Functional Ecology 2006
20, 226–232

Leaf endophytes affect mycorrhizal status and growth of co-infected and neighbouring plants

M. OMACINI*†, T. EGGERS‡, M. BONKOWSKI§, A. C. GANGE¶ and T. H. JONES**

*NERC Centre for Population Biology, Imperial College London, Silwood Park Campus, Ascot, Berkshire SL5 7PY UK, and *IFEVA/Facultad de Agronomía, Universidad de Buenos Aires, Argentina, ‡Faculty of Biology and Chemistry, University of Osnabrück, 49069 Osnabrück, Germany, §Institut für Zoologie, AG Tierökologie, D-64287 Darmstadt, Germany, ¶School of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX, UK, and **Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3TL, UK*

Summary

1. Fungal leaf endophytes and arbuscular mycorrhizal (AM) fungi are common constituents of natural grasslands. The simultaneous presence of these two grass plant symbionts is highly probable.

2. We describe the results of a glasshouse experiment investigating the outcome of dual infection of a cool-season grass species, *Lolium multiflorum*, by the fungal endophyte *Neotyphodium occultans* and three species of *Glomus* AM fungi.

3. Mycorrhizal colonization was investigated on monocultures of plants with or without leaf endophytes, and on mixtures of endophyte-infected and uninfected plants. In both scenarios, endophyte-infected plants had lower levels of mycorrhizal colonization, but in the endophyte mixtures the presence of endophyte-infected plants caused an increase in AM colonization in non-endophyte-infected conspecific neighbours.

4. Host-plant biomass, nutrient (nitrogen and phosphorus) accumulation, and competitive ability were increased by the presence of endophytes. AM fungi did not improve host performance or nutrient content (concentration or accumulation) in the presence or absence of the endophyte.

5. Interactions between host plants and AM fungi are mediated by fungal endophyte infection. The implications of such modified interactions for ecosystem dynamics and functioning are considered.

Key-words: indirect interaction, Italian ryegrass, mutualism, plant–microbial symbioses, soil feedback

Functional Ecology (2006) **20**, 226–232
doi: 10.1111/j.1365-2435.2006.01099.x

Introduction

How plant and microbial symbionts interact, and how these interactions influence plant communities, have been well explored (Gange, Brown & Sinclair. 1993; van der Putten & Peters 1997; Clay & Holah 1999). Micro-organisms induce phenotypic changes in host plants that have consequences for plant competition and herbivory (Hartnett *et al*. 1993; Gange & West 1994; van der Putten & Peters 1997). Although plants are typically simultaneously exposed to a huge diversity of micro-organisms, few empirical studies have considered how two or more potential mutualistic symbionts of the same host plant interact reciprocally and mediate other interactions within the community (but cf. Vicari, Hatcher & Ayres 2002). Most work has focused on the interaction between plant roots and symbiotic soil micro-organisms (Fitter 2001), not on common symbionts of above-ground plant tissues (Omacini, Chaneton & Ghersa 2005).

Although leaf endophytes and arbuscular mycorrhizal (AM) fungi are ubiquitous symbionts of many plant species, biologically they differ markedly. Endophytes of the genus *Neotyphodium* (Ascomycetes: Clavicipitaceae) grow intercellularly in leaf and stem tissue of most cool-season grasses. They cause asymptomatic infections that are transmitted exclusively through the host plant seeds, which can therefore represent the reproductive and dispersal unit of both plant and fungus (Clay & Schardl 2002). Endophyte-infected plants do produce uninfected seeds (transmission is not perfect), and this may explain the persistence of uninfected plants within an otherwise infected population (Ravel, Michalakis & Charmet 1997; Saikkonen, Jon & Gyllenberg 2002). Mycorrhizal fungi

†Author to whom correspondence should be addressed.
E-mail: omacini@ifeva.edu.ar

are common in natural grasslands and are able to colonize a broad range of plant species (Smith & Read 1997; van der Heijden & Sanders 2002). In contrast to leaf endophytes, which are restricted to vertical (maternal) transmission, mycorrhizal fungi are capable of horizontal spread through vegetative growth of hyphae between neighbouring plants colonizing new host roots.

Both forms of symbiosis offer potential benefits for all the interacting partners (Smith & Read 1997; Clay & Schardl 2002). The fungi obtain carbon from the host, while the plant primarily receives, via the endophyte, protection against herbivores (Bush, Wilkinson & Schardl 1997; but cf. Faeth & Sullivan 2003) and, via AM fungi, a supply of limiting mineral nutrients (Smith & Read 1997; but cf. Munkvold *et al*. 2004; Fitter 2005; Morgan, Bending & White 2005). Endophyte infection may also enhance host nutrient uptake and stress tolerance to different abiotic factors (Malinowski & Belesky 2000; Clay & Schardl 2002; Malinowski, Belesky & Lewis 2005). As both micro-organisms act as carbon sinks, dual infection could also, at least in some environments, result in competition for photosynthate in such a way that the cost of harbouring symbionts may exceed the benefits.

Empirical work has shown that plant–plant interactions may be modified by the presence of either leaf endophytes (Marks, Clay & Cheplick 1991; Faeth, Helander & Saikkonen 2004) or mycorrhiza (Hartnett *et al*. 1993). In addition, the plant–endophyte symbiosis can introduce changes in soil microenvironmental conditions (Malinowski & Belesky 2000) that affect the density and activity of different functional groups of soil organisms (Omacini *et al*. 2005; Popay & Bonos 2005). A few experiments have reported that leaf endophytes can reduce mycorrhizal sporulation and colonization of host roots (Chu-Chou *et al*. 1992; Guo, Hendrix & Ferriss 1992; Müller 2003). Such endophyte-driven changes in the density and behaviour of root-associated organisms can potentially affect their interaction with neighbouring plant roots.

This study sets out to determine experimentally the impact of endophyte infection of an invasive annual grass on AM colonization of roots, and the consequences of simultaneous infection by both symbionts for plant growth and competitive ability. We hypothesized that: (i) endophyte-free plants (–E) are inferior when competing against endophyte-infected plants (+E); (ii) AM fungi influence differentially the growth and nutrient content of +E and –E plants and (iii) also modify competitive interactions between +E and –E neighbouring plants; and (iv) endophyte infection reduces the capacity of AM fungi to colonize roots of +E plants (the effect of host endophyte infection) and that of –E plants when coexisting with +E plants (the effects of endophyte-infected neighbouring plants). To test these hypotheses, we used as a model system the widespread, natural association between the annual grass *Lolium multiflorum* Lam. and the endophyte *Neotyphodium occultans* (Moon *et al*. 2000; de Battista 2005) and *Glomus* species, the most frequent AM fungal species in grasslands where *L. multiflorum* presents typical levels of endophyte infection between 50 and 100% (Menéndez, Scervino & Godeas 2001; de Battista 2005).

Materials and methods

EXPERIMENTAL DESIGN

Seeds with a high level of endophyte infection (+E) had previously been collected from an old field dominated by *L. multiflorum* (Omacini *et al*. 2004). Fifty per cent of the seeds had been treated with the fungicide triadimenol (0·5 g active ingredient per 100 g seeds) to obtain endophyte-uninfected seeds (–E). Both seed batches were sown and *L. multiflorum* plants were raised under the same environmental conditions (outside plots with ≈1000 plants m^{-2}). Seeds of their F_1 generation were used for the experiment. Microscopic examination of 100 seeds of each type confirmed that 95 and 3% of +E and –E seeds, respectively, were infected with *N. occultans*.

Lolium multiflorum seeds (+E or –E) were seeded in soil either inoculated with (+M) or without (–M) mycorrhiza. Neighbouring plants in each pot were either with or without endophyte infection. The pots from each group were assigned randomly to six blocks of eight pots, with six replicates of each treatment (endophyte infection level; availability of AM inoculum; neighbour type).

Each experimental pot (16 cm diameter, 13 cm height) was filled with light sandy loam soil (≈2300 g per pot) that had been steam-sterilized (≈90 °C) to remove soil fauna. Prior to seeding, the soil was leached to reduce the nitrogen flush resulting from the sterilization process. The AM inoculum (20 g) was added in a layer 3–5 cm below the soil surface of each pot assigned to be mycorrhiza-containing (+M). The inoculum consisted of a mixture of *Glomus mosseae*, *Glomus caledonium* and *Glomus fasciculatum* spores (Vaminoc G; MicroBio Ltd, Herts, UK). For non-mycorrhizal treatments (–M), the same quantity of autoclaved inoculum was added. After 24 h, the pots were sown with either 12 seeds of the same endophytic infection level (monoculture) or six seeds each of both endophyte infection levels (mixture). Four groups of three seeds were sown at right angles to each other, and 7 days after germination four seedlings of uniform size were selected (one of each group), the remainder being weeded out. The experiment was performed under controlled temperature conditions in a glasshouse (25 ± 5 °C, 16/8 h day/night cycle). Each pot was watered to field capacity (≈500 ml per pot) three times per week.

MEASUREMENTS AND ANALYSES

Plants were grown for 8 weeks and the time of first flowering tiller was recorded. No plants died during the experiment, and after 8 weeks the biomass of vegetative shoots, flowering stems and roots (after oven-drying at

80 °C for 48 h) was determined as the mean value for plants harvested from each pot (four for monocultures and two from each endophyte level for mixtures). Dry weight biomass of reproductive tissue was used as a measure of plant reproductive output. Roots were sampled by taking 5-cm-diameter soil cores to pot depth with the plant at the centre of the core. Cores were carefully washed over a sieve (2-mm mesh), and extracted roots were then dried and weighed. This allowed separation of root samples from +E and –E plants, although the difficulties in extracting roots and estimating root biomass of neighbouring plants were recognized (Cahill 2002).

Mycorrhizal colonization of roots was determined by examining a random subsample of 20 cm root at ×200 using a Zeiss Axiophot epifluorescence microscope, fitted with a UV lamp and filters, giving a transmission of 455–490 nm in blue (Gange *et al*. 1999). Arbuscular colonization (percentage root length colonized, %RLC) was recorded using the cross-hair eyepiece method (McGonigle *et al*. 1990), with a minimum of 200 intersections per slide.

Total percentage C and N were determined on above-ground plant tissues of the six replicates of each treatment using a Fisons protein analyser conditioned for C, N, hydrogen and sulphur. Combustion products were separated on a chromatic column, eluted as N_2 and CO_2, and detected with a thermoconductivity detector (NRM Ltd, Bracknell, UK). The C : N ratio was calculated for each sample. Phosphorus content was obtained from the same plants, following a Kjeldahl acid digestion, using a colorimetric technique with a flow injection auto-analyser (Alpkem Corporation, Clackamas, OR, USA).

STATISTICAL ANALYSIS

The effects of host endophyte infection and neighbouring plant on mycorrhizal colonization were tested using two-way ANOVA including block as a non-interacting main effect. Potentially autocorrelated data such as biomass and nutrient content were analysed using multivariate analysis of variance (MANOVA) with endophyte (E: +E or –E), mycorrhiza (M: +M or –M) and neighbour (N: monoculture or mixture) as explanatory variables. Levene's tests for homogeneity of variance were applied prior to the analyses and, if necessary ($P < 0{\cdot}05$), data were transformed accordingly. For significant MANOVA results, multiple univariate tests were used (protected ANOVA, Scheiner 2001). Flowering frequencies were analysed using a log-linear model (*G*-test, Sokal & Rohlf 1995).

Results

COLONIZATION BY AM FUNGI

At harvest, all plants inoculated with AM spores were infected, irrespective of the presence of endophytes. Mycorrhizal colonization was, however, always markedly higher in the absence of the endophyte (endophyte effect for %RLC, E: $F_{1,27} = 47{\cdot}74$, $P < 0{\cdot}0001$; Fig. 1). Mycorrhizal colonization of endophyte-uninfected plants was dependent on the level of infection of the neighbouring plant (significant interaction with neighbour type for %RLC E × N: $F_{1,27} = 5{\cdot}16$, $P = 0{\cdot}038$). The –E plants had a higher degree of %RLC in pots with +E plants than in microcosms containing only endophyte-free plants (Fig. 1). The difference between %RLC of endophyte-uninfected and -infected plants was greater in mixture pots (33 ± 5·5, mean ± SE) compared with monocultures (18 ± 3·7). There was no colonization by arbuscules in the non-inoculated controls (–M pots).

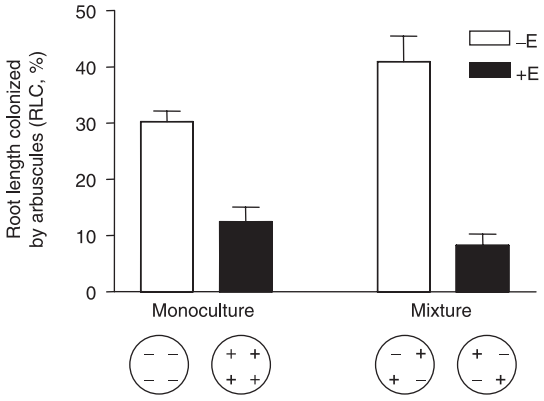

Fig. 1. Levels of arbuscule colonization, measured as mean percentage of root length colonized (%RLC ± SE, $n = 6$) for endophyte-infected and uninfected *Lolium multiflorum* plants (+E and –E, respectively) growing separately (monoculture) or together (mixture). Below the figure plus or minus symbols represent plants' endophyte infection (neighbouring plants in each treatment are in bold).

GROWTH, REPRODUCTION AND NUTRIENT STATUS OF PLANTS

The presence of the endophyte affected the performance of +E and neighbouring –E plants (Table 1). In the absence of endophytes, plants had a reduced shoot and reproductive biomass (ANOVA E: $F_{1,35} = 28{\cdot}7$, $P \leq 0{\cdot}0001$; $F_{1,35} = 110{\cdot}1$, $P \leq 0{\cdot}0001$, respectively; Fig. 2a,b). Differences in reproductive biomass between +E and –E plants were larger in mixtures than in monocultures (ANOVA E × N interaction: $F_{1,35} = 4{\cdot}4$, $P = 0{\cdot}044$; Fig. 2b). There was no difference in root biomass with respect to endophyte infection, mycorrhiza availability or neighbour plant type (Fig. 2c). Mycorrhizal fungi alone had no effect on either above-ground, reproductive or root biomass of *L. multiflorum* (Fig. 2a,b; Table 1). There was no interaction between the presence of mycorrhiza and host endophyte infection or neighbour type in terms of plant biomass responses (Table 1).

Endophyte infection stimulated flowering of *L. multiflorum* (E × N interaction: $G_1 = 16{\cdot}8$, $P < 0{\cdot}001$). In monocultures, 100 and 83% of pots with +E and –E plants, respectively, produced at least one plant with

Table 1. Summary of multivariate analyses of variance (MANOVA) of the effects of fungal endophytes, arbuscular micorrhizae and neighbour type on *Lolium multiflorum* final shoot, reproductive and root biomass

Treatment	df	*F* (Roy's greatest root)
Endophyte (E)	3, 33	49·36***
Mycorrhizae (M)	3, 33	0·46
Neighbour type (N)	3, 33	0·29
Block	5, 35	1·80
E × M	3, 33	0·45
E × N	3, 33	3·54*
M × N	3, 33	0·03
E × M × N	3, 33	0·73

*, $P = 0{\cdot}05$; ***, $P < 0{\cdot}001$.

Table 2. Summary of multivariate analyses of variance (MANOVA) exploring the effects of fungal endophytes, arbuscular mycorrhizae and neighbour type on the concentration (%) of nitrogen, carbon, phosphorus, the relationship between C and N, and total content of N and P in above-ground vegetative tissues of *Lolium multiflorum* plants

Treatment	df	*F* (Roy's greatest root)
Endophyte (E)	6, 30	6·99***
Mycorrhizae (M)	6, 30	0·93
Neighbour type (N)	6, 30	1·21
Block	5, 34	4·58
E × M	6, 30	0·75
E × N	6, 30	1·05
M × N	6, 30	0·41
E × M × N	6, 30	0·55

***, $P < 0{\cdot}001$.

seeds. In mixtures, in 50% of both +M and –M pots, –E plants failed to flower, but only in one pot did +E plants fail to produce seeds. Mycorrhiza had no effect on plant flowering ($G_1 = 0{\cdot}83$, $P = 0{\cdot}36$).

Endophytes affected nutrient concentration in host above-ground tissues (Fig. 3; Table 2). +E plants contained significantly lower percentage N and higher percentage C than –E plants (ANOVA E: $F_{1,35} = 29{\cdot}2$, $P < 0{\cdot}0001$; $F_{1,35} = 18{\cdot}3$, $P < 0{\cdot}0001$, respectively). Consequently, C : N ratio decreased from 62 (±3·3) in +E treatments to 43 (±2·7) in –E treatments ($F_{1,35} = 29$, $P < 0{\cdot}0001$). +E and –E plants contained the same level of percentage P ($F_{1,35} = 3{\cdot}8$, $P = 0{\cdot}06$; Fig. 3). Changes in total biomass and nutrient concentrations, however, resulted in 50% more P being accumulated in above-ground plant tissues of +E plants compared with –E plants ($F_{1,35} = 21{\cdot}3$, $P < 0{\cdot}0001$). In the absence of endophytes, total N in plant shoots tended to decrease ($F_{1,35} = 3{\cdot}74$, $P = 0{\cdot}056$). There were no significant differences in the concentration and total content of N and P with respect to neighbour type or mycorrhizal addition (Table 2).

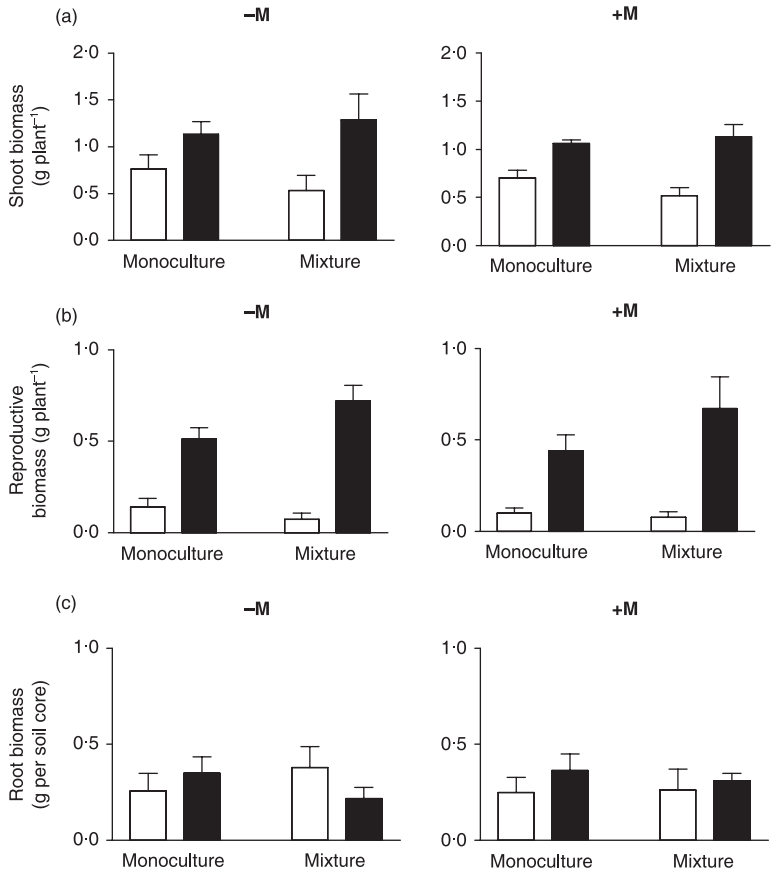

Fig. 2. Biomass production (g per plant, mean ± SE, $n = 6$) of infected and uninfected *Lolium multiflorum* plants (black and white bars, respectively) growing separately (monocultures) or together (mixtures) in pots where mycorrhizal inoculum was abundant (+M) or absent (–M).

Discussion

Most ecological studies tend to focus on visible above-ground components, although recognizing that below-ground processes, particularly at the microbial level, may control the abundance of plant species and the structure and diversity of plant communities (van der Heijden *et al.* 1998; Clay & Holah 1999; Fitter 2001, 2005; Borowicz 2002; Bonkowski & Roy 2005). We believe our study is among the first to take account of the effects of the interaction between two common microbial symbionts on both the performance of the host plant and its conspecific neighbours. The results demonstrate that endophyte effects can have important implications for colonization of plants by AM fungi. Future studies on either symbiont should consider including the endophyte–mycorrhizal interaction; to ignore this interaction potentially removes a naturally occurring modifier of community components and leads to conclusions that are at best unrealistic and at worst spurious.

When both symbionts occurred together, *Neotyphodium* infection suppressed mycorrhizal colonization, confirming observations in other endophyte–grass associations (Chu-Chou *et al.* 1992; Guo *et al.* 1992; Müller 2003). Contrary to our expectations, endophytes

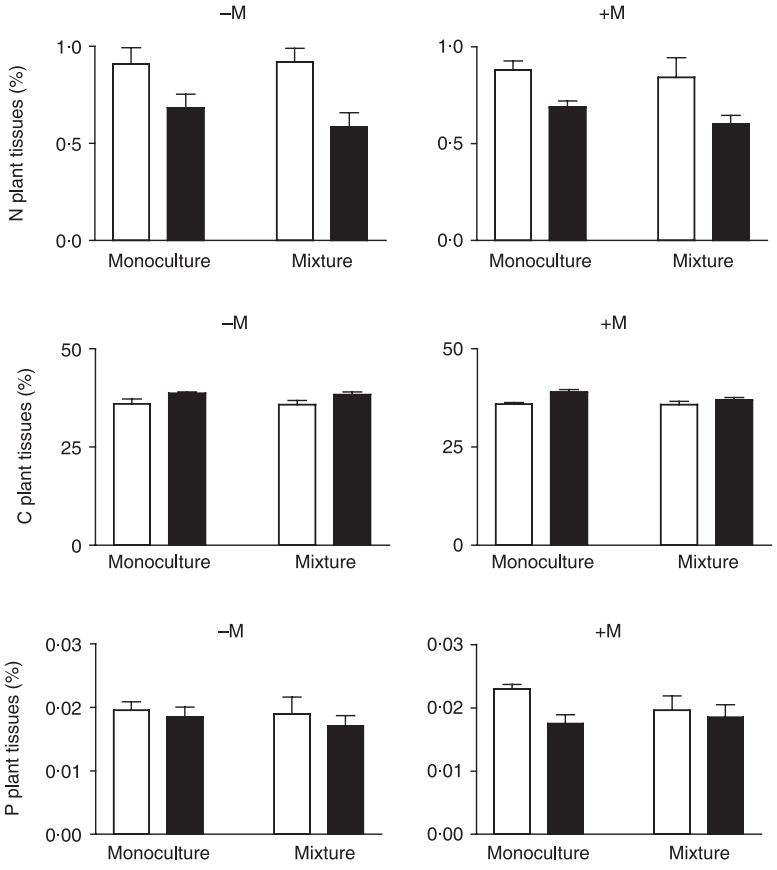

Fig. 3. Nutrient concentrations (% dry mass, mean ± SE, $n = 6$) of above-ground vegetative tissues of infected and uninfected *Lolium multiflorum* plants (black and white bars, respectively) growing separately (monocultures) or together (mixtures) in pots where mycorrhizal inoculum was abundant (+M) or absent (–M).

increased rather than reduced AM fungal colonization of uninfected neighbours, suggesting that inhibition occurs mainly by a systemic induction of resistance in endophyte-infected plants. As endophyte reproduction depends on the successful production of host seeds, natural selection may favour endophytes that improve host growth and reproduction by channelling resources to the leaf symbiont instead of to other potential competitors for resources, such as AM fungi. In addition, endophytes also have the potential to modify the level of mycorrhizal colonization in neighbouring plants. While previous studies have shown that mycorrhiza can behave in a mutualistic, neutral or parasitic way, depending on the species combination of host plant and AM fungal symbiont (Klironomos 2003), our results highlight the complexity of interactions that can occur between two functionally different groups of fungal plant symbionts.

In our experimental study, the competitive performance of *L. multiflorum* was mainly affected by endophytes. Endophyte-infected plants had higher reproductive biomass and suppressed flowering of endophyte-uninfected neighbouring plants, suggesting that endophytes strongly increased the competitive ability of their plant hosts. This indirect negative effect on the reproductive biomass and total nutrient content of –E plant neighbours was not compensated by the high mycorrhizal colonization levels detected in those plants. Another recent study (Müller 2003) found that *L. perenne* does not benefit from mycorrhizal colonization at different levels of endophyte infection. The competitive advantage obtained by some grasses through a symbiotic association with fungal endophytes has generally been related to resistance to herbivores (Clay & Schardl 2002; cf Faeth & Sullivan 2003). The present study demonstrates that there are other mutualistic contributions of *Neotyphodium* endophytes to the plant host (for review see Malinowski & Belesky 2000; Popay & Bonos 2005; Malinowski *et al*. 2005).

That mycorrhiza did not induce growth depression in *L. multiflorum* plants of either endophyte infection level indicated the absence of any parasitism-like effects. Endophyte infection increased plant growth, reproductive output, total nutrient content and competitive ability, but greater levels of mycorrhizal infection did not do so, even in the absence of endophytes. Such null results are not unusual (Gange & Ayres 1999), and do not suggest that AM fungi make no contribution to plant P uptake (Smith, Smith & Jakobsen 2004).

The results of this study demonstrate that endophyte effects can have important implications for the interpretation of other AM studies. Most competition-mycorrhiza studies (Davies & Graves 1998; Marler, Zabinski & Callaway 1999) have used pasture grasses, common hosts of *Neotyphodium* such as *Festuca* and *Lolium* spp., but without considering the presence or absence of the fungal endophyte. Additionally, experiments conducted to study the effects of mycorrhizae usually include use of the systemic fungicide benomyl to suppress the AM fungi (Marler *et al*. 1999; Smith, Hartnett & Wilson 1999), a fungicide that also effectively kills endophytes (Latch 1983).

In summary, our study demonstrates that interactions between plants and AM fungal communities are modified by endophytes, and that those changes do not necessarily feed back on the growth of plants. The exact mechanisms for mycorrhizal colonization rates between endophyte-infected and uninfected plants, and among plants under different competition conditions, have yet to be identified. Competition for photosynthates and the accumulation of secondary compounds in endophyte-infected plants, as well as any signals released by symbiosis tissues (Bush *et al*. 1997; Malinowski & Belesky 2000), may act to suppress other infecting microbial symbionts in the same plant host (Denison *et al*. 2003). The outcome of the interactions between plants and endophyte or mycorhrizal fungi may be dependent on soil nutrients (Lehtonen *et al*. 2005; Morgan *et al*. 2005). The current lack of information on interactions between different fungi hampers our understanding of how above- and below-ground microbial symbionts influence plant growth, competition and, consequently, community dynamics and ecosystem processes.

Acknowledgements

We thank Mark Bradford, Enrique Chaneton, Agustín Grimoldi, John Newington, Pablo Roset, George Tordoff and Andrea Uchitel for discussion. The work was funded, in part, by British Council-Fundación Antorchas, FOMEC and NERC grants.

References

Bonkowski, M. & Roy, J. (2005) Soil microbial diversity and soil functioning affect competition among grasses in experimental microcosms. *Oecologia* **143**, 232–240.

Borowicz, V. (2002) Do arbuscular mycorrhizal fungi alter plant–pathogen relations? *Ecology* **82**, 3057–3068.

Bush, L.P., Wilkinson, H.H. & Schardl, C.L. (1997) Bioprotective alkaloids of grass–fungal endophyte symbiosis. *Plant Physiology* **114**, 1–7.

Cahill, J. (2002) What evidence is necessary in studies which separate root and shoot competition along productivity gradients? *Journal of Ecology* **90**, 201–205.

Chu-Chou, M., Guo, B., An, Z.Q. *et al.* (1992) Suppression of mycorrhizal fungi in fescue by the *Acremonium coenophialum* endophyte. *Soil Biology and Biochemistry* **24**, 633–637.

Clay, K. & Holah, J. (1999) Fungal endophyte symbiosis and plant diversity in successional fields. *Science* **285**, 1742–1744.

Clay, K. & Schardl, C.L. (2002) Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* **160**, 99–127.

Davies, D. & Graves, J. (1998) Interactions between arbuscular mycorrhizal fungi and the hemiparasitic angiosperm *Rhinanthus minor* during co-infection of a host. *New Phytologist* **139**, 555–563.

de Battista, J. (2005) *Neotyphodium* research and application: current trends in South America. *Neotyphodium in Cool-Season Grasses* (eds C. Roberts, C.P. West & D. Spiers), pp. 65–71. Blackwell Scientific, Oxford, UK.

Denison, R., Bledsoe, C., Kahn, M., O'Gara, F., Simms, E. & Thomashow, L. (2003) Cooperation in the rhizosphere and the 'free rider' problem. *Ecology* **84**, 838–845.

Faeth, S. & Sullivan, T. (2003) Mutualistic asexual endophytes in a native grass are usually parasitic. *American Naturalist* **161**, 310–325.

Faeth, S., Helander, M.L. & Saikkonen, K.T. (2004) Asexual *Neotyphodium* endophytes in a native grass reduce competitive abilities. *Ecology Letters* **7**, 304–313.

Fitter, A.H. (2001) Specificity, links and networks in the control of diversity in plant and microbial communities. *Ecology: Achievement and Challenge* (eds M. Press, N. Huntly & S. Levin), pp. 95–114. Blackwell Scientific, Oxford, UK.

Fitter, A. (2005) Darkness visible: reflections on underground ecology. *Journal of Ecology* **93**, 231–243.

Gange, A.C. & Ayres, R.L. (1999) On the relation between arbuscular mycorrhizal colonization and plant 'benefit'. *Oikos* **87**, 615–621.

Gange, A.C. & West, H.M. (1994) Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. *New Phytologist* **128**, 79–87.

Gange, A.C., Brown, V.K. & Sinclair, G.C. (1993) Vesicular-arbuscular mycorrhizal fungi: a determinant of plant community structure in early succession. *Functional Ecology* **7**, 616–626.

Gange, A.C., Bower, E., Stagg, P., Aplin, D.M., Gillam, A.E. & Bracken, M. (1999) A comparison of visualization techniques for recording arbuscular mycorrhizal colonisation. *New Phytologist* **142**, 123–132.

Guo, B., Hendrix, J., An, Z.Q. & Ferriss, R. (1992) Role of *Acremonium* endophyte of fescue on inhibition of colonisation and reproduction of mycorrhizal fungi. *Mycologia* **84**, 882–885.

Hartnett, D., Hetrick, B., Wilson, G.W.T. & Gibson, D. (1993) Mycorrhizal influence on intra- and interspecific neighbour interactions among co-occurring prairie grasses. *Journal of Ecology* **81**, 787–795.

van der Heijden, M.G.A. & Sanders, I.R. (2002) Mycorrhizal ecology: synthesis and perspectives. *Mycorrhizal Ecology* (eds M.G.A. van der Heijden & I.R. Sanders), pp. 441–456. Springer-Verlag, Berlin, Heidelberg.

van der Heijden, M.G.A., Klironomos, J.N., Ursic, M. *et al.* (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**, 69–72.

Klironomos, J.N. (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* **84**, 2292–2301.

Latch, C.G.M. (1983) Incidence of endophytes in seed lines and their control with fungicides. *Proceedings of the New Zealand Grassland Association* **44**, 251–253.

Lehtonen, P., Helander, M. & Saikkonen, K. (2005) Are endophyte-mediated effects on herbivores conditional on soil nutrients? *Oecologia* **142**, 38–45.

Malinowski, D.P. & Belesky, D. (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. *Crop Science* **40**, 923–940.

Marlinowski, D.P., Belesky, D.P. & Lewis, G.C. (2005) Abiotic stresses in endophytic grasses. *Neotyphodium in Cool-Season Grasses Current Research & Applications* (eds C. Roberts, C.P. West & D. Spiers), pp. 187–200. Blackwell Scientific Publications, Oxford.

Marks, S., Clay, K. & Cheplick, G.P. (1991) Effects of fungal endophytes on interspecific and intraspecific competition in the grasses *Festuca arundinacea* and *Lolium perenne*. *Journal of Applied Ecology* **28**, 194–204.

Marler, M.J., Zabinski, C.A. & Callaway, R.M. (1999) Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass. *Ecology* **80**, 1180–1186.

McGonigle, T., Miller, M., Evans, D., Fairchild, G. & Swan, J. (1990) A new method which gives an objective measure of colonisation of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115**, 495–501.

Menéndez, A., Scervino, J. & Godeas, A. (2001) Arbuscular mycorrhizal populations associated with natural and cultivated vegetation on a site of Buenos Aires province, Argentina. *Biology and Fertility of Soils* **33**, 373–381.

Moon, C.D., Scott, B., Schardl, C.L. & Christensen, M.J. (2000) Evolutionary origins of *Epichloë* endophytes from annual ryegrasses. *Mycologia* **92**, 1103–1118.

Morgan, J., Bending, G. & White, P. (2005) Biological costs and benefits to plant–microbe interactions in the rhizosphere. *Journal of Experimental Botany* **56**, 1729–1739.

Müller, J. (2003) Artificial infection by endophytes affects growth and mycorrhizal colonisation of *Lolium perenne*. *Functional Plant Biology* **30**, 419–424.

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

Omacini, M., Chaneton, E.J., Ghersa, C.M. & Otero, P. (2004) Do foliar endophytes affect grass litter decomposition? A microcosm approach using *Lolium multiflorum*. *Oikos* **104**, 581–590.

Omacini, M., Chaneton, E.J. & Ghersa, C.M. (2005) A framework for understanding the ecosystem consequences of endophyte grass symbioses. *Neotyphodium in Cool-Season Grasses: Current Research and Applications* (eds C. Roberts, C.P. West & D. Spiers), pp. 141–161. Blackwell Scientific, Oxford, UK.

Popay, A.J. & Bonos, S.A. (2005) Biotic responses in endophytic grasses. *Neotyphodium in Cool-Season Grasses Current Research and Applications* (eds C. Roberts, C.P. West & D. Spiers), pp. 163–185. Blackwell Scientific, Oxford, UK.

Ravel, C., Michalakis, Y. & Charmet, G. (1997) The effect of imperfect transmission on the frequency of mutualistic seed-borne endophytes in natural populations of grasses. *Oikos* **80**, 18–24.

Saikkonen, K., Ion, D. & Gyllenberg, M. (2002) The persistence of vertically transmitted fungi in grass metapopulations. *Proceedings of the Royal Society of London B* **269**, 1397–1403.

Scheiner, S. (2001) MANOVA: multiple response variables and multispecies interactions. *Design and Analysis of Ecological Experiments* (eds S.M. Scheiner & J. Gurevitch), pp. 99–115. Oxford University Press, New York.

Smith, M., Hartnett, D.C. & Wilson, G.W.T. (1999) Interactive influence of mycorrhizal symbiosis and competition on plant diversity in tallgrass prairie. *Oecologia* **121**, 574–582.

Smith, S.E. & Read, D.J. (1997) *Mycorrhizal Symbioses*, 2nd edn. Academic Press, London.

Smith, S.E., Smith, A. & Jakobsen, I. (2004) Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist* **162**, 511–524.

Sokal, R. & Rohlf, F. (1995) *Biometry*, 3rd edn. WH Freeman, San Francisco, CA, USA.

van der Putten, W.H. & Peters, B.A.M. (1997) How soil-borne pathogens may affect plant competition. *Ecology* **78**, 1785–1795.

Vicari, M., Hatcher, P.E. & Ayres, P. (2002) Combined effect of foliar and mycorrhizal endophytes on an insect herbivore. *Ecology* **83**, 2452–2464.

Received 14 September 2005; revised 28 November 2005; accepted 9 December 2005
Editor: J. Cresswell