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Specificity and resilience in the arbuscular mycorrhizal fungi of a natural woodland community

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SUMMARY

1. While tThe composition of communities of arbuscular mycorrhizal (AM) fungi can have a large effect on the performance of their plant hosts, but role of individual fungal species in shaping this response is as yet unresolved.
2. We have used the fungicide benomyl to alter the community of AM fungi in undisturbed monoliths of soil in a natural community. Changes in the community were characterised by root colonisation (%RLC), cloning, sequencing and tRFLP of a partial SSUrDNA fragment. Eleven plant species were sufficiently abundant in the monoliths to be examined.
3. In the highly mycorrhiza-dependent perennial herb *Ajuga reptans*, phosphate concentration was significantly reduced after benomyl treatment over a full growing season. The other plant species showed low colonisation and no significant difference in phosphate concentration after benomyl treatment.
4. Although colonisation in *A. reptans* was reduced, many mycorrhizal fungi survived in the roots. Some became more abundant following fungicide treatment, suggesting competitive release. Fungi that increased were generalists that have been identified in field samples from published studies colonising a wide range of plant species. Those that declined were specialists with a narrow host range; five types had not been recorded previously in field samples.
5. AM fungi in this study differed greatly in their response to perturbation, independent of the identity of the host plant. If such functional diversity is widespread, then elucidating the part played by AM fungal diversity in regulating plant community structure will be key to our understanding and management of ecosystems.

Keywords: arbuscular mycorrhizal fungi, *Ajuga reptans*, host range, Glomeromycota, phosphorous, SSUrDNA

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INTRODUCTION

Uncultured micro-organisms dominate soil ecosystems (Fitter 2005). One key group in which many species may be unculturable is the Phylum Glomeromycota that forms arbuscular mycorrhizas (AM). These fungi are among the most abundant and ecologically important symbionts on earth, forming mycorrhizas with around two-thirds of all plant species and occurring in virtually all ecosystems. The Glomeromycota were traditionally viewed as a species-poor phylum. Relatively few AM fungal species have been successfully grown in culture, with all of them observed only in symbiosis with a host plant. Almost all AM fungi are non-specific symbionts, readily colonising the roots of most plant species they encounter (Smith & Read 1997). This fact, combined with the apparent low diversity of this taxon (150-200 described species, Morton & Benny 1990) and its enormous host range (>2 x 10^5 plant species; Fitter & Moyersoen 1996), has led to the view that AM fungi are non-specific. If so, we would predict that AM communities would not show a high degree of differentiation among host plants. However, results of spore-based morphological studies (Sanders & Fitter 1992; Bever *et al.* 1996; Eom *et al.* 2000) and molecular analyses of the occurrence of AM fungi within the root systems of plants collected in natural communities (Clapp *et al.*1995; Helgason *et al.* 2002; Husband *et al.* 2002; Vandenkoornhuyse *et al.* 2003; Öpik *et al.* 2003; Johnson *et al.* 2004; Santos *et al.* 2006) demonstrate not only that the genetic diversity of these fungi is much greater than the morphological taxonomy suggests, but also that different plant species often harbour quite distinct AM fungal communities. This suggests that even where AM fungi display a high degree of non-specificity, other factors are influencing community structure. Since the majority of the fungi revealed by molecular techniques are apparently unknown in culture, the question has been raised as to

whether these uncharacterised species play distinct ecological roles and may be responsible for some of the profound ecological impacts of AM fungi (Newsham *et al.* 1994; van der Heijden *et al.* 1998; Hartnett & Wilson 1999; Dhillion & Gardsjord 2004).

Moora *et al.* (2004) recently showed that different communities of AM fungi had large effects on the performance of two closely-related, co-existing plant species. What remains to be resolved is the extent to which particular members of the AM fungal community associated with a plant species have distinctive impacts on plant performance. The null hypothesis must be that all the fungi act in the same way, transporting P to the plant, protecting it from pathogens, binding roots to soil and hence promoting drought resistance, and performing any of the other functions that have been ascribed to mycorrhizas (Newsham *et al.* 1995). However, many of these activities are mutually incompatible: enhancing P uptake depends on growth of the extra-radical mycelium outside the rhizosphere (Avio *et al.* 2006, Oliveira *et al.* 2006), promoting drought resistance requires mycelial growth in the rhizosphere, and pathogen protection must depend on the internal mycelium. It seems inherently more likely, therefore, that co-existing fungi have distinct functional capabilities (van der Heijden *et al.* 2003; Oliveira *et al.* 2006). Those fungal taxa that display a degree of specificity towards particular host plants are likely to offer those plant species the greatest symbiotic benefit, since there is likely to have been a co-evolutionary response by both partners (Fitter 2005).

In an earlier laboratory study (Helgason *et al.* 2002), we showed that plants from a semi-natural woodland varied both in their overall response to AM colonisation, and to specific AM fungi. The same study suggested that AM fungi display a range of colonisation strategies. In particular, the taxa that were able to colonise all the host

plants were those that resulted in a consistent but small benefit to the plant, measured as P acquisition; these we identified as essentially opportunist taxa. Here, we have used the fungicide benomyl to alter the fungal community colonising roots of the same natural plant community used previously by Helgason *et al* (2002). We hypothesised that the fungicide would not affect all fungi equally, and that the fungi most likely to recover quickly from this environmental perturbation would be *r*-selected fungi that display "weedy" characteristics of broad host range and weak symbiotic effectiveness. We also predicted that a change in the fungal community favouring generalist types would be accompanied as before by a reduction in plant phosphate uptake, at least in the most mycorrhiza-dependent plants, because the less effective symbionts would be more abundant.

MATERIALS AND METHODS

Experimental Design

The experiment was conducted at Pretty Wood near York, UK (grid ref. SE 732 867, altitude 50 m), under a mature canopy of *Quercus petraea* (Mattuschka) Liebl. and sycamore *Acer pseudoplatanus* L**.** We used the fungicide benomyl ([1-[(Butylamino)carbonyl]-1H-benzimidazol-2-yl] carbamic acid methyl ester) at a concentration of 1.125 g l^{-1} to disrupt the AM fungal community associated with the ground flora in this woodland. Benomyl has been widely used to reduce root colonisation by AM fungi (Fitter & Nichols 1988; Newsham & Fitter 1994; Hartnett & Wilson 1999; Smith *et al.* 2000; Smilauer & Smilauerova 2000; Callaway *et al.* 2004). However, it penetrates soil poorly and we adapted the technique we previously used at the same site (Merryweather & Fitter 1996) in which Entire

monoliths of soil (with growing plants) were lifted intact, placed in mesh baskets, replaced *in situ* and raised regularly for immersion in a bath of fungicide for 3 mins, found to be sufficient time for the monolith to be thoroughly drenched. Control baskets were immersed in water at the same time.

The experiment was set up during September and October 1996. Twenty-four cubic monoliths of soil (30 x 30 x 30 cm; volume 27 l) were created in three blocks of eight in adjacent sites in the wood, no more than 100 m apart, selected to represent distinct sub-communities to allow for variation in plant community structure and to ensure that some plant species were well represented in the experiment. Each basket contained between 3 and 6 plant species; block 1 all had *Primula vulgaris* Huds. and *Glechoma hederacea* L., block 2 all had *Ajuga reptans* L. and *Teucrium scorodonia* L., and block 3 all had *Mercurialis perennis* L. Across all 24 baskets, the most abundant species were *G. hederacea* and *Poa trivialis* L. (17/24), *A. reptans* (18/24), *Oxalis acetosella* L. (13/24) and *Lysimachia nummularia* L. (12/24). The treatments (plus and minus fungicide) were allocated randomly but equitably within these subsets of 8 baskets, to give 12 of each (4 in each sub-community). Treatments were applied monthly from 18 March 1997 to 18 June 1998.

Leaf samples of the principal species were taken on three occasions (28 May 1997; 21 May 1998; 20 July 1998), dried for 48 h at 70°C and a subsample digested for phosphorus concentration assay by the molybdenum blue method (Allen 1974). Root samples were taken at the final harvest (20 July 1998); part of each sample was analysed for P as above and part stained with 0.01% acid fuchsin (Kormanik & McGraw 1982) (using a phenol-free modification) and percentage root length colonised by AM fungi (%RLC) assessed using the magnified intersection method (McGonigle *et al.* 1990). Soil phosphate concentration was measured by extracting

1g soil in 20ml deionised water for 30 minutes in a rotating shaker; P concentration in the supernatant was measured after centrifugation as above. Leaf N concentration was measured on ground material using a CHN NA2100 Brewanalyser (CE Instruments, Milan Italy). There were no treatment effects on leaf nitrogen or soil phosphorus (data not shown).

Molecular Methods

DNA was extracted from sub-samples of the final harvest roots using a PEX extraction method (Edwards *et al.* 1997). As only one species, *A. reptans,* showed either a high degree of root colonisation (%RLC) in the controls, or a significant decrease in %RLC and tissue P concentration, only *A. reptans* roots were analysed in detail. 18 root samples, 9 from control and 9 from benomyl treated monoliths were analysed. Partial SSU DNA fragments (c.550 base pairs) were amplified using a universal eukaryotic primer NS31 (Simon *et al.* 1993) and a fungal primer AM1 (Helgason *et al.* 1998). PCR products were cloned and digested with the restriction enzymes *Hinf* I and *Hsp92*II (Promega, Southampton UK) after Helgason *et al.* (2002). The abundance of each Restriction Fragment Length Polymorphism (RFLP) profile was scored from 2% agarose gels. Representative clones were sequenced; sequencing was performed by Lark Technologies Ltd (Essex, UK), using ABI BigDye™ chemistry (Applied Biosystems, Warrington UK).

Those samples from all host species sampled that gave a sufficient product yield in the initial PCR were amplified using NS31 and AM1 primers labelled with WellRED Beckman Dye D4-PA and D3-PA respectively (Sigma-Proligo, Gillingham UK). The NS31-D4-PA primer was diluted 1:3 with unlabelled NS31 to compensate for the dye's greater intensity, resulting in equivalent peak heights in the subsequent tRFLP analysis. PCR products were checked on an agarose gel and 5µl aliquots were

digested with *Hsp92*II as described above. Digested PCR products were resuspended in deionised formamide following ethanol precipitation. A 15µl aliquot was added to 30µl SLS loading solution (containing 0.16 µl Size Standard600; Beckman Coulter UK Ltd. High Wycombe UK). Fluorescently labelled fragments were separated by capillary electrophoresis and detected by laser induced fluorescence using the CEQ 8000 automated gene sequencer (Beckman Coulter, UK Ltd, High Wycombe UK). Samples were denatured at 90 ºC for 2 min before injection at 2 kV for 25 s and separation for 65 mins. Fragments were compared to the internal standard using CEQ 8000 software for fragment size analysis. Samples were rejected if maximum peak height was outside a range of peak height was between 10-100K fluorescence units; 4 samples exceeded the maximum and were diluted 1:1 with loading solution and re-run. Each sample was scored for the presence/absence of two peaks diagnostic for specific AMF groups: Acaulosporaceae and *Glomus intraradices/G .mosseae* (Table 2).

Fungal host range

In order to identify the host ranges, a reference sequence of each type was used as a query in a BLAST search (Altschul *et al*. 1997), and the nearest hits used to identify published studies where the sequences have been identified from field roots. Sequences from unpublished studies were excluded because the methodology could not be verified from the database entries alone. Laboratory experiments using cultured isolates and trap cultures using field soils as inoculum were likewise excluded on the grounds that these represent artificial situations (additional information in Table S1 and Table S2 of supplementary information).

Statistical Methods

Data of root colonisation by AM fungi were arcsine-square root transformed and tested using the GLM command for bivariate analysis of variance for blocked design of unbalanced data using SPSS 11. All other data were tested using standard bivariate analysis of variance for a blocked design. Raw sequence data was checked by eye for quality and forward and reverse sequences assembled and checked using Lasergene v.6 software (DNASTAR Inc. Madison WI, USA). Alignments were generated using ClustalX alignment (Thompson *et al.* 1997), and NJ tree building (Saitou & Nei 1987). Correlations were performed using SPSS 11.

RESULTS

The intensity of mycorrhizal colonisation was unusually low in this community: only one species, *A. reptans*, was heavily colonised. Four other species had >10% root length colonised in control plots and in all these benomyl reduced colonisation, though not significantly (Table 1). Benomyl application reduced the mean community root length colonised by AM fungi, averaged across 11 plant species, from 15% to 4% at the final harvest ($F_{1,101}$ = 8.73, P=0.004). Among individual species the reduction was only significant for *A. reptans*, which had the highest colonisation in control plots (45 ± 12% of root length), reduced to 12 ± 7% after benomyl treatment. The phosphorus (P) concentration of shoots and roots was affected by benomyl treatment only in *A. reptans* (Fig. 1), where it was reduced by 37-40% except at harvest 1; at the final harvest, overall leaf P concentration was reduced by one-third in benomyl-treated *A. reptans* (1.8 *vs* 2.7 mg g^{-1}). There was no significant relationship between shoot P concentration and root length colonised, either for all plants together, or for *A. reptans* on its own (Fig. S1 supplementary

information). Although benomyl treatment reduced colonisation of roots by AM fungi, that reduction was not simply responsible for the marked decline in P concentration in *A. reptans*.-

Morphological analyses can give only limited information on the species of fungi involved. We therefore obtained 408 clones from 18 roots of *A. reptans*, from both control and benomyl plots (mean 22.7 clones per root). RFLP analysis of the clones revealed a total of 26 RFLP types using *Hinf*I and *Hsp*92II. Sequencing confirmed that RFLP types sampled by the NS31-AM1 PCR could be grouped into 24 sequence types. Of these, one was a group of *Fusarium*-like Ascomycetes, one was a mix of Archaeosporaceae and an *Acaulospora* sp. that could not be separated using this combination of restriction enzymes, and the remaining 23 were AM fungal sequence types that could be unambiguously resolved (Glomeromycota: Table 2). Individual roots had between 2 and 12 sequence types (mean 7.2). This rich diversity of AM fungi from a single plant species at one site is comparable to that recorded in other recent detailed studies (Vandenkoornhuyse *et al.* 2003; Wirsel 2004).

Most of the fungi (17/24; 71%) increased in frequency in the benomyl treated plots, including the Ascomycete group. There was great variation in the response of the AM fungi to benomyl treatment: while some types were almost eliminated, others were more abundant than in the control. The variable response was reflected in a lack of correlation between the abundance of each sequence type in the control and treated plots.

Five sequence types that were abundant in *A. reptans* had not been previously identified in any other host species in the field, either at this site or others; only one of those is known in culture (*Glomus spurcum*). The values of the host range, defined as the number of genera from which the sequence type had been recorded,

were examined in relation to the abundance of each type in the controls and benomyl treated plots. While there was no significant correlation between host range and abundance in control plots (Fig 2a), the treated plots showed a significant correlation between the abundance of sequence type and host range (Fig 2b). Sequence types with wide host range were, on average, more resilient to this treatment, probably reflecting an ability to recover quickly and colonise any available root.

In the tRFLP analysis, low colonisation resulted in poor amplification yields, and only 35 roots yielded sufficient PCR product for tRFLP analysis (Fig. 3). The abundance of the two AM group-specific fragments showed a response to the treatment similar to that observed in *A. reptans* alone. The proportion of roots showing Acaulosporaceae and *G. intraradices/G. mosseae* fragments was similar in the control treatment. However, the Acaulosporaceae were reduced whereas *G. intraradices/G. mosseae* doubled in frequency after benomyl application, suggesting that the response of the fungi to the treatment is independent of the identity of the host plant.

DISCUSSION

There has been much debate about the ecological role of individual AM fungal taxa (Hart *et al.* 2003). Molecular studies of AM associations in natural communities have begun to reveal that AM fungal taxa vary greatly in specificity and host range (Clapp *et al.*1995; Helgason *et al.* 2002; Husband *et al.* 2002; Vandenkoornhuyse *et al.* 2003; Öpik *et al.* 2003; Klironomos 2003; Johnson *et al.* 2004). We show here that this variation can be linked to the biology of the fungi. Treating *A. reptans* growing in a natural community with the fungicide benomyl reduced both P concentration and colonisation, but there is not a straightforward correlation between colonisation and tissue P concentration, as would be predicted if all AM fungi were functionally

equivalent. The fungal taxa least affected by the fungicide were widespread taxa that have been recorded with many other plant genera, not only at this field site but elsewhere (Öpik *et al.* 2006). The rare and specific taxa, some of which have not been recorded from field samples previously, may therefore have been principally responsible for promoting P uptake in *A. reptans*. Cultures of *Glomus sp*. UY1225 (Glo3) and *G. hoi*, two of the widespread taxa identified in *A. reptans* roots in this study (Fig 2b), have been used as symbionts in a previous laboratory experiment with *A. reptans* and several other co-occurring plant species from the same field site. Both fungal taxa were successful colonisers, and both resulted in only a small increase in P uptake by *A. reptans* relative to uncolonised controls (Helgason *et al.* 2002). The same effect was seen with most other plant-fungus pairs in the experiment. *G.mosseae* and *G. intraradices*, two commonly cultured taxa, are also among the most commonly identified sequence types in field studies. *G. mosseae* increased in frequency, and *G. intraradices* remained the most abundant AM type after benomyl treatment, at the expense of *Acaulospora* spp. That result is consistent with Fig 2b (open symbols) and reinforces the view that these fungi are indeed generalists. This interpretation of course depends on demonstrating that the generalist taxa are less able to promote uptake of P, something this study was not able to do. It is possible that this response is due to a differential effect of benomyl on the AM fungi that may not necessarily reflect a difference in function. Clearly, the next step is to determine whether or not there is any functional diversity among taxa in the field. Diversity in P-uptake has been demonstrated even within a single species in laboratory experiments (Munkvold *et al.* 2004), so it would be reasonable to predict such diversity in a field community.

There are a number of underlying assumptions made in equating relative abundance of clones with abundance of root colonisation e.g. that all targets amplify equally and in proportion at all abundances (Helgason *et al*. 1999). It is notable that no AM taxa found in the control samples are absent in the treatment, suggesting that the increased diversity of taxa may be a function of the reduced dominance of the abundant AM sequence types. Whether or not this is the case, the fact remains that the overall abundance of AM sequence types relative to other fungi declines and a number of taxa with apparently restricted host range decreased more. Our data provide the framework then for explicit testing of the hypothesis that host range is linked to the biology of the fungus, in this case to disturbance in the form of fungicide application.

Benomyl treatment does not eliminate fungi in the Glomeromycota but it can be used to control them by regular treatment (Fitter & Nicholls 1988), as undertaken here. It is likely that benomyl kills the extra-radical mycelium which is then regenerated from mycelium surviving in roots. This interpretation is consistent with the finding that taxa which we have found consistently to produce extensive external mycelium (e.g. *G. intraradices, G. hoi*) recovered most effectively when regularly treated with benomyl, as compared with *Acaulospora* spp. It is also likely that taxa that are most effective at producing and maintaining a large extra-radical mycelium will have a large host range, since they will encounter a wider variety of roots. Host range is therefore likely to be linked, as shown here, with other aspects of fungal biology.

Most studies of AM fungi are conducted with a limited number of species, such as *Glomus intraradices, G. mosseae* and *Gigaspora margarita*, because they are easy to culture and will colonise almost any host (Smith & Read 1997). Even some of these species display preferential patterns of colonisation when offered different host plants

(van der Heijden *et al.* 1998; Helgason *et al.* 2002; Hart *et al.* 2003). However, AM fungal species that are abundant in the field, whether revealed by spore collections (Merryweather & Fitter 1995) or molecular techniques (Helgason *et al.* 2002), may be difficult or impossible to bring into culture. Our data imply that these uncultured species may be responsible for important symbiotic functions. AWhile an increasing number of studies have shown that AM fungi are not randomly distributed among t hosts in natural communities (e.g. Sanders and Fitter 1992; Clapp *et al.*1995; Bever *et al.* 1996; Eom *et al.* 2000; Helgason *et al.* 2002; Husband *et al.* 2002; Vandenkoornhuyse *et al.* 2003; Öpik *et al.* 2003; Johnson *et al.* 2004; Öpik *et al.* 2006), but none of these have shown whether this pattern is caused by host specificity. An alternative explanation would be control by environmental factors. Abiotic niche differentiation among the fungi might lead to these patterns since the plant species will also respond differentially to the same factors. This study demonstrates that AM fungi differ in both host-range (i.e. the biotic niche) and symbiotic function, and that these two characteristics may be linked. Therefore, the discovery of high diversity of AM fungi in a wide range of natural communities, and notably in the roots of single plant species (Helgason *et al.* 2002; Vandenkoornhuyse 2003; Wirsel 2004) as here, seems likely to reflect diversity both in function (Newsham *et al.* 1995) and host-range, which will play a major role in controlling the diversity of the plant communities with which they interact (Hart *et al.* 2003).

This study represents evidence from a natural ecosystem that the high levels of AM fungal diversity found associated with individual plant species allow diversity in functional properties of individual co-existing AM fungal taxa. This functional diversity may provide the mechanism by which AM diversity might regulate plant

community diversity: plant species may be expected to persist best in communities where their specialist symbionts occur, an outcome that would have large implications for the conservation of endangered species, for the ability of communities to respond to rapidly changing environments, and for our ability to manage invasive plant species. A key priority in rhizosphere biology is therefore to elucidate these patterns of diversity, colonisation, and function in natural communities.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Table S1 *Host range data and references*
Table S2 *Identified host genera: habitats and study locations*
Figure S1. *Relationship between leaf P concentration and mycorrhizal colonisation of* A. reptans.
This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2745.XXXX.XXXXX.x

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Table 1. Colonisation of roots by arbuscular mycorrhizal fungi in the principal species sampled in the plots. Only a single plant of *M. perennis* was sampled from a control basket. The only species in which the reduction in colonisation was significant was *A. reptans*: $F_{1,14} = 9.54$, P=0.008

Species	Control (%RLC)	Benomyl (%RLC)
Poa trivialis	1.4	3.0
Primula vulgaris	2.3	2.4
Ajuga reptans	44.6	12.3
Lysimachia nummularia	13.0	2.7
Teucrium scorodonia	16.7	6.8
Oxalis acetosella	0.0	6.7
Glechoma hederacea	16.1	1.7
Mercurialis perennis	(19.0)	3.0
Silene dioica	0.0	0.0
Circaea lutetiana	2.8	0.0
Urtica dioica	0.0	0.0

Table 2. Sequence types identified in *A. reptans* roots using NS31-AM1 PCR.

	Fragment sizes†		Clone numbers (relative abundance)					Nearest
Identity*	*Hinf*I	*Hsp*92II	Control		Benlate		Host Range‡	similar sequence (%)§
Ascomycete	503	90,146,267	29		41			U32414 100
Archaeosporaceae	478,23,5	237,269	10		22			AF131054 98
Archaeosporaceae	474,23,5	147,90,260,5	0		1			AJ563887(96)
Archaeo/Acau17	474,23,5	147,90,265	0		4			AJ563887(96); AF074346(96)
Archaeosporaceae	301,177,22,6	102,137,267	0		1			AF131054 (93)
Archaeosporaceae	485,26,5	246,270	10		14			AJ563866 (-)
Archaeosporaceae	485,26,5	238,260,5	16		13			AJ563887 (98)
Archaeosporaceae	419,55,23,5	147,90,260,5	0		1			AJ563887 (95)
Acau11	**77,428,5**	**148,90,23,249**	0	**0.0**	1	**0.008**	**1**	**AJ716002 (99)**
Acau2	**121,384,5/ 505,5**	**148,90,23,249**	25	**0.197**	6	**0.050**	**1**	**AF074346 (99)**
Acau7	**121,384,5**	**148,90,272**	11	**0.087**	11	**0.092**	**7**	**Y17633 (99)**

G.etunicatum	**261,249**	**148,90,272**	4	**0.031**	0	**0.0**	**3**	**Y17644 (99)**
G.hoi	**503,5**	**144,93,271**	9	**0.071**	20	**0.167**	**11**	**AJ716012 (100)**
G.intraradices	**120,383,5**	**95,142,271**	33	**0.260**	23	**0.192**	**22**	**AJ309462 (99)**
G.mosseae	**260,244,5**	**95,143,271**	3	**0.024**	8	**0.067**	**14**	**AY635833 (100)**
G.spurcum	**120,141,249**	**148,90,271**	0	**0.0**	9	**0.075**	**1**	**AJ315525 (99)**
Glo14	**120,49,90,241,5**	**237,268**	0	**0.0**	1	**0.008**	**5**	**AF437664 (100)**
Glo18	**168,90,244,5**	**143,93,271**	1	**0.008**	2	**0.017**	**8**	**AF437675 (99)**
Glo2	**169,331,5/120,49,331,5**	**90,147,268**	24	**0.189**	6	**0.050**	**11**	**AJ418882 (99)**
Glo3(UY1227)	**120,49,334,5**	**237,271**	10	**0.079**	19	**0.016**	**15**	**AF437719 (100)**
Glo4	**168,90,243,5**	**236,270**	0	**0.0**	5	**0.042**	**10**	**AJ716005 (99)**
Glo54(new)	**120,380,5**	**237,268**	7	**0.055**	1	**0.008**	**1**	**AJ496085 (97)**
Glo55(new)	**502,5**	**237,270**	0	**0.0**	3	**0.025**	**1**	**AY129612 (97)**
Glo56(new)	**168,333,5**	**235,271**	0	**0.0**	5	**0.042**	**1**	**AJ716007 (96)**

* Sequence types were given names derived from the isolates with greatest similarity. The 3 sequence types with low similarity to databank sequences (Glo54-56; the numbering is consistent with previous publications) were judged to be new types.

† RFLP fragment sizes reflect the order in which they occur, and are given for a single representative sequence without primers; they may vary within a group by 1-3 bp in total length. Acau2 and Glo2 have 2 RFLP patterns as the resulting sequences do not resolve into 2 groups. Only sequence types that fell into the groups resolved by NS31-AM1 PCR (in bold), i.e. the Diversisporales and Glomerales (Schüßler *et al.* 2001) were assessed for host range, as amplification of the Archaeosporales and Paraglomales is unpredictable with this primer pair. Underlined fragments are those used in the t-RFLP analysis.

‡ Host range was estimated by the number of plant genera in which the sequence type has been found in other published studies and database entries. This was restricted to studies using field material (Helgason *et al.* 1999; Daniell *et al.* 2001; Helgason *et al.* 2002; Husband *et al.* 2002; Kowalchuk *et al.* 2002; Vandenkoornhuyse *et al.* 2002; Regvar *et al.* 2003; Scheublin *et al.* 2004; Whitfield *et al.* 2004; Wirsel 2004; Oba *et al.* 2004; Vallino *et al.* 2006; Santos *et al.* 2006). (Table S1 and Table S2, supplementary information)

§ % similarity is given where a full alignment was generated. Representative sequences have been deposited in EMBL; accession numbers AJ854081-AJ854105.

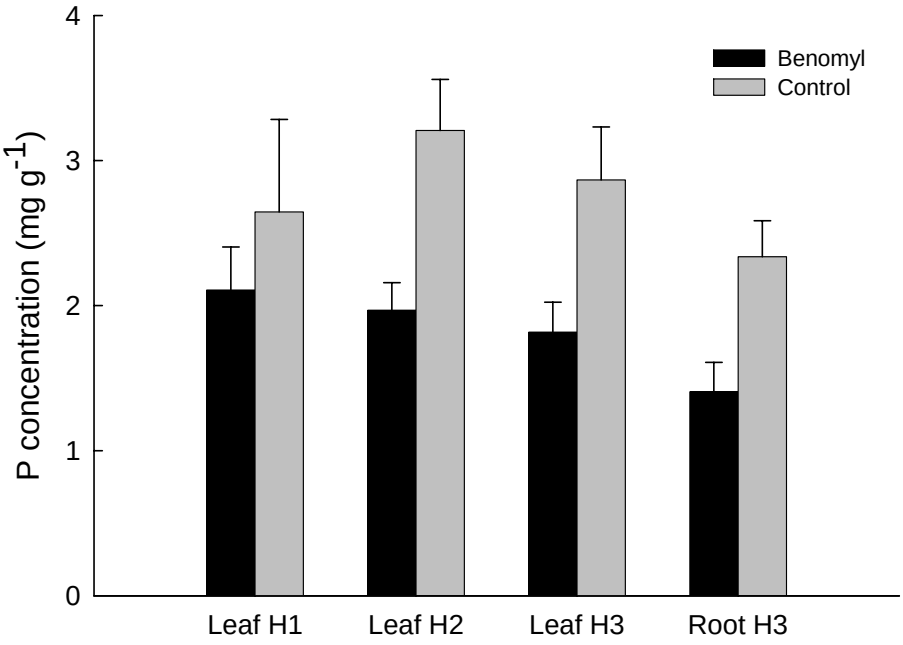

Figure 1. Leaf and root P concentrations of *A. reptans* in benomyl-treated and control baskets. H1-H3 are harvests 1-3

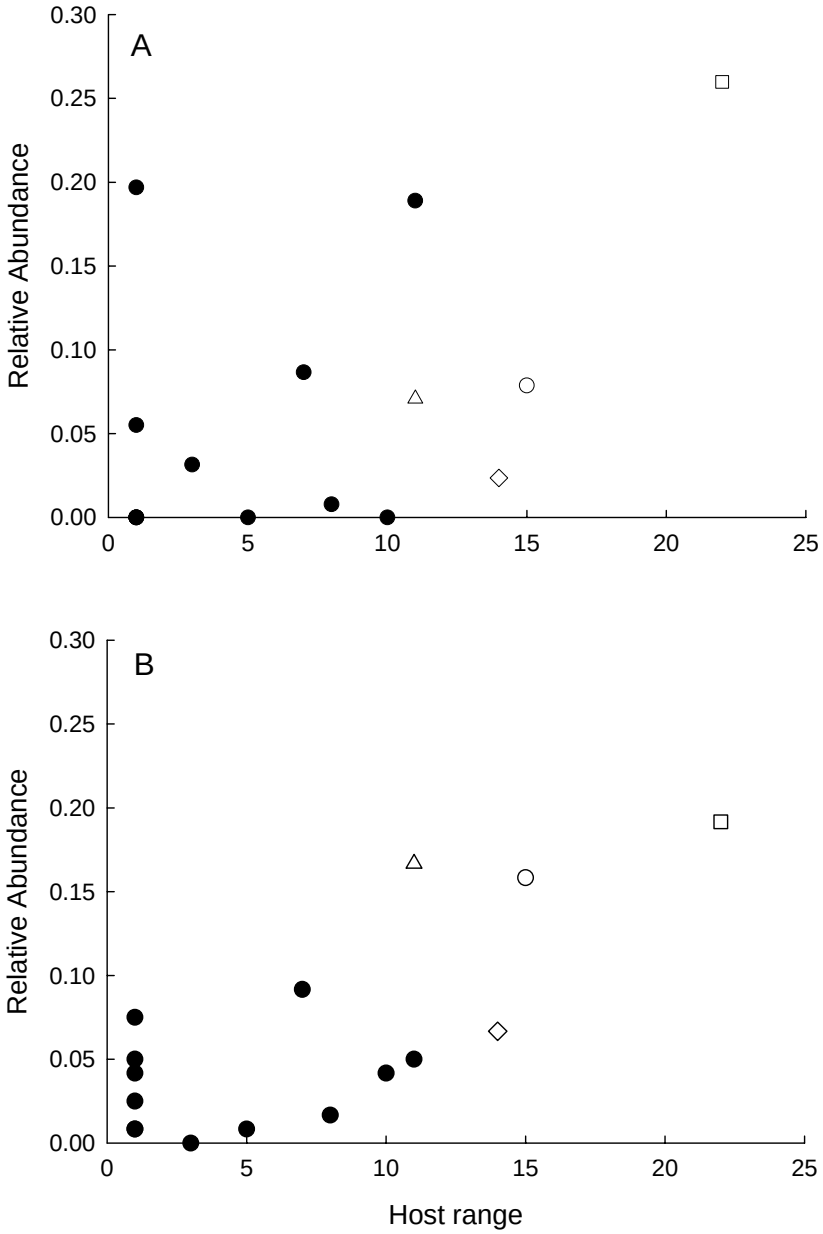

Figure 2. (a) Relationship between relative abundance of clones of each AM sequence type isolated from roots of *A. reptans* in the control baskets and their host range. Total number of AM clones (n = 127) was used as the reference point, (Kendall's tau correlation = 0.365, p>0.05; number of clones used rather than RA). (b) Relationship between number of clones of each AM sequence type isolated from

roots of *A. reptans* in the benomyl treated baskets and their host range. Total number of AM clones (n = 120) was used as above, Kendall's tau correlation = 0.450, $p<0.05$; clone numbers used as above Open symbols are taxa used in the studies discussed in the text, Δ - *G. hoi*, ◊ - *G. mosseae*, □ - *G. intraradices*, ○- *Glomus* sp. Glo3.

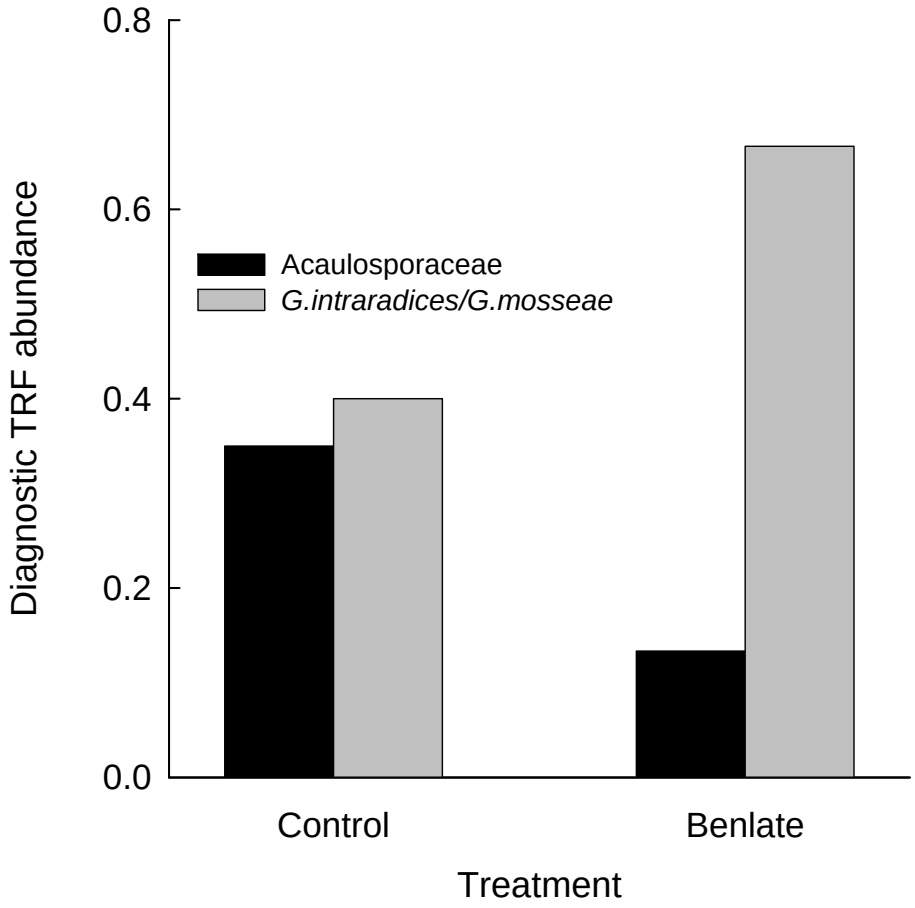

Figure 3. Proportion of Terminal Restriction Fragments (TRF) that could be ascribed to *Acaulospora* spp. and *G. intraradices/G. mosseae* on the 35 roots from 7 species from which amplification was obtained, viz. 8 (control)/ 6 (benlate) *A. reptans*, 2/1 *G.*

hederacea, 0/2 *Circaea lutetiana* L., 3/1 *M. perennis*, 3/3 *L. nummularia*, 1/2 *O. acetosella*, 3/0 *P. trivialis*; 20 plants from control treatments and 15 from benlate.