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Author(s): K. K. Newsham, A. H. Fitter and A. R. Watkinson

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# Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field

K. K. NEWSHAM,\* A. H. FITTER and A. R. WATKINSON†

Department of Biology, University of York, York YO1 5DD and †School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK

## Summary

**1** Seedlings of the annual grass *Vulpia ciliata* ssp. *ambigua* were inoculated in the laboratory with a factorial combination of the cosmopolitan root pathogen *Fusarium oxysporum* and an arbuscular mycorrhizal (AM) fungus (a *Glomus* sp.) before being planted out into a natural population of *V. ciliata* at Mildenhall, UK, from which both fungi had been isolated.

**2** At both 62 and 90 days after transplantation, inoculation with *Glomus* sp. had not increased plant P concentrations, but had protected the plants from the deleterious effects of *F. oxysporum* infection on shoot and root growth, apparently by suppressing pathogen development in roots. The effects of *Glomus* sp. on plant performance were negligible in the absence of *F. oxysporum*.

**3** After transplantation, comparisons made of the root-infecting mycofloras of uninoculated plants and plants inoculated only with *Glomus* sp. showed that the latter developed fewer naturally occurring infections of *F. oxysporum* and *Embellisia chlamydospora*, two species of fungi which are correlated with reductions in fecundity in natural populations of *V. ciliata*.

**4** These results confirm conclusions from previous experiments that the main benefit supplied by AM fungi to *V. ciliata* is in protection from pathogenic fungi, rather than improved P uptake, and indicate that AM colonization significantly alters the root-infecting mycoflora of *V. ciliata*. We propose that AM fungi may confer similar benefits in other plant species, which may account for the difficulty in demonstrating a benefit of AM fungi to the P nutrition of host plant species under natural conditions.

**Keywords:** arbuscular mycorrhizal fungi, *Fusarium oxysporum*, *Glomus* sp., plant population dynamics, root-infecting mycoflora, root pathogenic fungi, *Vulpia ciliata* ssp. *ambigua*

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## Introduction

Although arbuscular mycorrhizal (AM) fungi are probably the commonest symbiotic organisms associated with higher plants, their role in plant ecology is poorly defined. At present, their principal role is thought to be in the facilitation of phosphorus (P) uptake by plants. This assumption stems both from theoretical calculations of P mobility in soil (Sanders & Tinker 1973) and from the large number of research papers that have demonstrated positive effects of AM colonization in roots on plant P nutrition. However,

these studies have typically been made on plants grown in the glasshouse or laboratory under contrived conditions where elevated levels of other nutrients are applied to ensure that P is limiting. Field experiments in strongly P-limited soils, such as those found in the tropics, have shown that AM fungi can improve plant P nutrition (Howeler *et al.* 1983; Howeler *et al.* 1987), but field evidence for a role of these fungi in plant P nutrition in temperate ecosystems is at best conflicting (Fitter 1985) or at worst absent (Sanders & Fitter 1992). This may be because plants growing in soils in temperate regions are not P-limited, and if this is the case, then it is plausible that AM fungi may confer other benefits on plant species growing in these regions. However, the possibility that AM fungi may provide benefits to plants other than in P uptake has

\*Present address and correspondence: ITE Monks Wood Research Station, Abbots Ripton, Huntingdon, Cambs. PE17 2LS, UK.

not been widely addressed, although they are known to be capable of reducing pathogenic infection (Schönbeck 1979; Dehne 1982), increasing the uptake of poorly mobile nutrients such as zinc (Gildon & Tinker 1983) or possibly improving plant water relations (Allen *et al.* 1981; Allen & Allen 1986).

In recent years, we have investigated the contribution of AM fungi to the fitness of *Vulpia ciliata* ssp. *ambigua* (Le Gall) Stace & Auquier (hereafter *V. ciliata*), an annual grass of north-western Europe. The results of these studies show conclusively that there is no relationship between the level of AM colonization in the roots of *V. ciliata* and the species' P nutrition under field conditions (Carey *et al.* 1992; West *et al.* 1993; Newsham *et al.* 1994). This is probably because *V. ciliata* possesses a relatively efficient root system, with many fine roots which allow the exploitation of a relatively large volume of soil and reduce the dependence of the species upon AM fungi for P uptake. However, although *V. ciliata* does not benefit from the association with AM fungi through enhanced P uptake, the roots of the species are consistently colonized by these fungi in the wild (Carey *et al.* 1992; West *et al.* 1993; Newsham *et al.* 1994). This raises the question as to what benefits the association confers on *V. ciliata*, and whether the symbiosis is mutualistic.

The application of fungicides to natural populations of *V. ciliata* significantly reduces AM colonization of roots but typically has no effect on plant performance (Newsham *et al.* 1994). This lack of response apparently arises from the simultaneous reduction in both AM and pathogenic fungi in roots, and is consistent with correlative evidence which indicates that AM fungi may protect *V. ciliata* against the substantial deleterious effects of the pathogenic fungus, *Fusarium oxysporum* Schlecht. on seed production (Newsham *et al.* 1994). Although AM fungi are known to protect horticultural plants from fungal root pathogens under controlled conditions (Dehne & Schönbeck 1979a; Davis & Menge 1980), no studies have apparently been made to demonstrate this effect conclusively in plants growing under natural conditions with specific combinations of root fungi. Furthermore, the extent to which AM fungi can protect plants from naturally occurring root mycofloras remains unresolved. Most studies have concentrated on the ability of AM colonization to reduce infection by specific root pathogens, most notably *formae speciales* of *F. oxysporum* (e.g. Dehne & Schönbeck 1979a; Hwang *et al.* 1992). However, given the wide diversity of root-infecting fungi (Harley & Waid 1955; Garrett 1970), it is necessary to assess the extent of this protective effect other than in monocultures in the glasshouse or laboratory.

Here we test the ability of an AM fungus (a *Glomus* sp.) to protect *V. ciliata* from the deleterious effects of *F. oxysporum* by the use of a transplant technique in which plants were inoculated with a factorial com-

bination of both fungi in the laboratory before being transplanted into a natural population of *V. ciliata*. This approach enabled the establishment of controlled levels of AM and pathogenic fungi in roots prior to subjecting the plants to nutrient- and water-stressed field conditions, which are likely to induce marked responses to root pathogens (Campbell & Hendrix 1974). In addition, to provide a wider view of the role of AM fungi in protecting plants from root-infecting fungi, we tested whether the presence of AM colonization in roots significantly altered the abundance of naturally occurring root pathogenic fungi.

## Materials and methods

### STUDY SPECIES

*V. ciliata* is a winter annual grass restricted to sandy soils in eastern and southern England and northern France (Cotton & Stace 1976), which are not usually P-limited. Germination occurs in the autumn and early winter and flowering in the following spring and early summer. Seeds remain dormant over the summer and there is no permanent bank of seed (Carey & Watkinson 1993). Plant mortality is low in the vegetative phase, and so the species normally exhibits Deevey type one mortality (Carey 1991). AM colonization in roots is low in the winter months (Carey *et al.* 1992; West *et al.* 1993) but reaches 40% of the root length of *V. ciliata* in the spring and early summer (West *et al.* 1993).

### SITE DESCRIPTION

The study site was an open grassland located at Mildenhall, Suffolk, UK (National Grid reference TL 728 758). The soil is a humic sandy gley with a pH of 7.3 (in 10-mM CaCl<sub>2</sub>), nitrate-N (extracted in H<sub>2</sub>O), P (H<sub>2</sub>O) and potassium (NH<sub>4</sub>OAc) concentrations of 12.4, 11.0 and 53.8 mg kg<sup>-1</sup>, respectively, and an organic matter content of 3.8% (Newsham *et al.* 1994). The main vascular plant species at the site were *Cerastium fontanum* Baumg., *Erodium cicutarium* (L.) L'Her., *Saxifraga granulata* L., *Sedum acre* L., *Trifolium micranthum* Viv. and *V. ciliata* (Newsham *et al.* 1994).

### CULTURE OF AM FUNGI

The culture of *Glomus* sp. used in the study was obtained from trap cultures of *V. ciliata* plants taken from Mildenhall in June 1992. This is registered in the European Bank of Glomales as BEG 6 and voucher cultures are held in the Department of Biology at the University of York, UK. Plants from the site were transferred into double-autoclaved sand (2 × 30 min at 121 °C on two successive days) in sterile 6-cm-deep pots and were watered with sterile (20 min at 121 °C)

deionized water from above every day. A sterile filter paper circle in the base of each pot prevented the loss of sand. The pots were kept in a growth cabinet held at 60% RH with a 16-h cycle of light (at a temperature of 20 °C) and 8 h of darkness (17 °C). Cores of roots taken regularly from the pots were examined for AM fungal spore production; after 30 weeks, 160 spores of the same morphotype were removed from one plant. These spores were surface sterilized in 10% sodium hypochlorite solution for 6 s and were washed five times in sterile deionized water in a sterile watch glass. Wefts of sterile cotton wool held the spores in place as solutions were added and removed with sterile Pasteur pipettes. Twenty spores were then used to inoculate each of eight surface sterilized seedlings of *V. ciliata* in 6-cm-deep pots containing sterile sand. A further eight surface sterilized *V. ciliata* seedlings were planted in 6-cm-deep pots of sterile sand.

Surface sterilized seedlings of *V. ciliata* were obtained by shaking seeds collected from Mildenhall in 15 mL of 10% sodium hypochlorite solution in sterile McCartney bottles for 30 s on a wrist action shaker at 60 beats s<sup>-1</sup>. The solution was decanted from the bottles and the seeds were then washed in three further changes of 15 mL sterile deionized water for 2 min, again on a wrist action shaker at 60 beats s<sup>-1</sup>. The seeds were germinated on 0.75% w/v technical agar (Oxoid no. 3) to detect any contamination by bacteria or fungi and uncontaminated seedlings were picked from the agar after 4 days.

The 16 seedlings of *V. ciliata* were replaced in the growth cabinet, were watered as above and occasionally fed with quarter-strength Rorison's nutrient solution.

#### CULTURE OF ROOT PATHOGENIC FUNGI

The culture of *F. oxysporum* used in the study was isolated from *V. ciliata* roots collected from Mildenhall in May 1992 in the experiments described by Newsham *et al.* (1994). The fungus was sustained on SNA medium (Nirenberg 1981; 15% agar w/v) on slants and was routinely subcultured. A Koch's Postulate experiment in the laboratory indicated that this was an aggressive isolate of *F. oxysporum* with the ability to reduce shoot and root biomass of *V. ciliata* (K. K. Newsham unpublished data). To obtain sufficient inoculum for the experiment, the isolate was grown on 15 mL of SNA in eight 9 cm non-vented Petri dishes for 14 days at room temperature. Ten 10-mm discs were cut from the growing margin of each culture and were added to double-autoclaved mixtures of 190 g of sieved sand (obtained from an embankment area at Mildenhall), 10 g of crushed wheat and 30 mL of deionized water in eight cotton-wool-plugged, 500-mL flasks (Skipp & Christensen 1982). A further eight flasks were inoculated with 10 discs of SNA medium minus *F. oxysporum*. The flasks were incubated in the dark at 30 °C and were watered

regularly under a sterile hood with 5 mL of sterile deionized water to avoid desiccation.

The density of *F. oxysporum* propagules in the sand in each flask was estimated after 4 weeks by the Warcup (1950) soil-plate method. Sand (30–40 mg dry wt) from each flask was added to each of three 9 cm non-vented Petri dishes from a sterile microspatula and cooled (*c.* 40 °C) Czapek–Dox nutrient agar medium (12 mL) was added after dispersing the sand evenly in the dishes. The Petri dishes were kept in the light at room temperature and were examined after 3–4 days when *F. oxysporum* colonies were counted. This procedure also confirmed the absence of contaminants in the cultures. Sterile sand was used to adjust the inoculum density of *F. oxysporum* in each flask to 5 × 10<sup>3</sup> propagules g<sup>-1</sup>, equivalent to field inoculum density of the species in *V. ciliata* rhizosphere sand (K. K. Newsham unpublished data).

#### INOCULATION OF SEEDLINGS WITH FUNGI

Seedlings of *V. ciliata* were grown with a factorial combination of *F. oxysporum* and *Glomus* sp. in seed trays measuring 21 cm × 17 cm × 5 cm. Two replicate trays were prepared for each treatment under a sterile hood with a plastic divider inserted into each tray. *F. oxysporum* inocula consisted of 200 g of sand and wheat mixture from each *F. oxysporum*-inoculated or uninoculated flask, added to the bottom of the trays on either side of each divider. The *F. oxysporum* inocula were covered with a further 1200 g of sterile sand. *Glomus* sp. inocula consisted of 200 g of chopped roots and sand from the *Glomus*-inoculated or uninoculated *V. ciliata* plants added to either side of each divider into the sterile sand. Seedlings of *V. ciliata* from Mildenhall were surface sterilized as described above and 15 were planted into the wetted sand on either side of each divider on 31 January 1994. Water extracts (5 mL) from the *Glomus*-inoculated pots of *V. ciliata* were passed through a 5-µm Millipore filter and were added to each tray to ensure the same microfungal and bacterial complement in each treatment. The eight trays were returned to the growth cabinet and were watered as before (minus Rorison's solution) for 40 days. Between 14 February 1994 and 3 March 1994, the temperature of the cabinet was lowered for the light and dark cycles by 1 °C day<sup>-1</sup> in an attempt to vernalize the plants. The final light and dark cycle temperatures were 4 °C and 1 °C, respectively.

#### EXPERIMENTAL DESIGN AND SAMPLING

The seedlings of *V. ciliata* were taken from the laboratory to Mildenhall on 12 March 1994, where they were carefully extracted from the sand in each tray and were planted out into a randomized block experimental design. The vegetation into which the plants were placed was sparse, so little disturbance to the

community was necessary. Four blocks were defined in which each plant was separated from its neighbour by 20 cm and each of the four blocks were separated by 1 m. There were 32 plants in each block. Sixteen plants per treatment were retained as a time zero measurement. Four plants from each treatment within each block were subsequently sampled on 13 May and 10 June 1994 after 62 and 90 days of growth in the field.

#### PLANT GROWTH DETERMINATIONS

Shoots were dried at 80 °C for 48 h prior to weighing and were subsequently digested in a mixture of HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> and HClO<sub>4</sub> (10:1:1). P concentrations were determined by the molybdenum blue method with ascorbic acid (Allen 1989). Root length was measured by a modified line intersect method (Marsh 1971).

#### MICROBIAL DETERMINATIONS

Root-infecting fungi were quantified by a modification of the Harley & Waid (1955) root-plating method within 3 days of returning the plants to the laboratory. Three roots from each plant were stripped of their side-branches, washed in water, surface sterilized for 6 s in 10% sodium hypochlorite solution and were aseptically transferred to 5 mL of sterile deionized water in a McCartney bottle. Bottles were agitated on a wrist-action shaker for 2 min at 60 beats s<sup>-1</sup> and each root was blotted dry on sterile filter paper and cut into 2 mm sections with a sterile scalpel under a sterile hood. Five sections of each root were then plated equidistantly into Czapek–Dox agar medium and three plates were prepared per plant. Czapek–Dox medium was used to encourage the growth of a broad spectrum of fungal species (although the isolation of all fungi on the media was unlikely), with rose bengal (1:15 000) added to slow the growth of faster-growing species of fungi and to reduce the growth of bacteria (Warcup 1950). The plates were incubated in the light at room temperature and were examined after 3 days for colonies of faster-growing species of fungi which were excised, and then over a further 18 days to isolate slower-growing species of fungi. Isolation frequencies of root-infecting fungi were expressed as the number of colonies isolated per plant, i.e. per 30 mm of root.

The remaining roots of each plant were bulked, washed in water and cleared in 10% KOH at 90 °C for 5 min, using the batch method described by Grace & Stribley (1991). The roots were rinsed in three changes of water, acidified in cold 1% HCl for 15 min and stained in 0.01% acid fuchsin at 90 °C for 15 min. After destaining in lactoglycerol overnight at room temperature, the roots of each plant were mounted in lactoglycerol on a slide. The abundance of AM and pathogenic fungi in the roots was quantified by epifluorescence microscopy (Merryweather & Fitter

1991) at × 250 magnification using the intersection method described by McGonigle *et al.* (1990) with a minimum number of 100 intersections per slide. Mycorrhizal colonization was defined as typically aseptate hyphae (> 5 µm in diameter), forming arbuscules and vesicles, and *F. oxysporum* infection defined as septate hyphae (< 5 µm in diameter) with frequent production of chlamydospores (Booth 1977).

#### STATISTICAL ANALYSES

To test for main and interactive effects of AM and pathogenic fungi on plant performance, the shoot biomass, root length, P concentration and percentage septate hyphal infection data were subjected to a three-way generalised linear model and a Fisher's pairwise comparisons test in the MINITAB 8.21 package. Shoot biomass and percentage hyphal infection data were ln and arcsine square root transformed, respectively, prior to analysis. Correlations were also performed in the MINITAB 8.21 package. Owing to the distribution of the data on fungal abundances in roots, statistical analyses using simple correlations were not appropriate and so an Olmstead and Tukey's corner test for association was used to test for a relationship between the percentage frequencies of AM and septate hyphae in roots (Sokal & Rohlf 1981).

The numbers of colonies of each individual fungal species and of the total numbers of fungal and bacterial colonies isolated after 62 and 90 days of growth in the field from each uninoculated plant and each *Glomus*-inoculated plant were compared at each harvest by an analysis of deviance test in the GENSTAT 5.0 package. The deviance (the maximum value of the log-likelihood ratio) was used to test the goodness of fit of a model, by measuring the discrepancy between the data and the fitted values derived from the model (Dighton *et al.* 1986).

## Results

#### PLANT GROWTH

Microbial analyses prior to transplantation showed that the two fungal taxa occurred only in the appropriately inoculated plants: there was apparently no cross-contamination between treatments. A maximum of two (and a minimum of one) *F. oxysporum* colonies were isolated per 30 mm of root from plants inoculated with the pathogen and the mean level of AM colonization in the roots of *Glomus*-inoculated plants prior to transplantation was 6% of root length. After 40 days of growth with *F. oxysporum* and *Glomus* sp. in the laboratory, i.e. prior to transplantation into the field, neither of the fungi affected shoot P concentration or root length of *V. ciliata*. However, shoot biomass was affected: there were significant ( $F_{1,12} = 11.7$ ,  $P < 0.01$ ) reductions in



**Table 1** Main and interactive effects of a factorial combination of *Fusarium oxysporum* (Fus) and *Glomus* sp. (Glm) on root length, shoot biomass, leaf P concentration and septate fungal infection of roots of *V. ciliata* plants sampled from the field 62 and 90 days after transplantation. Data from both harvests were pooled and analysed with a three-way generalised linear model in the MINITAB 8.21 package. Shoot biomass and percentage pathogenic infection data were ln and arc sine square root transformed prior to analysis respectively. Sampling × Fus and sampling × Glm interactions have not been included in the table as none were significant

Main and interactive effects	Root length	Shoot biomass	Leaf P concentration	Septate hyphal infection
Sampling	NS	$F_{1,111} = 10.6^{**}$	NS	NS
Fus	$F_{1,114} = 9.0^{**}$	$F_{1,111} = 17.3^{***}$	NS	NS
Glm	$F_{1,114} = 9.4^{**}$	NS	NS	$F_{1,114} = 14.8^{***}$
Fus × Glm	$F_{1,114} = 9.0^{**}$	$F_{1,111} = 4.8^{**}$	$F_{1,111} = 9.7^{**}$	$F_{1,114} = 3.9^{**}$
Sampling × Fus × Glm	NS	$F_{1,111} = 6.5^{**}$	NS	NS

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

ln shoot biomass values in response to *F. oxysporum* inoculation, significant ( $F_{1,12} = 16.1$ ,  $P < 0.01$ ) increases in shoot biomass values in response to *Glomus* sp. inoculation and a significant ( $F_{1,12} = 9.2$ ,  $P < 0.05$ ) *F. oxysporum* × *Glomus* sp. interaction: the shoots of plants grown with both fungi were heavier than those of plants grown with *F. oxysporum* alone.

After 62 and 90 days of growth in the field, main effects suggested that *Glomus* sp. increased root length and that *F. oxysporum* decreased both root length and shoot biomass, but there was a significant *F. oxysporum* × *Glomus* sp. interaction for both variables (Table 1): *Glomus* sp. exerted significant impacts on the performance of *V. ciliata* only when plants had been grown with *F. oxysporum* prior to transplantation and the effects of *Glomus* sp. on plant performance were negligible in the absence of the pathogen (Fig. 1a,b). There was a significant sampling effect for shoot biomass, as *Glomus* sp. did not appear to protect the plants from the effects of *F. oxysporum* after 90 days of growth in the field (Table 1; Fig. 1b), which was further indicated by the significant sampling × *F. oxysporum* × *Glomus* sp. interaction (Table 1).

There were no significant effects of the two fungal taxa individually at 62 and 90 days after transplantation on shoot P concentrations (Table 1; Fig. 2a). However, when data for both harvests were analysed together, there was a significant *F. oxysporum* × *Glomus* sp. interaction (Table 1). Leaf P concentration was not correlated with the frequency of either AM ( $F_{1,119} = 1.78$ ,  $P > 0.05$ ,  $r^2 = 0.02$ ) or septate hyphae ( $F_{1,119} = 0.00$ ,  $P > 0.05$ ,  $r^2 = 0.00$ ) in roots over both harvests.

FUNGAL COLONIZATION

Inoculation of plants with *Glomus* sp. reduced the percentage of root length infected by septate hyphae over both harvests: plants grown with both fungi were infected by significantly fewer septate hyphae than plants grown with *F. oxysporum* alone prior to transplantation (Table 1; Fig. 2b). An Olmstead and

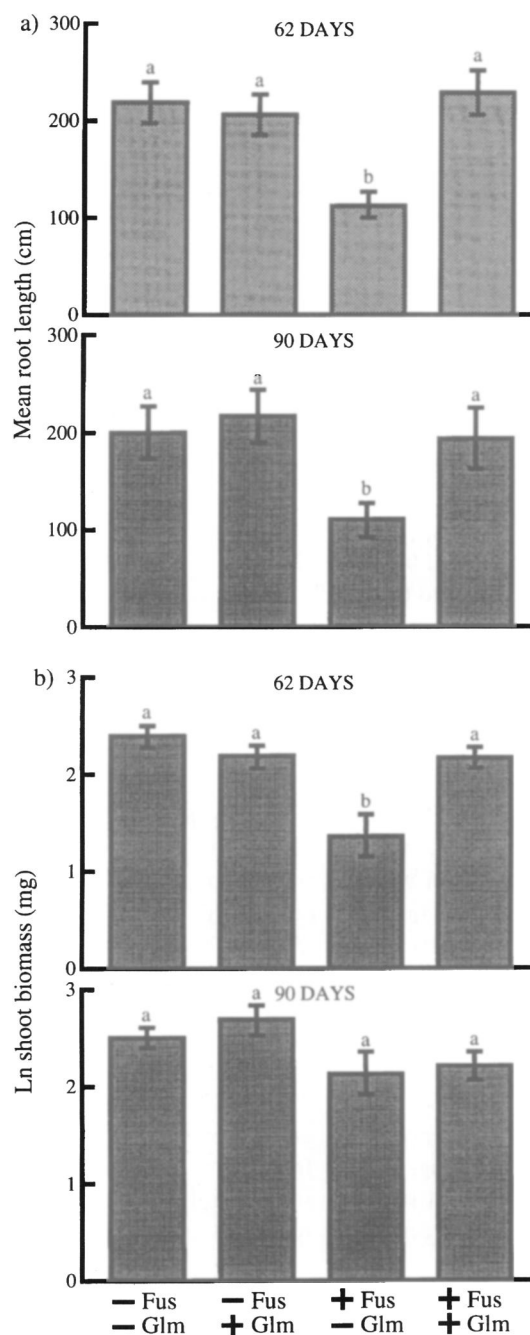
Tukey's corner test also indicated that there was a significant ( $P < 0.01$ ) relationship between the intensity of AM colonization in roots and the infection of root tissues by septate hyphae: roots were rarely heavily colonized by both taxa (Fig. 3). The frequency of septate hyphae in roots over both harvests was a negative function of both shoot biomass ( $F_{1,119} = 8.34$ ,  $P < 0.01$ ,  $r^2 = 0.12$ ) and root length ( $F_{1,122} = 13.46$ ,  $P < 0.001$ ,  $r^2 = 0.08$ ).

Twenty different fungal taxa were isolated from the roots of *V. ciliata* plants sampled from Mildenhall, the majority of which were hyphomycetes. The most commonly isolated organism was *F. oxysporum* (c. 40% of all isolates): other commonly isolated fungal species (> 5% frequencies of occurrence) were *Embellisia chlamydospora* (Hoes, Bruehl & C.G. Shaw) E. Simmons, *Fusarium equiseti* (Corda) Sacc., *Fusarium* sp., *Penicillium* spp., sterile isolates m7 and m14, *Truncatella truncata* (Lev.) Steyaert and *Ulocladium atrum* (Preuss), which are typical of the fungi isolated from the roots of Mildenhall *V. ciliata* plants (Newsham *et al.* 1995). Bacteria were also isolated from the roots, but these were not identified to specific taxa.

The roots of *V. ciliata* plants which had been inoculated with only *Glomus* sp. in the laboratory before transplantation supported significantly fewer colonies of *F. oxysporum* and *E. chlamydospora* after 62 and 90 days of growth in the field, when compared with plants uninoculated with any fungi (Fig. 4). The roots of AM-inoculated plants were more frequently infected by sterile isolate m7 after 62 days of growth in the field and by *Penicillium* spp. after 90 days of growth in the field (Fig. 4). The total numbers of fungal colonies (all taxa) isolated from the roots was significantly ( $P < 0.05$ ) lower in *Glomus*-inoculated plants, but total bacterial colonization of the roots was apparently unaffected by AM colonization.

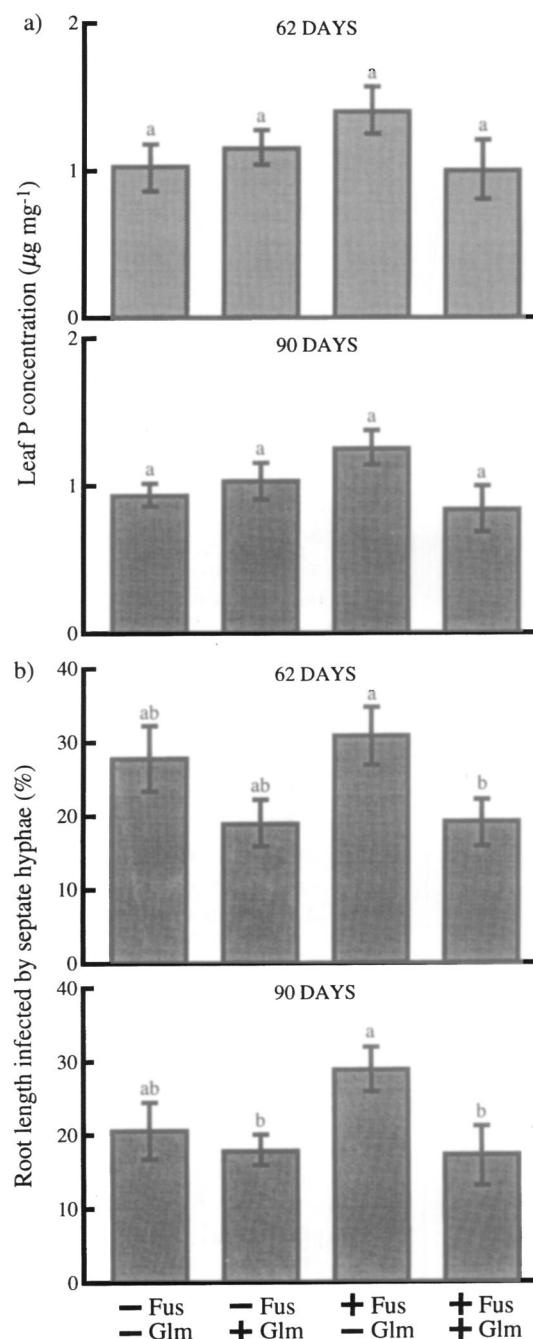
Discussion

The results from our study are one of the few clear demonstrations that AM fungi benefit plant performance in the natural environment and the first to



**Fig. 1** The effects of a factorial combination of *Fusarium oxysporum* (Fus) and *Glomus* sp. (Glm) on (a) root length and (b) ln shoot biomass of *V. ciliata* plants sampled from the field 62 and 90 days after transplantation. Values are means of 16 replicates  $\pm$  SEM. Values distinctly superscripted differ at  $P < 0.05$ . See Table 1 for main and interactive effects of treatments.

show that this benefit is unrelated to P nutrition. They show unequivocally that a species of *Glomus* benefits a plant species in the wild by protecting it from the deleterious effects of a cosmopolitan root pathogenic fungus. Roots of *V. ciliata* were up to twice as long when plants had been grown with *Glomus* sp. and *F. oxysporum* prior to transplantation compared with plants grown with *F. oxysporum* alone, corroborating the conclusions drawn by Carey *et al.* (1992) that the



**Fig. 2** The effects of a factorial combination of *Fusarium oxysporum* (Fus) and *Glomus* sp. (Glm) on (a) leaf P concentration and (b) septate fungal infection of roots of *V. ciliata* plants sampled from the field 62 and 90 days after transplantation. Values are means of 16 replicates  $\pm$  SEM. Values distinctly superscripted differ at  $P < 0.05$ . See Table 1 for main and interactive effects of treatments.

benefit of AM fungi to *V. ciliata* in protection from pathogens was probably greater than the 31% estimated in their study. The results of our study also confirm the conclusions drawn by Carey *et al.* (1992), West *et al.* (1993) and Newsham *et al.* (1994) that AM fungi play no part in the P nutrition of *V. ciliata*.

The plants used in our experiment were apparently not fully vernalized prior to transplantation and did not flower. However, since seed number is a function

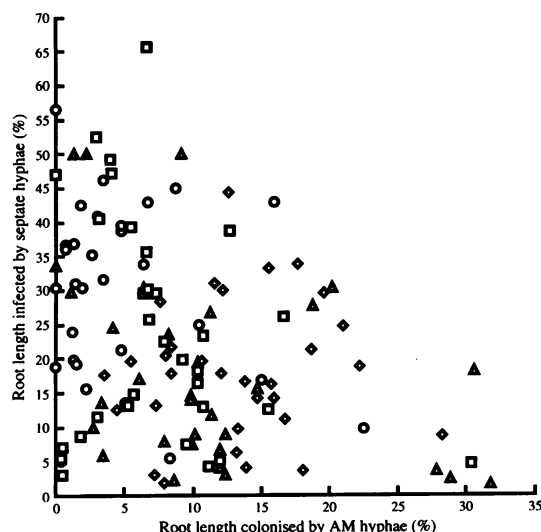


Fig. 3 Percentage of root length of *V. ciliata* infected by septate hyphae as a function of percentage root length colonized by arbuscular mycorrhizal (AM) hyphae after 62 and 90 days of growth in the field: (□) -Fus-Glm; (◇) -Fus + Glm; (○) +Fus-Glm; (△) +Fus + Glm.

of shoot biomass and root biomass in *V. ciliata* ( $F_{1,29} = 26.8$ ,  $P < 0.001$ ,  $r^2 = 0.49$  and  $F_{1,29} = 19.5$ ,  $P < 0.001$ ,  $r^2 = 0.41$ , respectively; data from the experiments described by Newsham *et al.* 1994), the results of our experiment imply a large benefit of AM fungi on seed output in the species, which is

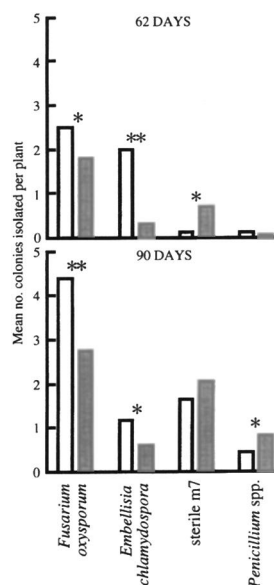


Fig. 4 Fungi isolated from the roots of *V. ciliata* grown in the laboratory (□) without (-Fus-Glm) and (■) with (-Fus + Glm) *Glomus* sp. inocula before being transplanted into the field and sampled after 62 and 90 days. Only those species of fungi which responded significantly to treatment are shown. Significant differences between means are indicated by \* $P < 0.05$  and \*\* $P < 0.01$ . Values are means of 16 replicates. Error bars are not shown as these data were binomially distributed. The mean number of colonies of each species were compared between treatments by an analysis of deviance test in the GENSTAT 5.0 package.

corroborated by correlative evidence in Newsham *et al.* (1994) that AM fungi apparently protect *V. ciliata* from the substantial (up to 50%) reductions in fecundity associated with root pathogenic fungi. In an annual plant species such as *V. ciliata*, in which abundance is directly related to fecundity (Carey 1991), AM fungi may therefore play an important role in controlling the dynamics of natural plant populations, as much as endophytic clavicipitaceous fungi do in natural grass populations (Clay 1988).

A typical criticism of studies made on the role of AM fungi in plant ecology is that the organisms used are not those normally encountered by plants in the field, which may result in unrealistic plant responses to fungal inocula. This was not the case in our study, either for the AM fungus or the pathogen: both were isolated from the roots of Mildenhall *V. ciliata* plants. The abundances of fungi in roots were also realistic in our study: the maximum density of AM colonization in the roots of *V. ciliata* was 32%, which is comparable to that of field-grown plants from Mildenhall at the end of a typical growing season (West *et al.* 1993). In addition, the inoculum density of *F. oxysporum* used in our study was equivalent to that encountered in *V. ciliata* rhizosphere sand, and prior to transplantation, a maximum of two colonies of *F. oxysporum* were isolated per 30 mm of root taken from individual plants, compared with a maximum of five colonies of *F. oxysporum* isolated from 30 mm of root from *V. ciliata* plants growing at Mildenhall in March 1992 (Newsham *et al.* 1995).

The phenomenon of AM fungi protecting plants from fungal root pathogens is known from studies made on some horticultural and agricultural species: for example, tomato (*Lycopersicon esculentum* Mill.) plants colonized by AM fungi have been shown to be infected by fewer hyphae of *F. oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen in roots and stems and to display fewer *Fusarium* wilt symptoms than uncolonized plants (Dehne & Schönbeck 1979a). A similar situation exists in alfalfa (*Medicago sativa* L.), which exhibits fewer wilt symptoms associated with *Verticillium albo-atrum* Reinke & Berthold and *F. oxysporum* f. sp. *medicaginis* (Weimer) Snyder & Hansen infection when roots are colonized by AM fungi (Hwang *et al.* 1992). What makes our data novel is that they are the first conclusive evidence that this phenomenon occurs in the field, and that AM fungi can protect plants from pathogens in a situation where their effects on plant P nutrition can be discounted.

The protective effects conferred on *V. ciliata* by the *Glomus* sp. were apparently not due to the enhancement of shoot P concentrations by the mycorrhiza, which was suggested as the mechanism for the protection of sweet orange (*Citrus sinensis* [L.] Osbeck) plants from *Phytophthora parasitica* Dast. by *Glomus fasciculatus* (Thaxter) Gerd. & Trappe (Davis & Menge 1980). The interactive effects of the two fungi in roots in our study appear to have been due to a



reduction in septate hyphal infection as a result of AM colonization: the roots of plants grown with both fungi were infected by fewer septate hyphae compared with plants grown with *F. oxysporum* alone. Although we are not certain as to how AM fungi protect *V. ciliata* against fungal root infections, the large portions of roots which are typically uncolonized by either AM or pathogenic fungi suggest that the protective effect is indirect, i.e. from the AM fungus *via* the plant, such as by the induction of chitinase activity (Dehne & Schönbeck 1978), the increased lignification of cell walls (Dehne & Schönbeck 1979b) or the production of phytoalexins (Wyss *et al.* 1991) and not by simple spatial pre-emption as suggested by Muchovej *et al.* (1991). In light of this, there are therefore considerable ecological advantages to an annual plant species such as *V. ciliata* of allowing early colonization of roots by AM fungi that will defend the plant against pathogens which may become abundant later in the life-cycle of the plant (Fig. 5).

The data reported here on the effects of AM colonization on a wide range of root-infecting fungal taxa are apparently also the first indication that AM fungi can alter the root pathogenic fungal communities of wild plants. This has always been implicit in other studies which have shown AM fungi to offer protection from individual fungal pathogens, but this phenomenon has apparently not been hitherto demonstrated for a wide range of fungal species in plant roots. It is significant that those species of root-infecting fungi which were reduced in abundance in *V. ciliata* roots by AM colonization (*F. oxysporum* and *E. chlamydospora*) are species associated with reduced fecundity in natural populations of the plant species (Newsham *et al.* 1994).

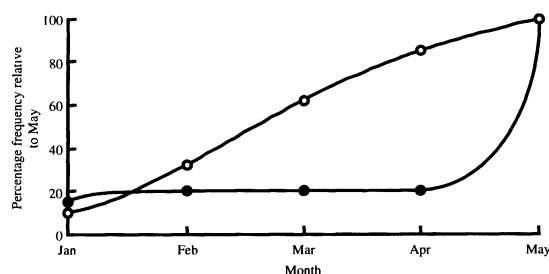
The evolutionary events that have led to AM associations offering protection to plants from root pathogens are likely to have been driven by the development of plant root systems. Early land plants had either rootless rhizomes (Kidston & Lang 1921) or poorly developed root systems with few lateral branches (Rayner 1984) and all must have needed assistance from soil fungi in nutrient capture (Pirozynski

& Malloch 1975), as is demonstrable in present-day plant species with poorly developed roots (Merryweather & Fitter 1995). Indeed, the rhizomes of early land plants were colonized by fungi morphologically similar to present-day AM fungi (Remy *et al.* 1994) and recent estimates (based on molecular sequence data) place the origin of AM fungi at 353–462 Myr ago, coincident with the evolution of land plants (Simon *et al.* 1993). However, with the evolution of highly branched root systems with root hairs, as, for example, in the modern grasses, the needs of plants for mycorrhizal assistance in nutrient capture has demonstrably diminished. The persistence of AM fungi in many such species argues for some additional benefit conferred by these fungi in roots. The most likely benefit would have been in protection from pathogens, since branched root systems also offer greater opportunities for pathogenic attack (Marx & Davey 1969; Garrett 1970). It has been suggested that enhanced water relations may be an important benefit of AM colonization to plants (Allen *et al.* 1981; Allen & Allen 1986), but these are a less likely candidate in this scenario, as plant root systems will also have become more efficient at water uptake as they became more branched. Therefore we propose that AM fungi might still remain ubiquitous in the roots of plant species with highly branched root systems such as *V. ciliata*, as much as because they confer benefits in terms of protection from pathogens, as because they improve plant nutrient relations. This implies a continuum of mycorrhizal benefit, with plant species with poorly developed root systems benefiting from the association in terms of nutrient uptake, and species with more highly branched roots benefiting in terms of protection from pathogens.

The demonstrable interaction between AM and pathogenic fungi in the roots of *V. ciliata* represents a considerable step forward in our understanding of the interactions between soil micro-organisms and higher plants. Given the abundance and apparent low specificity of AM fungi in the field (Harley & Smith 1983) and the abundance of *Fusarium* species and other fungal pathogens in plant roots (Harley & Waid 1955; Garrett 1970), such interactions may well occur more widely and could represent a largely overlooked yet potentially very important aspect of plant ecology.

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**Fig. 5** Percentage colonisation frequency of arbuscular mycorrhizal fungi (○) and isolation frequency of *Fusarium oxysporum* (●) from *Vulpia ciliata* roots at Mildenhall between January and May 1992. Mycorrhizal colonization data are from the experiments described in Newsham *et al.* (1994) and *F. oxysporum* data are from Newsham *et al.* (1995).

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