0.0001	12.10	0.0001
	AMF vs sterile	1, 54	7.73	0.007	6.38	0.01
	Among AMF	4, 54	28.81	0.0001	13.53	0.0001
Anthoxanthum	Treatment	5, 53	6.52	0.0002	2.85	0.02
	AMF vs sterile	1, 53	0.13	NS	13.56	0.0001
	Among AMF	4, 53	8.12	0.0001	0.17	NS
Plantago	Treatment	5, 59	5.64	0.0003	3.89	0.004
	AMF vs sterile	1, 54	12.54	0.0008	13.11	0.0006
	Among AMF	4, 54	3.91	0.007	1.58	NS
Rumex	Treatment	5, 54[3]	3.16	0.01	1.43	NS
	AMF vs sterile	1, 54[3]	1.24	NS	1.95	NS
	Among AMF	4, 54[3]	3.64	0.01	1.31	NS
Veronica	Treatment	5, 54	5.44	0.0004	5.07	0.0007
	AMF vs sterile	1, 54	7.76	0.007	14.14	0.0004
	Among AMF	4, 54	4.86	0.002	2.80	0.03

[1]Contrasts in this table are as described for Table 1.
[2]Numerator degrees of freedom, denominator degrees of freedom.
[3]Denominator degrees of freedom for roots is 24.
NS, not significant.

a comparison of plants infected with different fungi (and excluding uninfected plants) plant shoot weights were found to be variably influenced by the associations of plants with different species of AM fungi (i.e. there was a significant plant species × among AM fungal species interaction; Table 1). Analyses of data for individual species demonstrated that treatment significantly affected *Allium*, *Anthoxanthum*, *Plantago*, and *Veronica* shoot and root weights (Table 2). By contrast, treatment significantly affected only *Rumex* shoot (and not root) weights (Table 2).

Associations of plant species with particular species of AM fungi were evaluated by comparing the growth of plants infected with the species of AM fungus with that of uninfected plants (Figs 2, 3). Significant differences were common; infected shoots and roots were generally larger than uninfected shoots and roots (for example, consider the growth of *Allium*, *Plantago* and *Veronica* with *A. trappei*). In a few cases (and in only one species) infected shoots and roots were significantly smaller than uninfected shoots and roots (*Anthoxanthum* shoots infected with *G. gigantea*, and *Anthoxanthum* roots infected with any AM fungus, were significantly smaller than uninfected *Anthoxanthum*).

Associations of plant species with different AM fungi were evaluated by comparing growth among plants infected with different fungi (Fig. 2). Plants grew best with different species of fungi; for example, *Allium* shoots and roots were heaviest with *A. trappei* but *Plantago* shoots and roots were equivalently heavy with *A. trappei*, *A. morrowiae*, and *S. pellucida*. In a few cases the benefits to one plant associating with two fungi were exactly reversed for a second plant associating with the same fungi: *Anthoxanthum* shoots were significantly heavier with *Glomus* 'white' vs *G. gigantea*, but *Rumex* shoots were significantly heavier with *G. gigantea* in the same comparison.

Cerastium fitness

Treatment had significant effects on both capsule ($F_{5,49} = 2.36$, $P = 0.05$) and seed ($F_{5,49} = 6.57$, $P \leq 0.0001$) production, although direct comparisons between pairs of treatments were not significant when capsule data were analyzed (Fig. 4). Effects on capsule and seed production differed. While plants associated especially with *S. pellucida* or *G. gigantea* were likely to produce fewer capsules than control plants, control plants produced significantly fewer seeds per capsule than any plant associated with a fungus. Plants associated with *A. trappei* and *Glomus* 'white' were likely to produce high numbers of capsules and seeds.

Reproductive and biomass data for field plants

Significant predictors of plant performance at harvest included initial size at transplanting and experimental block, as well as interactions between initial size at transplanting and plant species, and plant species and AM fungal species (Table 3). Initial size was a significant predictor of growth in the field, but plants were very small at transplant (e.g. individual *Plantago* had on average one to two leaves) and the vast majority of growth took place after individuals were placed in the field (AP, personal observation). The significance of the plant species by AM fungal species interaction demonstrates

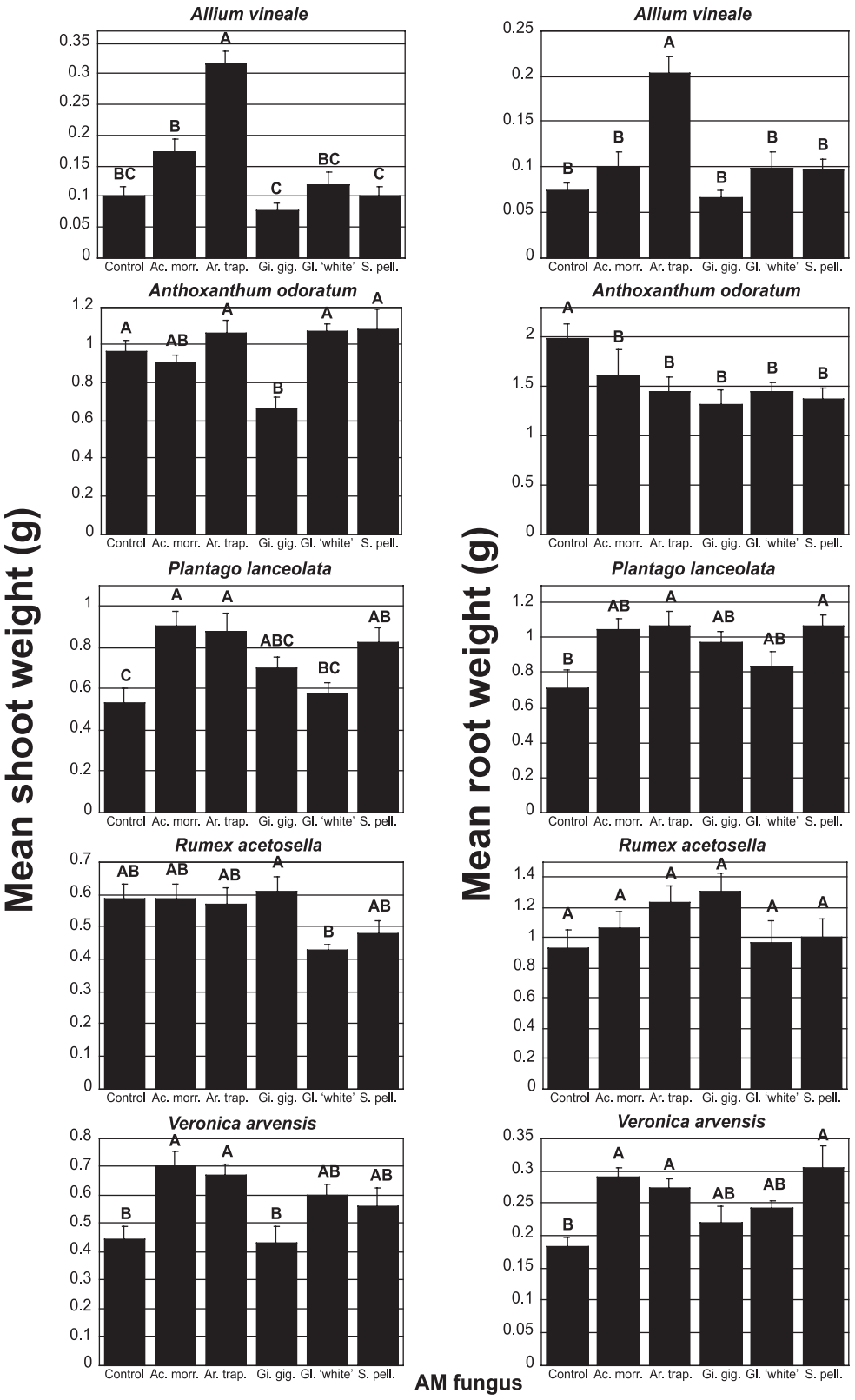

Fig. 2 Average shoot and root biomass of uninfected plants and plants infected with diverse arbuscular mycorrhizal (AM) fungi; laboratory data. AM fungal species abbreviations: control, uninfected plants; Ac. morr., *Acaulospora morrowiae*; Ar. trap., *Archaeospora trappei*; Gi. gig., *Gigaspora gigantea*; Gl. 'white', *Glomus* sp.; S. pell., *Scutellospora pellucida*. Standard errors are given for each plant and fungal combination; letters indicate significant differences between treatments.

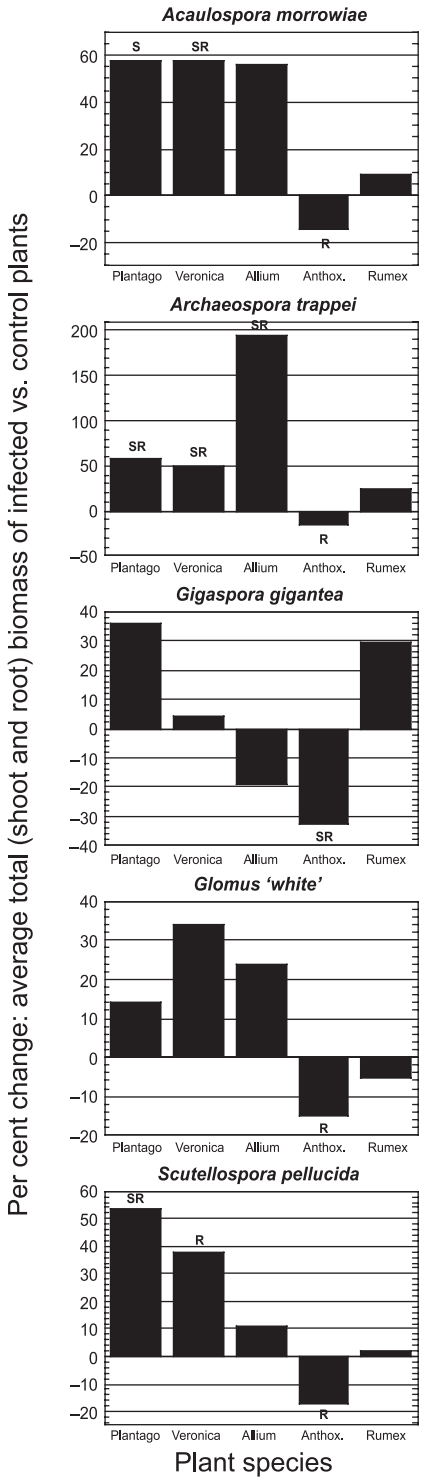

Fig. 3 Total biomass of plants infected with diverse arbuscular mycorrhizal (AM) fungi, grouped by AM fungus; laboratory data. The average (shoot and root) biomass of plants infected with a single fungus was standardized to the weight of uninfected plants by dividing infected weight by control weight and multiplying by 100. Therefore, departures from the zero line indicate a gain or loss of biomass (relative to uninfected plants). An S or R above a bar indicates that either shoot or root biomass was significantly different from control shoot or root biomass.

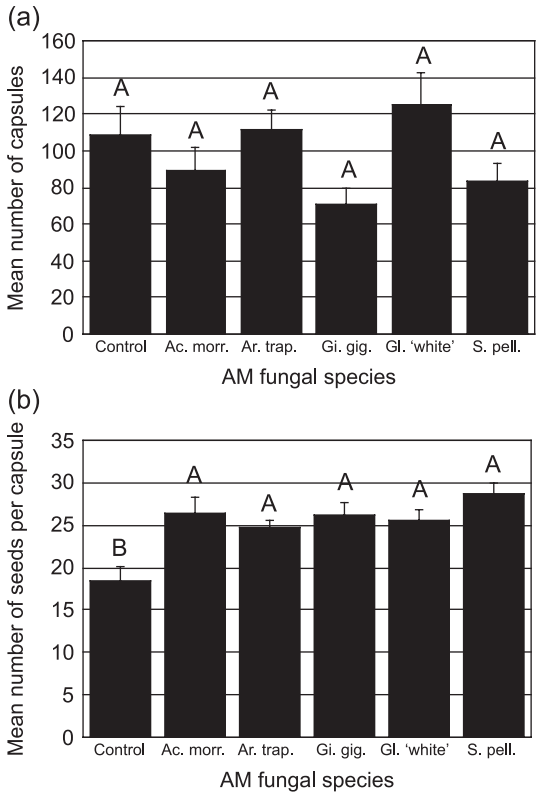

Fig. 4 Fitness estimates for *Cerastium glomeratum*. Arbuscular mycorrhizal (AM) fungal species abbreviations: control, uninfected plants; Ac. morr., *Acaulospora morrowiae*; Ar. trap., *Archaeospora trappei*; Gi. gig., *Gigaspora gigantea*; Gl. 'white', *Glomus* sp.; S. pell., *Scutellospora pellucida*. Standard errors are given for each plant and fungal combination; letters indicate significant differences between treatments. Note that different estimates of fitness (number of capsules vs seeds per capsule) give different kinds of information on the effectiveness of AM fungi (see also Nuortila *et al*., 2004).

that plant species differed in their responses to infection by AM fungal species, as is illustrated by the growth of *Allium* and *Plantago* or *Rumex* (Tables 3, 4, Fig. 5). *Plantago* and *Rumex* grew equally well with every species of AM fungus, but *Allium* grew best with *A. trappei*. Finally, the interaction of fungal species and block was not significant, indicating that environmental heterogeneity within the experimental area did not substantially modify plant response to different AM fungi. Further analyses focused on the data for individual plant species.

The growth of *Anthoxanthum*, *Plantago* and *Rumex* plants was significantly influenced by both initial size at transplanting and experimental block, but was unaffected by infection with AM fungal species (although data for *Anthoxanthum* inflorescence length approached significance) (Table 4).

Allium bulb weights were significantly influenced by initial size at transplanting, experimental block and AM fungal treatment (Table 4, Fig. 5). *Allium* plants infected with

Table 3 Effects of plant species, initial size, block and arbuscular mycorrhizal (AM) fungal species on fitness[1] of field plants (ANOVA of ranked data)

Effect of:	d.f.	*F*	*p*
Plant species	4	1.2	NS
Initial size	1	30.65	0.0001
Block	19	4.67	0.0001
AM fungal species	4	1.64	NS
Plant species × block interaction	66	1.26	NS
Initial size × plant species interaction	4	7.86	0.0001
Block × AM fungal species interaction	76	1.02	NS
Plant species × AM fungal species interaction	16	2.27	0.004

[1]Fitness was estimated from harvest weights for *Allium*, *Plantago* and *Rumex* plants, and from reproductive data for *Anthoxanthum* and *Veronica* plants. The error degrees of freedom is 225. NS, not significant.

Table 4 Effects of initial size, block and arbuscular mycorrhizal (AM) fungal species on fitness[1] of individual plant species grown in the field (ANOVA of ranked data of individual species)

	Effect of:	d.f.[2]	*F*	*p*
Allium	Initial size	1 (35)	15.53	0.0004
	Block	9 (35)	2.38	0.03
	AM fungal species	4 (35)	4.42	0.005
Anthoxanthum	Initial size	1 (74)	5.33	0.02
	Block	19 (74)	1.79	0.04
	AM fungal species	4 (74)	2.21	0.07
Plantago	Initial size	1 (68)	5.26	0.02
	Block	19 (68)	2.35	0.005
	AM fungal species	4 (68)	1.08	NS
Rumex	Initial size	1 (56)	20.95	0.0001
	Block	19 (56)	2	0.02
	AM fungal species	4 (56)	1.35	NS
Veronica	Initial size	1 (68)	37.79	0.0001
	Block	19 (68)	2.46	0.004
	AM fungal species	4 (68)	6.45	0.0002

[1]Fitness was estimated from harvest weights for *Allium*, *Plantago* and *Rumex* plants and from reproductive data for *Anthoxanthum* and *Veronica* plants.
[2]Error degrees of freedom are reported in parentheses. NS, not significant.

A. trappei grew significantly better than *Allium* plants infected with *A. morrowiae*, *G. gigantea*, or *S. pellucida*.

Veronica capsule number was also significantly influenced by initial size at transplanting, experimental block and AM fungal treatment (Table 4, Fig. 5). In contrast to the data for *Allium*, *Veronica* plants infected with *G. gigantea* grew significantly fewer capsules than plants infected with other AM fungi.

In this North Carolina field the roots of single plants are difficult to separate from a surrounding matrix of other plant roots and soil, and AM fungi were not isolated from the roots of plants used in the field experiment. However, it is unlikely that anything other than the AM fungal infections drove our results. First, the correlations between laboratory and field data (see below) suggest that secondary infections did not overwhelm the initial inoculation with target species of AM fungi. Moreover, to invoke secondary infections as the cause of observed patterns of plant growth would require the same microbe to have infected a particular combination of plant and fungus in each of the 20 replicates blocked across the North Carolina field; most soil microbes have patchy distributions (Ettema & Wardle, 2002) and this seems unlikely.

Comparison of field and growth chamber data

The growth of plant species associated with different AM fungi in the field was predicted by their growth with the same fungi in the laboratory, at least in some cases. The observed effects of AM fungal species on *Allium* in the field were strongly predicted by our observations of *Allium* associated with the same fungi in the growth chamber (Table 5, Fig. 6). To a lesser degree, the performances of *Veronica* and *Plantago* in the field were also predicted by their growth with AM

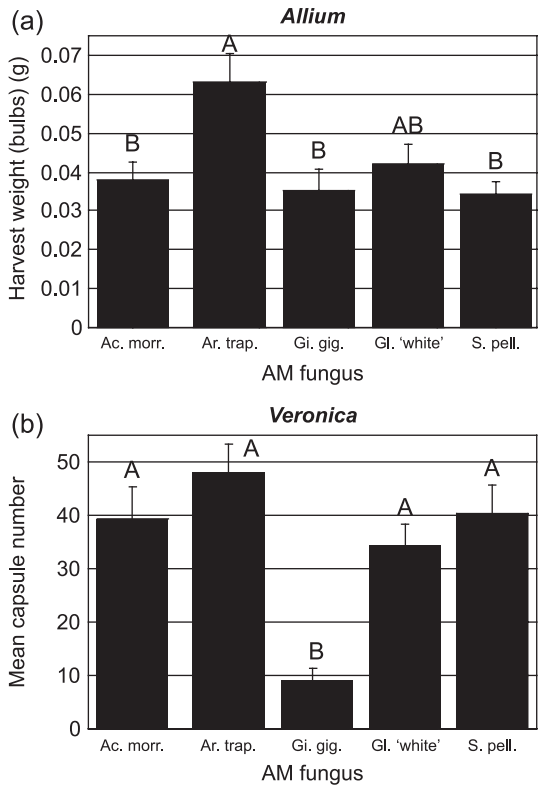

Fig. 5 Data from the field experiment. Standard errors are given for each plant and fungal combination; letters indicate significant differences between treatments. Arbuscular mycorrhizal (AM) fungal species abbreviations: Ac. morr., *Acaulospora morrowiae*; Ar. trap., *Archaeospora trappei*; Gi. gig., *Gigaspora gigantea*; Gl. 'white', *Glomus* sp.; S. pell., *Scutellospora pellucida*.

Table 5 Comparison of field and laboratory data

Plant	d.f.	r^2
Allium	4	0.912**
Anthoxanthum	4	0.007
Plantago	4	0.618†
Rumex	4	0.155
Veronica	4	0.659*

The table shows regression statistics of mean growth response when associated with individual fungal species in the field as predicted by growth of plant species with the same fungal species in the growth chamber. There is a strong correlation between the strength of these regressions, as measured using the r^2 statistic, and the general responsiveness of the same species to inoculation with any arbuscular mycorrhizal (AM) fungus, as measured using *F*-statistics of the contrasts between control and inoculated plants (see the Results). Significance: **, $P < 0.01$; *, $P < 0.05$; †, $P < 0.06$ (using one-sided tests).

fungal species in the laboratory (Fig. 6), but there was no relationship between the field and growth chamber data collected for *Anthoxanthum* or *Rumex* (Table 5).

Parallels between field and growth chamber data were predicted by the average response of a plant species to laboratory inoculations by any AM fungus. While *Allium* was greatly influenced by associations with any AM fungus (see 'AMF vs sterile' contrasts for root and shoot data, Table 2), *Anthoxanthum* and *Rumex* were the two species that did not normally show a response to inoculation with AM fungi in the growth chambers (Table 2; note the difference between *Anthoxanthum* shoot and root analyses). More generally, the *F*-statistics of the 'AMF vs sterile' contrasts for total plant biomass and the r^2 of the regressions of field and growth chamber data (Table 5) were strongly correlated (Kendall's $\tau = 1.0$, d.f. = 4, $P = 0.014$).

Discussion

Experiments were used to explore the benefits derived by plants in association with AM fungi, both in the laboratory and in the field, and the data can be used to draw three general conclusions. First, data confirm that the effects of a mycorrhizal association vary dramatically as a single plant species is grown with different species of mycorrhizal fungi. Second, although it is clear that both the plant and the fungus must be identified to determine if an association will provide a benefit to the plant, as for example *A. trappei* may benefit *Allium* but parasitize *Anthoxanthum* (Fig. 3), it is also true that a single fungal species that provides (or does not provide) a benefit to one plant species is likely to provide (or not provide) a benefit to other species. Despite its parasitism of *Anthoxanthum* roots, *A. trappei* was among the most beneficial mycorrhizal fungi in each experiment for every plant species (Fig. 3).

Finally, the data provide evidence that the identity of the mycorrhizal fungi associated with plants in the field does matter to plant success in nature, at least for some plant species. One limitation of these data is that colonization data were not obtained for plants as they were moved from the growth chamber to the field, and different intensities of infection may have a played a role in our results. However, the data available suggest that in many cases patterns observed in the laboratory translate to the field. The ability of glasshouse experiments to predict dynamics in nature is correlated with the general responsiveness of plant species to infection with AM fungi (often discussed as 'mycotrophy' or 'mycorrhizal dependence'; see Janos, 2007). Those plant species that are most responsive to infection are also the species for which correlations between growth chamber and field data are strongest.

The effects of a mycorrhizal association vary as different combinations of plants and fungi are tested; nonetheless, there are patterns to be found

There is a tension in the literature between the classification and use of AM fungal species as either mutualists or parasites

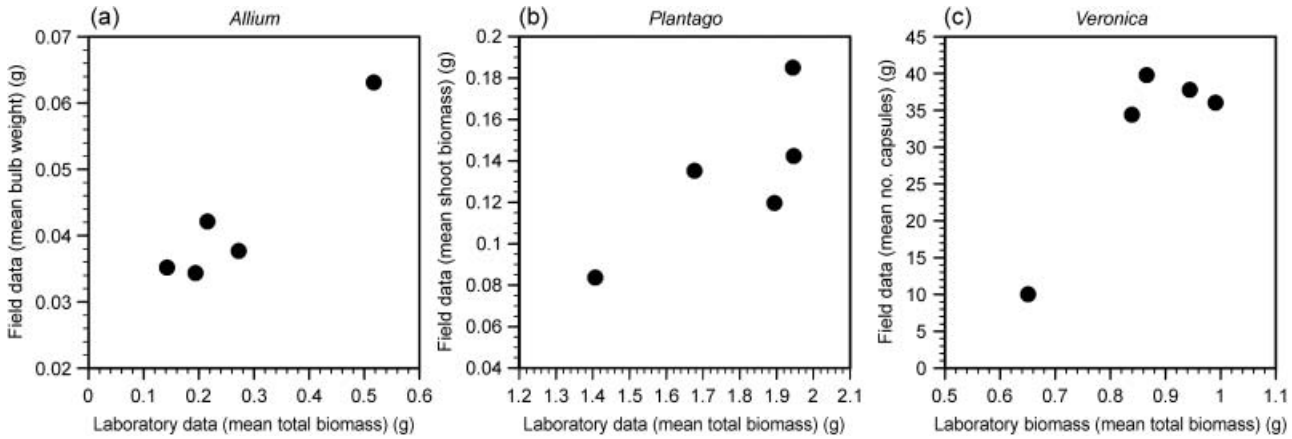

Fig. 6 Plots of field and laboratory data. Each panel shows means for either biomass or reproductive data, as noted on axes, and the data within each panel are grouped according to arbuscular mycorrhizal (AM) fungal species so that, for example, growth of *Allium* with *Archaeospora trappei* in the field is compared to growth of *Allium* with *A. trappei* in the laboratory. Field data are given using least-squares means as these disentangle the effects of initial size at planting from AM fungal effects, and provide data on size or reproduction at harvest as driven by the different AM fungi. Data are shown for plant species where laboratory and field data were significantly correlated (see Table 5).

(e.g. Smith & Smith, 1996), and an explicit rejection of AM fungal identity as a predictor of function (Pringle, 2001; Klironomos, 2003; see Johnson *et al.*, 1997 and Kiers & van der Heijden, 2006 for discussions of the genetic basis of the symbiotic phenotype). There is no doubt that the same fungal species or culture can be more or less effective when combined with different plant species (Helgason *et al.*, 2002). Klironomos (2003) found the range of effects to be greatest when fungi and plants were isolated from the same habitat. This variability is used to predict plant and fungal community dynamics; for example, the positive and negative feedbacks that may control biodiversity (Bever, 2002; Bever *et al.*, 2002; Castelli & Casper, 2003; Kardol *et al.*, 2006). In our own experiments, statistical interactions of the effects of fungal species among plant species were significant, and confirm that a single fungal species affects diverse plant species differently.

However, there was also a degree of consistency in the effects of AM fungal species, as is evident from both the significant 'among AMF' contrast of the laboratory experiment (Table 1) and a visual inspection of the data (Figs 2 and 3). For example, *A. trappei* generally promoted the growth of plants. Plants infected with *G. gigantea* or *Glomus* 'white' were more often than not no different from uninfected controls. AM fungi are used as organic fertilizers (Gianinazzi & Vosatka, 2004; Schwartz *et al.*, 2006), and when sold consistency is an implicit assumption (i.e. the fungus X will provide a benefit to any plant). Other experiments with diverse *Glomus*, *Acaulospora*, *Gigaspora* and *Scutellospora* isolates also suggest that fungal identity can be used to predict which species will effectively promote the growth of different plants over a range of edaphic conditions (Syliva *et al.*, 1993; Graham *et al.*, 1996; Graham & Abbott, 2000). Smith & Smith (1996) synthesized several lines of evidence to suggest that *S. calospora* is typically a parasite. Different aspects of fungal biology are known to be constant across environments; for example, phenology – *G. gigantea* sporulates in late fall and winter in both the North Carolina field and in Rhode Island sand dunes (Pringle & Bever, 2001). Perhaps the benefits provided to plants will correlate with these other aspects of fungal biology that are also a constant feature of the species or family; for example, a larger mycelium within plant roots (Hart & Reader, 2002). Of course, plant species themselves are often described as 'mycotrophic' or 'not mycotrophic', suggesting that particular plants either do or do not benefit from infection, regardless of the fungal symbiont. Aspects of our laboratory experiment support that idea (see discussion below).

But there are clear limits to the constancy of AM fungal phenotypes. Data from other groups of fungi make clear that mutualism, parasitism, and saprotrophy are labile characters (Hibbett & Donoghue, 1995; Hibbett *et al.*, 2000), and an AM fungal culture isolated from a single spore will evolve in the laboratory as it is propagated from pot to pot (Feldman, 1998; Malcová *et al.*, 2003; JDB, unpublished). The cultures that result may provide a very different level of benefit as compared with the original culture. In nature, single morphological species allopatrically isolated across Europe, and between Europe and South America, have evolved very different behaviors as tested in association with the cucumber plant (*Cucumis sativus*; Munkvold *et al.*, 2004); further evidence for the lability of function within an AM fungus. Even at a single site there are both phenotypic differences associated with the genetic diversity within a morphological species (Koch *et al.*, 2004) and intraspecific differences in the benefit provided by a morphological species (Castelli & Casper, 2003; Koch *et al.*, 2006).

A resolution of these questions – is the symbiotic phenotype (mutualism vs parasitism) labile and genetically based, or controlled by plant genetics and environmental cues; which aspects of fungal biology are constant and which are not? – will require manipulative experiments in which cultures are artificially selected for different effects on plants, and the evolutionary capacity of various fungal species explicitly tested.

Parasitism and patterns of plant growth within the laboratory

The classification of AM fungal species according to phenotype would be analogous to the classification of plant species or families as more or less responsive or dependent on mycorrhizal fungi (Janos, 2007). In fact our experiments were designed to include a diversity of plants more or less likely to experience mutualism or parasitism, as based on Francis & Read's (1995) observations. Results generally support their hypotheses. As expected, *Allium* and *Plantago* (Liliaceae and Plantaginaceae; mutualism) derived a benefit from associations with AM fungi. Although adult *Rumex* (Polygonaceae; antagonism) plants were largely unperturbed by mycorrhizal associations, *Rumex* seedlings experienced significant mortality when grown with AM fungi. However, in this experiment, neither *Anthoxanthum* nor *Cerastium* (Graminae and Caryophyllaceae; neutralism) interacted entirely neutrally with AM fungi. Roots of *Anthoxanthum* were generally parasitized in mycorrhizal associations, and *Cerastium* derived a slight benefit in the same associations. Finally, contrary to the prediction of Francis & Read (1995), *Veronica* (Scrophulariaceae; antagonism) appeared to derive a benefit from its associations with mycorrhizal fungi, both in the laboratory and in the field. Francis & Read's (1995) hypotheses emerge as a useful guide and an illustration of the potential for generalizations across taxa, but as with the AM fungi, individual cases must be tested before mutualism or parasitism is assumed.

In the laboratory parasitism was rare and involved a single species. The roots of *Anthoxanthum* did not grow as well when associated with mycorrhizal fungi, although shoots were generally unaffected (Table 2, Fig. 2). *Anthoxanthum* is abundant in the grassland community, which suggests either that AM fungi offer *Anthoxanthum* an alternative benefit in nature (e.g. defense against fungal pathogens; Borowicz, 2001) or that *Anthoxanthum* is remarkably tolerant of AM fungal infection.

Parallels between laboratory and field experiments

Field and laboratory data were broadly comparable. First, the death of *Rumex* in the field mirrors the early mortality experienced by *Rumex* seedlings in the laboratory experiment; in both cases, *Rumex* infected by *S. pellucida* experienced disproportionate fatalities. Moreover, the growth of *Allium*, *Plantago*, and *Veronica* when associated with different species of AM fungi in the field was correlated to their growth with the same fungal species in the growth chamber (Figs 2, 5, 6, Table 5). These data reflect a more general result; plant species found to be highly responsive to inoculation with any AM fungus in the laboratory were more likely to show analogous effects among laboratory and field responses to individual species.

Other data on AM fungal effects in the field are concentrated within the agricultural and restoration ecology literature. Two separate meta-analyses of the agricultural literature arrive at somewhat different conclusions: McGonigle (1988) concluded that direct evidence for a benefit from AM fungi in the field is weak. Lekberg & Koide (2005) were more optimistic about the possibility of AM fungi functioning as mutualists in the field, and concluded that even a weakly significant relationship between mycorrhizal colonization and yield or biomass is strong evidence for the real potential for mutualism. Neither of the meta-analyses explicitly discussed whether different AM fungal species are more or less effective across field trials.

The literature on land restoration generally demonstrates that AM fungi facilitate the growth of plants after disturbance (e.g. Aldon, 1975 on a coal mine spoil; Smith *et al.*, 1998 in a tall grass prairie restoration; Allen *et al.*, 2003 and Allen *et al.*, 2005 in a seasonal tropical forest). This literature is also focused on the presence or absence of mycorrhizal fungi, rather than the different effects of different species.

Our data suggest that AM fungal identity influences plant growth and reproduction in the field, and that finding may have implications for both agriculture (Kiers *et al.*, 2002; Gosling *et al.*, 2006) and restoration ecology. For example, later successional species are more likely to be responsive to AM fungi (Janos, 1980; Reynolds *et al.*, 2003) and are often the focus of restoration efforts. Because the identity of the AM fungi used in inoculations appears to matter most when plant species are generally responsive to inoculation, inoculation with a single species of fungus known to provide a benefit may be more useful than inoculation with a mixture of untested species when late successional plants are targeted. Care should also be taken when choosing AM fungal species as fertilizers for agricultural crops known to be highly responsive to inoculation, because in these cases AM fungal identity is likely to have a great influence on yield.

Acknowledgements

The authors would like to thank J. Bronstein, J. Fink, B. Morris and L. Moyle for advice and help with the manuscript. AP would like to thank D. Johnson, the Antonovics Laboratory, M. Chin and P. Chin-Henkel, L. Jancaitis, R. Kehrer, L. Moyle and M. Sipes for aid in data collection; and B. E. Mills for completing the P analyses. AP gratefully acknowledges the support of the National Science Foundation, the Miller

Institute for Basic Research in Science, and Harvard University. JDB acknowledges the support of the National Science Foundation and a Harvard Forest Charles Bullard Fellowship in Forest Research.

References

Aldon EF. 1975. *Endomycorrhizae enhance survival and growth of fourwing saltbush on coal mine spoils. USDA Forest Service Research Note RM-294*. Fort Collins, CO, USA: Rocky Mountain Forest and Range Experiment Station.

Allen EB, Allen MF, Egerton-Warburton L, Corkidi L, Gómez-Pompa A. 2003. Impacts of early- and late-seral mycorrhizae during restoration in seasonal tropical forest, Mexico. *Ecological Applications* **13**: 1701–1717.

Allen MF, Allen EB, Gómez-Pompa A. 2005. Effects of mycorrhizae and nontarget organisms on restoration of a seasonal tropical forest in Quintana Roo, Mexico: Factors limiting tree establishment. *Restoration Ecology* **13**: 325–333.

Bever JD. 1992. Ecological and evolutionary dynamics between plants and their soil communities. PhD thesis, Durham, NC, USA: Duke University.

Bever JD. 1999. Dynamics within mutualism and the maintenance of diversity: inference from a model of interguild frequency dependence. *Ecology Letters* **2**: 52–62.

Bever JD. 2002. Negative feedback within a mutualism: Host-specific growth of mycorrhizal fungi reduces plant benefit. *Proceedings of the Royal Society of London* **269**: 2595–2601.

Bever JD, Morton JB. 1999. Heritable variation of spore shape in a population of arbuscular mycorrhizal fungi: suggestions of a novel mechanism of inheritance. *American Journal of Botany* **86**: 1209–1216.

Bever JD, Morton JB, Antonovics J, Schultz PA. 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *Journal of Ecology* **84**: 71–82.

Bever JD, Pringle A, Schultz PA. 2002. Dynamics within the plant-arbuscular mycorrhizal fungal mutualism: testing the nature of community feedback. In: Van der Heijden MGA, Sanders I, eds. *Mycorrhizal ecology*. Berlin, Germany: Springer-Verlag, 267–292.

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.

Borowicz VA. 2001. Do arbuscular mycorrhizal fungi alter plant-pathogen relations? *Ecology* **82**: 3057–3068.

Castelli, JP, Casper BB. 2003. Intraspecific AM fungal variation contributes to plant-fungal feedback in a serpentine grassland. *Ecology* **84**: 323–336.

Clay K. 1982. Ecological and genetic consequences of cleistogamy in the grass *Danthonia spicata*. PhD thesis, Durham, NC, USA: Duke University.

Cook BD, Jastrow JD, Miller RM. 1988. Root and mycorrhizal endophyte development in a chronosequence of restored tall grass prairie. *New Phytologist* **110**: 355–362.

Ettema CH, Wardle DA. 2002. Spatial soil ecology. *Trends in Ecology and Evolution* **17**: 177–183.

Feldman F. 1998. The strain-inherent variability of arbuscular mycorrhizal effectiveness: II. Effectiveness of single spores. *Symbiosis* **25**: 131–143.

Fitter AH. 1985. Functioning of vesicular-arbuscular mycorrhizas under field conditions. *New Phytologist* **99**: 257–265.

Fitter AH. 1990. The role and ecological significance of vesicular-arbuscular mycorrhizas in temperate ecosystems. *Agriculture, Ecosystems and Environment* **29**: 137–151.

Fowler NL. 1978. Competition and coexistence in a North Carolina grassland. PhD thesis, Durham, NC, USA: Duke University.

Fowler NL, Antonovics J. 1981. Competition and coexistence in a North Carolina grassland I. Patterns in undisturbed vegetation. *Journal of Ecology* **69**: 825–841.

Francis R, Read DJ. 1995. Mutualism and antagonism in the mycorrhizal symbiosis, with special reference to impacts on plant community structure. *Canadian Journal of Botany* **73** (Suppl. 1): 1301–1309.

Gianinazzi S, Vosatka M. 2004. Inoculum of arbuscular mycorrhizal fungi for production systems, science meets business. *Canadian Journal of Botany* **82**: 1264–1271.

Gosling P, Hodge A, Goodlass G, Bending GD. 2006. Arbuscular mycorrhizal fungi and organic farming. *Agriculture, Ecosystems and Environment* **113**: 17–35.

Graham JH, Abbott LK. 2000. Wheat responses to aggressive and non aggressive arbuscular mycorrhizal fungi. *Plant and Soil* **220**: 207–218.

Graham JH, Drouillard DL, Hodge NC. 1996. Carbon economy of sour orange in response to different *Glomus* spp. *Tree Physiology* **16**: 1023–1029.

Hart MM, Reader RJ. 2002. Host plant benefit from association with arbuscular mycorrhizal fungi: variation due to differences in size of mycelium. *Biology and Fertility of Soils* **36**: 357–366.

Hart MM, Reader RJ, Klironomos JN. 2003. Plant coexistence mediated by arbuscular mycorrhizal fungi. *Trends in Ecology and Evolution* **18**: 418–423.

Hayman DS, Mosse B. 1971. Plant growth responses to vesicular-arbuscular mycorrhiza. I. Growth of endogone inoculated plants in phosphate deficient soils. *New Phytologist* **70**: 19–27.

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH. 2002. Selectivity and functional diversity in arbuscular mycorrhizas of co-occuring fungi and plants from a temperate deciduous woodland. *Journal of Ecology* **90**: 371–384.

Hetrick BAD, Bockus WW, Bloom J. 1984. The role of vesicular-arbuscular mycorrhizal fungi in the growth of Kansas winter wheat. *Canadian Journal of Botany* **62**: 735–740.

Hibbett DS, Donoghue MJ. 1995. Progress toward a phylogenetic classification of the polyporaceae through parsimony analysis of mitochondrial ribosomal DNA sequences. *Canadian Journal of Botany* **73** (Suppl. 1): S853–S861.

Hibbett DS, Gilbert L, Donoghue MJ. 2000. Evolutionary instability of ectomycorrhizal symbioses in basidiomycetes. *Nature* **407**: 506–508.

Janos DP. 1980. Mycorrhizae influence tropical succession. *Biotropica* **12**: 56–64.

Janos DP. 2007. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. *Mycorrhiza* **17**: 75–91.

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

Kardol P, Martijn Bezemer T, van der Putten WH. 2006. Temporal variation in plant-soil feedback controls succession. *Ecology Letters* **9**: 1080–1088.

Kelley SE. 1985. The mechanism of sib competition for the maintenance of sexual reproduction in *Anthoxanthum odoratum* L. PhD thesis, Durham, NC, USA: Duke University.

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

Kiers ET, West SA, Denison RF. 2002. Mediating mutualisms: farm management practices and evolutionary changes in symbiont co-operation. *Journal of Applied Ecology* **39**: 745–754.

Klironomos JN. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* **84**: 2292–2301.

Koch AM, Croll D, Sanders IR. 2006. Genetic variability in a population of arbuscular mycorrhizal fungi causes variation in plant growth. *Ecology Letters* **9**: 103–110.

Koch AM, Kuhn G, Fontanillas P, Fumagalli L, Goudet I, Sanders IR.

2004. High genetic variability and low local diversity in a population of arbuscular mycorrhizal fungi. *Proceedings of the National Academy of Sciences, USA* **101**: 2369–2374.

Koide RT, Shumway DL, Mabon SA. 1994. Mycorrhizal fungi and reproduction of field population of *Abutilon theophrasti* Medic. (Malvaceae). *New Phytologist* **126**: 123–130.

Lekberg Y, Koide RT. 2005. Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytologist* **168**: 189–204.

Malcová R, Rydlová J, Vosátka M. 2003. Metal-free cultivation of *Glomus* sp. BEG 140 isolated from Mn-contaminated soil reduces tolerance to Mn. *Mycorrhiza* **13**: 151–157.

McGonigle TP. 1988. A numerical analysis of published field trials with vesicular-arbuscular mycorrhizal fungi. *Functional Ecology* **2**: 473–478.

Miller RM, Jarstfer AG, Pillai JK. 1987. Biomass allocation in an *Agropyron smithii-Glomus* symbiosis. *American Journal of Botany* **74**: 114–122.

Moloney KA. 1986. Determinants of species distributions: *Danthonia sericea* as a model system. PhD thesis, Durham, NC, USA: Duke University.

Mosse B, Hayman DS. 1971. Plant growth responses to vesicular-arbuscular mycorrhiza. II. In unsterilized field soils. *New Phytologist* **70**: 29–34.

Munkvold L, Kjøller R, Vestberg M, Rosendahl S, Jakobsen I. 2004. High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist* **164**: 357–364.

Newsham KK, Fitter AH, Watkinson AR. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal of Ecology* **83**: 991–1000.

Nuortila C, Kytoviita MM, Tuomi J. 2004. Mycorrhizal symbiosis has contrasting effects on fitness components in *Campanula rotundifolia*. *New Phytologist* **164**: 543–553.

Pendleton RL. 2000. Pre-inoculation by an arbuscular mycorrhizal fungus enhances male reproductive output of *Cucurbita foetidissima*. *International Journal of Plant Science* **161**: 683–689.

Pringle A. 2001. Ecology and genetics of arbuscular mycorrhizal fungi. PhD thesis, Durham, NC, USA: Duke University.

Pringle A, Bever JD. 2001. Divergent phenologies may facilitate the coexistence of arbuscular mycorrhizal fungi in a North Carolina grassland. *American Journal of Botany* **89**: 1439–1446.

Read DJ. 2002. Towards ecological relevance – progress and pitfalls in the path towards an understanding of mycorrhizal functions in nature. In: Van der Heijden MGA, Sanders IR, eds. *Mycorrhizal ecology*. Berlin, Germany: Springer, 3–24.

Reynolds H, Packer A, Bever JD, Clay K. 2003. Grassroots ecology: Plant–microbe-soil interactions as drivers of plant community structure and dynamics. *Ecology* **84**: 2281–2291.

Sanders IR, Koide RT. 1994. Nutrient acquisition and community structure in co-occurring mycotrophic and non mycotrophic old-field annuals. *Functional Ecology* **8**: 77–84.

SAS Institute Inc. 1996. *SAS version 6.12*. Cary, NC, USA: SAS Institute Inc.

Schultz PA. 1996. Arbuscular mycorrhizal species diversity and distribution in an old field community. PhD thesis, Durham, NC, USA: Duke University.

Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A. 2006. The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecology Letters* **9**: 601–616.

Smith MR, Charvat I, Jacobson RL. 1998. Arbuscular mycorrhizae promote establishment of prairie species in a tallgrass prairie restoration. *Canadian Journal of Botany* **76**: 1947–1954.

Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. San Diego, CA, USA: Academic Press.

Smith FA, Smith SE. 1996. Mutualism and parasitism: diversity in function and structure in the 'arbuscular' (VA) mycorrhizal symbiosis. *Advances in Botanical Research* **22**: 1–43.

Streitwolf-Engel R, Boller T, Wiemken T, Sanders IR. 1997. Clonal growth traits of two *Prunella* species are determined by co-occurring arbuscular mycorrhizal fungi from a calcareous grassland. *Journal of Ecology* **85**: 181–191.

Syliva DM, Wilson DO, Graham JH, Maddox JJ, Millner P, Morton JB, Skipper HD, Wright SF, Jarstfer AG. 1993. Evaluation of vesicular-arbuscular mycorrhizal fungi in diverse plants and soils. *Soil Biology and Biochemistry* **25**: 705–713.

Van der Heijden MGA, Boller T, Wiemken A, Sanders IR. 1998a. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* **79**: 2082–2091.

Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR. 1998b. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69–72.

Vogelsang KM, Reynolds HL, Bever JD. 2006. Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytologist* **172**: 554–562.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Initial plant size as influenced by different arbuscular mycorrhizal (AM) fungal species and growth chamber.

Please note: Blackwell Publishing are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.