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The specificity of arbuscular mycorrhizal fungi in perennial ryegrass–white clover pasture

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Abstract

For nitrogen to be transferred from a legume to grass through hyphae of arbuscular mycorrhizal fungi (AMF), as reported for a number of grass/legume combinations, the two species should be linked by a common hyphal network, which gives rise to the question of host–fungus preference. To examine the relative preference of perennial ryegrass and white clover for co-existing AMF, two experiments, adopting a 'spreader–receiver' system, were carried out in a glasshouse. Both experiments demonstrated quantitative host preference by perennial ryegrass and white clover for AMF associated with their own rhizospheres. However, the effect seemed to be temporal, as, by Week 17, in Experiment 2, AMF not associated with the roots of the spreader may have had an opportunity to infect the receiver. Differences in the population of AMF spores around the roots of the two plant species also suggest differences in preference by the two hosts. The study confirms that white clover is more highly mycotrophic than grass. It is concluded that quantitative differences in preference by perennial ryegrass and white clover for specific AMF may reduce the ecological and agricultural significance of N transferred from clover in the field compared with laboratory estimates. ©2000 Elsevier Science B.V. All rights reserved.

Keywords: Arbuscular mycorrhiza; Ecological specificity; Pasture; N transfer; Northern Ireland

1. Introduction

Nitrogen transfer between a legume and accompanying grass species via arbuscular mycorrhizal fungal hyphae has been demonstrated in both glasshouse and field studies (e.g., Van Kessel et al., 1985; Haystead et al., 1988; Hamel and Smith, 1992). Other studies have failed to demonstrate hyphal-mediated transfer, either in the glasshouse (Rogers, 1993) or in the field (Hamel et al., 1991a), or results have been ambiguous (Hamel et al., 1991b). Interplant transfer of N requires that both plant species be connected via shared AMF hyphae.

It has been generally accepted that arbuscular mycorrhizal fungi often have little, or no, host specificity (Gianinazzi-Pearson et al., 1985); however, a host exposed to a mixture of AMF could be colonized preferentially by one or more strains, suggesting host–endophyte preference or ecological specificity (McGonigle and Fitter, 1990). Schenck and Kinloch (1980) reported that crop species can exert a selective

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effect in determining which AMF species become predominant in a mixed indigenous population, and Hayman (1982) pointed out that the distribution of AMF in cultivated soils can be greatly affected by the plant species present. Therefore, these findings provide indirect evidence that some ecological specificity of AMF and host plant associations can occur in the field.

In a field investigation of endomycorrhizas in a hay meadow, McGonigle and Fitter (1990) found that the grass *Holcus lanatus* was predominantly infected by *Glomus tenue*, whereas the roots of three herbaceous species were colonized mainly by other mycorrhizal endophytes. Sanders and Fitter (1992) argued that the differences they detected in spore production indicated that AMF responded differently according to the host species. Other evidence for host–mycorrhizal specificity has been provided in rice (Dhillion, 1992a), native grasses (Dhillion, 1992b) and forage legumes (Giovannetti and Hepper, 1985). In contrast, using root inoculum, Sanders (1993) failed to provide any obvious evidence for mycorrhizal specificity.

Although compatibility is usually determined by the ability of the fungus to infect the host, the host may, or may not, demonstrate a benefit when in association with the fungus, the former being known as 'functional compatibility'. This has been demonstrated with *Glomus invermaium* in association with cucumber, flax and wheat. The three crop species are compatible with *G. invermaium*, but the fungus enhances uptake of P only when in association with flax (Ravnskov and Jakobsen, 1995).

Perennial ryegrass and white clover co-exist in temperate permanent grassland and are usually highly infected with AMF in the field. The existence of even a small degree of AMF specificity could have important consequences on AMF function in this plant community and nutrient dynamics within it. Preliminary work on these plant species has indicated that perennial ryegrass responds differentially to AMF inocula produced from trap cultures with either a grass or clover host plant (Rogers et al., 1994). However, an experiment including all combinations of host species and inoculum sources was needed. The purpose of the present study was to examine the differential infection of both grass and clover plant roots to indigenous AMF, obtained either by grass or clover trap cultures or directly from the rhizosphere of naturally growing grass or clover in an established sward, and on the growth responses of the two host plant species to the different sources of AMF inoculum.

2. Materials and methods

2.1. Experiment 1: study of preference of perennial ryegrass and white clover for AM fungi from their own and reciprocal trap cultures when each plant species is a receiver and spreader for the inoculum

For the production of AMF inocula, 7.5 cm diameter plastic pots were filled with a mixture of sterilized sand and soil (1 : 1 by volume) and planted with 5 white clover (*Trifolium repens* cv Huia) or 5 perennial ryegrass (*Lolium perenne* cv Talbot) plants. Plants were grown in a heated glasshouse (minimum temperature 12°C) from November to April, supplemented by mercury vapour lamps supplying a maximum of $400\,\mu\mathrm{mol\,s^{-1}}$ at plant height from mid-September to the end of the experiment. At harvest, the shoots were excised and the soil was dried out for 1 week. The pot contents were removed as a root ball, stored in a sealed plastic bag in a cold room at 4°C for 2 weeks, and then used as inoculum.

Perennial ryegrass and white clover seedlings propagated in sterilized sand were transplanted into split square pots of side 10 cm (Fig. 1), the design of which was based on the cuvette system developed by Schüepp et al. (1987). The 2 cm wide central compartment was packed with sterilized sand, and the pore size of the mesh separating the central compartment from the rest of the rooting medium was 35 μm.

Table 1 lists all combinations of inoculum sources and host plant species. Only the spreader plants were inoculated. 50 g fresh soil and 0.5 g fresh roots from the trap cultures were put immediately around the root system. In the case of the control, 10 g of *Glomus geosporum* inoculum was procured from the European Bank of the Glomales (BEG 11, supplied by Dr. J.C. Dodd, University of Kent).

The plants were grown in the glasshouse, described above, from May to October. At harvest, the whole plant was divided into shoot and root, and dry weights were determined after drying at 70°C overnight.
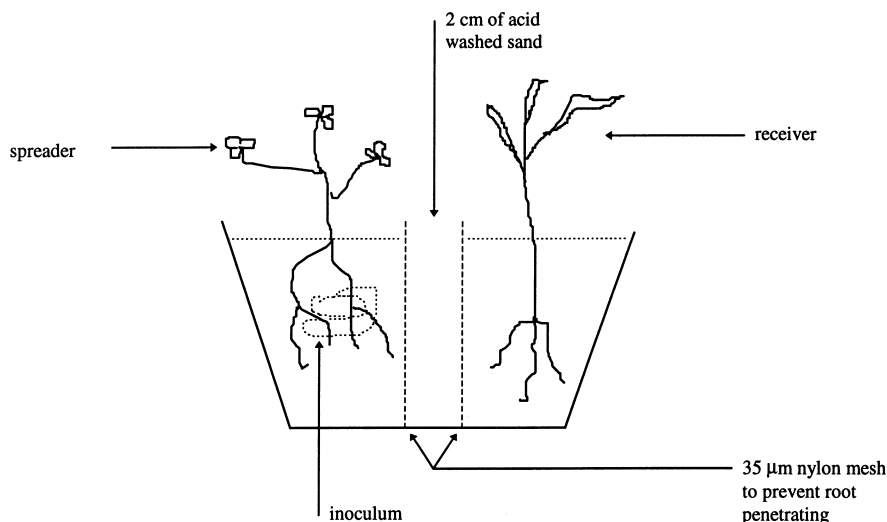
Fig. 1. The split-root system used in experiment.

Table 1
Combinations of host plant and AM inoculum sources in Experiment 1

Treatment No.	Inoculum source	Spreader	Receiver
1	CTI[a]	grass	grass
2	CTI	grass	clover
3	CTI	clover	clover
4	CTI	clover	grass
5	GTI[b]	grass	grass
6	GTI	grass	clover
7	GTI	clover	clover
8	GTI	clover	grass
9	G. geosporum	grass	grass
10	G. geosporum	grass	clover
11	G. geosporum	clover	clover
12	G. geosporum	clover	grass

[a] Clover trap culture used as inoculum.
[b] Grass trap culture used as inoculum.

2.2. Experiment 2: to investigate the infection of perennial ryegrass and white clover with AM fungi when exposed to naturally occurring 'spreaders' of the same and reciprocal species

The principle behind the set-up of this experiment was to use spreaders from typical areas within a sward in the field and test the reception of the AM fungi by receivers of the same or reciprocal species within a thimble of mesh that allowed hyphae of AMF to pass through, but was too narrow for roots to enter.

An assembly was set up, in which a thimble of 35 μm mesh was inserted into a 7.5 cm diameter pot, both the thimble and the pot being filled with a 1 : 1 (by volume) loam soil/sand mixture, and with the thimble protruding 1.5 cm above the surface of the medium in the pot. The soil had been irradiated with 0.1 kGy γ-radiation and the sand autoclaved at 121°C for 1 h.

Two 'spreader plants' were planted diametrically opposite each other in pots outside the thimble and a 'receiver plant' was planted within the thimble. Spreader plants were either rooted grass tillers or rooted stolon tips taken from predominantly grass and clover areas, respectively, in a grass/white clover sward which was in a long term grassland nitrogen epxerimental site at the Agricultural Research Institute of Northern Ireland, Hillsborough, Co Down. The plot had been sown down to perennial ryegrass cv Talbot and white clover cv Huia in 1987, and after being subject to conservation management in 1988, had been continuously stocked with steers during the growing season of each year thereafter.

The spreaders were planted on 28 October 1994, and seeds of the receiver plants, sterilised with hypochloric acid, were sown within the thimbles on 12 October. Two seeds were sown per thimble and thinned to one 6 weeks later. Receivers were the same cultivars as those of the spreaders.

Fourteen pots of each spreader/receiver combination were set up, six harvested from each treatment 11

weeks after planting and eight 6 weeks later. The plants were grown in a heated glasshouse so that temperature did not fall below ~10°C with supplementary light supplying a maximim of about 400 μmol m^{-2} s^{-1}. The pots were placed on saucers. Whereas plants were watered liberally, care was taken to prevent water accumulating in the saucers.

At the first harvest, soil was collected from around the roots of spreader plants and stored at 4°C to provide material from which AMF spores would be isolated and counted. At both harvests, roots were washed free and shoots were harvested and dried. Roots were cut into lengths of 10–15 mm, mixed and sampled, and the remainder dried and weighed.

2.3. Experiments 1 and 2

Roots were cleared with 5% KOH for 1 h at 90°C, rinsed and immersed in 1% HCl overnight (Phillips and Hayman, 1970). The cleared roots were stained in 0.05% Trypan blue for 15 min at 60°C and then transferred to distilled water in a Petri dish.

The roots were cut into bundles about 2 cm long and separated into segments. A magnified intersection method (McGonigle et al., 1990) was used to determine the mycorrhizal infection rates. Root segments were aligned parallel to the long axis of the slide. The field of view under 200× magnification was moved one by one perpendicular to the long axis of the slide. All intersections between the vertical eyepiece crosshair and roots were counted as either negative or positive for the presence of arbuscules, vesicles or hyphae. Usually, 90–120 intersections were counted.

Differences between means were tested for significance by analysis of variance using the Genstat package (Genstat 5 Committee, 1994).

3. Results

3.1. Experiment 1

Table 2 lists infection rates of roots with AMF. Clover spreaders were more highly infected than grass spreaders, taken over all infection and receiver treatments. The significant inoculum × spreader interaction was because of infection of clover spreader roots by CTI being twice as high as that of grass roots, whereas both species as spreaders were infected equally by GTI. Grass spreaders were infected more highly with GTI than CTI but the converse was the case for clover spreaders. *G. geosporum* infected clover spreaders more highly than grass spreaders, level of infection being lower than for the other inocula. Spreader clover plants were considerably more highly infected than grass plants irrespective of inoculum source.

The infection of receiver plants was affected by both spreader species and inoculum sources of spreader

Table 2

Percentage of root length infected with different AM inocula in Experiment 1 (statistical analysis was performed on arcsin transformed data)

Infection of spreaders				
Spreader	Grass		Clover	
Receiver	Grass	Clover	Grass	Clover
CTI	25.6	25.2	42.6	43.4
GTI	30.2	35.0	34.9	35.7
G. geosporum	5.4	2.9	16.7	14.7
Sems.[a]	inoc. 1.49***; sp. 1.22***; rec. 1.22 NS; inoc. × sp. 2.11**; inoc. × rec. 2.11 NS; sp. × rec. 1.72 NS; inoc. × rec. × sp. 2.98 NS			
Infection of recievers				
CTI	9.8	32.9	5.6	26.5
GTI	12.5	30.1	2.7	15.9
G. geosporum	2.0	1.8	1.8	1.8
Sems.	inoc. 1.83***; sp. 1.49**; rec. 1.49***; inoc. × sp. 2.59**; inoc. × rec. 2.59**; sp. × rec. 2.11NS; inoc. × rec. × sp. 3.66 NS			

[a] inoc., inoculum; sp., spreader; rec., receiver; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; NS, not significant.

Table 3
Root and shoot dry weights (g) of spreader plants harvested in Experiment 1

Shoot weight

Spreader	Grass		Clover	
Receiver	Grass	Clover	Grass	Clover
CTI	3.00	4.56	6.65	8.45
GTI	2.76	3.99	7.45	8.88
G. geosporum	2.51	4.02	2.48	2.36
Sems. [a]	inoc.0.205***; sp. 0.167***; rec. 0.167***; inoc. × sp. 0.290**; inoc. × rec. 0.290 NS; sp. × rec. 0.237 NS; inoc. × rec. × sp. 0.410 NS			

Root weight

	Grass		Clover	
CTI	3.71	5.85	2.30	3.12
GTI	3.77	5.59	2.40	2.91
G. geosporum	4.23	5.52	0.69	0.66
sems.	inoc. 0.159***; sp. 0.130***; rec. 0.130***; inoc. × sp. 0.225***; inoc. × rec. 0.225 NS; sp. × rec. 0.184***; inoc. × rec. × sp. 0.319 NS			

[a] inoc., inoculum; sp., spreader; rec., receiver; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; NS, not significant.

plants. Clover, as a receiver, was more highly infected, over all treatments, than grass. However, infection rates of receiver plants with grass as spreader, were significantly higher than those with clover as spreader. Grass receivers were infected similarly by the two-trap culture inocula, but clover receivers were more highly infected by CTI than GTI. Spreaders receiving *G. geosporum* did not infect the receivers.

Table 3 shows shoot and root biomass of spreader plants. Spreader shoots were affected by the inoculum applied, the receiver associated with the spreader and the species of spreader. Clover shoots were generally heavier than those of grass. Heavier spreader shoots of both grass and clover resulted from the trap culture rather than *G. geosporum* inocula, and especially when associated with clover receivers. Grass spreader roots were heavier than clover spreaders, *G. geosporum* inoculum resulting in very light clover roots and roots of grass spreaders were positively affected by clover as receivers. Table 4 lists receiver plants' biomass. Receiver shoots were heaviest in the *G. geosporum* inoculum treatment mainly because of the promotive

Table 4
Shoot and root dry weights (g) of receiver plants harvested in Experiment 1

Shoot weight

Spreader	Grass		Clover	
Receiver	Grass	Clover	Grass	Clover
CTI	3.06	2.56	3.09	2.26
GTI	2.79	2.99	3.16	2.81
G. geosporum	2.76	3.49	4.47	4.01
Sems.[a]	inoc.0.183**; sp. 0.149 NS; rec. 0.149 NS; inoc. × sp. 0.258*; inoc. × rec. 0.258 NS; sp. × rec. 0.211 NS; inoc. × rec. × sp. 0.365 NS			

Root weight

	Grass		Clover	
CTI	4.96	0.70	4.79	0.57
GTI	4.24	0.79	4.42	0.67
G. geosporum	5.14	1.03	5.18	1.03
Sems.	inoc. 0.180*; sp. 0.147 NS; rec. 0.147***; inoc. × sp. 0.254 NS; inoc. × rec. 0.254 NS; sp. × rec. 0.208 NS; inoc. × rec. × sp. 0.360 NS			

[a] inoc., inoculum; sp., spreader; rec., receiver; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; NS, not significant.

Table 5
Percentage of root length infected with AMF 11 and 17 weeks after sowing in Experiment 2

Spreader	Grass		Clover	
Receiver	Grass	Clover	Grass	Clover
Infection of spreader plants				
Week 11	17.2	14.6	46.7	50.0
Sems:	sp. 0.75***; rec. 0.75 NS; sp. × rec. 1.07*			
Week 17	24.7	23.4	54.8	57.9
Sems.	sp. 1.24***; rec. 1.24 NS; sp. × rec. 1.75 NS			
Infection of receiver plants				
Week 11	14.9	14.8	31.5	45.5
Sems.	sp. 2.10***; rec. 2.10*; sp. × rec. 2.98*			
Week 17	27.1	40.0	45.8	55.7
Sems.	sp. 2.11***; rec. 2.11***; sp. × rec. 2.98 NS			

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS, not significant.

effect of the clover spreader in that inoculum treatment. Roots of grass receivers were much heavier than those of clover and grass, and clover receivers associated with spreaders receiving *G. geosporum* inoculum were heavier than those in the trap-culture treatment.

3.2. Experiment 2

Clover spreader plants had higher infection rates than grass at both harvests, although the difference between the two species was higher at the first than at the second harvest (Table 5). At the first harvest, spreaders associated with the same species of receiver had a higher colonisation percentage than those with reciprocal receivers. However, the ecological significance of this difference may be marginal.

Clover receivers had a higher infection level than grass, and both species had higher levels when they were associated with clover spreaders at both harvests. The particularly high colonisation level of clover receiver with a clover spreader at the first harvest resulted in a significant interaction between effect of receivers and spreaders on colonisation of receivers.

Dry weight data are not presented, but grass spreaders and receivers had significantly heavier shoots and roots than clover's at both harvests, receiver type having no effect on spreaders and vice versa.

Spore types were classified according to colour, the clear/white types ranging in diameter from 0.05 to 0.2 mm; yellow/brown types, from 0.075 to 0.2 mm;

Table 6
Number of spores of different colours in the total population of 50 g of soil surrounding spreader plants 11 weeks after sowing in Experiment 2

Spreader	Clear/white	Yellow/brown	Black
Grass	85	96	160
Clover	245	90	141
Sems.	35.1*	13.7 NS	16.6 NS

*$p < 0.05$; NS, not significant.

and black, being the largest, ranging from 0.075 to 0.3 mm. Very few green and red spores were recorded. Clover spreaders had a higher proportion of clear/white spores, whereas grass spreaders had a higher proportion of black spores than clover at 11 weeks (Table 6). No significant differences were detected in contribution of spore types to the total spore population of receivers at the second harvest, nor did absolute numbers of spores differ significantly between any of the treatments.

4. Discussion

Arbuscular mycorrhizal associations generally lack specificity, some species of AM fungi being able to form mycorrhizal associations with diverse plant groups. Similarly, some plants often associate with many AM fungi. Nevertheless, caution is needed in generalising the lack of specificity in AM associations, because most plant species and habitats remain unexamined for VAM, and only a few experimental cross inoculations have been made between AM fungi and hosts. In the present study, the infection of receivers of both species by spreaders of both species confirms a lack of absolute specificity between perennial ryegrass and white clover for particular AMF (Rogers et al., 1994). This finding is in agreement with a recent study by Douds et al. (1998), suggesting the existence of a degree of specificity between AM fungi and host. At the second harvest, in Experiment 2, clover receivers were well infected by grass spreaders and the reciprocal also occurred, suggesting that specificity is temporal. Temporary differences in level of colonisation in grasses has been reported by Sanders and Fitter (1992). Sanders (1993) showed that although *Trifolium pratense* had relatively stable levels of infection throughout the season, grass had low infection levels in January,

increasing by March and were also recorded to be high in September. This suggests that environmental conditions or differing levels of competition may influence the degree of AM fungal colonisation of different species.

In the present experiments, all possible combinations of host and inocula were made, with both plant species being used as receivers and spreaders. In Experiment 1 and in Experiment 2, at Week 11, there was an indication that receiver type influenced spreader infection rate, spreaders having a higher infection rate if grown with receivers of the same species. Level of colonisation can be influenced by the other species growing with the host.

In Experiment 2, differences in the number and proportion of spore types in association with different species of spreader contribute to the hypothesis that host preference is an issue in ecosystems. Examples of different AMF differing around host species in the same environment have been found in a study in which agronomic crops were grown on a woodland site. *Gigaspora* spp. were found in abundance around soybean, and *Glomus* and *Acaulospora* predominated around the roots of monocotyledonous crops. (Schenck and Kinloch, 1980). The particularly high content of the small clear, or white, spores in the vicinity of white clover roots suggests that a different species of AMF was preferred by clover relative to grass, which, in contrast, encouraged sporulation of the larger black type. However, it is of interest to note that the differences in spore type in spreaders did not have an effect on the spores found around receivers by Week 15 in Experiment 2. Also, in a correlation analysis between infection of receivers and that of spreaders after treatment effects had been taken out (correlation of residuals), infection of receivers was correlated with degree of colonisation of spreaders, suggesting that it is hyphal spread, rather than spore production, that is responsible for the infection of receivers by spreaders as a source of inoculum. Inoculum density decreases lag time for infection (Wilson and Trinick, 1983). Therefore, the same argument may apply to the intensity of colonisation of spreaders determining the ease and speed of colonisation of associated receivers, within the constraints of host preference for AMF.

The effect of receiver plant species on dry weight of spreaders can be explained by nitrogen transfer from the clover receiver to the spreader increasing both shoot and root dry weight in spreaders in Experiment 1. The poor performance of clover spreaders when inoculated with *G. geosporum* is difficult to explain. Infection levels were low and so it is possible that clover was dependent on AMF infection to grow well.

In Experiment 2, impurity of the inoculum around the spreaders may have resulted in the eventual build-up of AMF, which was preferred by the receiver species, irrespective of spreader species, and so masked the effects of host preference detected at the first harvest. The eventual colonisation of the receiver may not necessarily have been by the AMF which infected the spreader, but by contact at the mesh barrier between the host root and spores or hyphae of the preferred AMF (Douds et al., 1996).

Evidence of different host preferences for specific AMF raises the possibility that hyphal links between plant species may not be a common phenomenon under field conditions. In fact, it is possible that some of the apparent demonstrations of functional hyphal links between legume and grass are a consequence of penetration by grass roots of experimental mesh barriers, believed to be capable of allowing only hyphae to pass and used to separate the effects of hyphal and root connection between plant species, and so allowing the grass access to the N-rich rhizosphere of the adjacent legume. Although in some studies mesh sizes of 45 to 60 μm have been used in the belief that they were impenetrable by roots (Haystead et al., 1988; Frey and Schüepp, 1992), Rogers (1993) has shown that fine grass roots can pass through mesh pores in this size range.

White clover is highly mycotrophic in comparison with grass (e.g., Warner and Mosse, 1982) and this was confirmed in both experiments. In addition to grass having more root hairs, a factor considered to reduce AMF infection is that grass has heavier roots than white clover, and if this is taken as an indication of root density, it is possible that the low infection rate was the result of relatively low inoculum loading relative to the amount of root, and thereby lowering the proportion of the root being colonised compared with white clover. However, Warner and Mosse (1982) found that colonisation by *Glomus fasiculatum* was greater in white clover than fescue despite the higher root density of the latter.

Therefore, taking account of the quantitative preference of grass and white clover for different AM fungi, and the very low levels of nutrient transfer recorded in the experiments, some of which may not be demonstrations of functional hyphal linkage, the likelihood of hyphal links between plant species playing an agriculturally or ecologically significant role in long term or permanent grassland would seem to be remote. Results obtained in this study also provide some explanation of variation in inter-plant N transfer, as reported elsewhere.

Acknowledgements

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