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The proliferation of fungal hyphae in soils supporting mycorrhizal and non-mycorrhizal plants

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Abstract This study investigated the impact of mycorrhizal plants, non-mycorrhizal plants and soil organic matter on the relative abundance of soil hyphae perceived to belong to indigenous arbuscular mycorrhizal (AM) plants. The mycorrhizal plants corn (*Zea mays* L.) and barley (*Hordeum vulgare* L.) and a non-mycorrhizal plant, canola (*Brassica napus* L.), were grown in unsterilized soil in pots inoculated with mycorrhizal corn root fragments. The abundance of hyphae was measured after 5 weeks and the response of fungal growth to the addition of corn residues in the absence of plants was assessed. The abundance of hyphae was higher in the presence of the mycorrhizal plants than in the other treatments. AM hyphae present under mycorrhizal plants accounted for more than 83% of the measured hyphae. The levels of root colonization of 32% in corn and 27% in barley confirmed the mycorrhizal status of the experimental plants. Only a few points of entry were observed in canola, the non-host plant. The percentage of mycorrhizal colonization was positively related ($R^2 = 0.85$) to the abundance of soil hyphae, indicating that AM hyphae were the major component of the soil hyphae in the presence of mycorrhizal plants in this study.

Key words Arbuscular mycorrhizal fungi · Soil hyphae · Soil fungi · Host plant · Non-host plant

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Introduction

While few plant species are incompatible with arbuscular mycorrhizal fungi (AMF) (Newman and Reddell 1987; Vierheilig et al. 1995), a broad range of plants naturally form a mutualistic association with AMF (Harley and Harley 1987; Crush 1973). Arbuscular mycorrhizal fungi colonize host plant roots and their extraradical hyphae proliferate within the soil to acquire mineral nutrients. The main effect of these hyphae is an increase in the absorptive surface area of the host plant root system (Miller et al. 1987) and a supply of host plant photosynthates to the fungal symbionts (Mosse et al. 1982). The plant demand for mineral nutrients, especially P, determined by plant genes (Krishna et al. 1985) and soil fertility status (Hayman 1982), probably regulates the proliferation of AMF hyphae in soil. Non-mycorrhizal plants do not support AMF hyphae but may encourage the proliferation of other non-symbiotic fungal mycelium through release of carbon compounds in the vicinity of the roots (Bowen and Rovira 1991). Saprophytic hyphal proliferation is also stimulated by the addition of organic residues to soils (Broder and Wagner 1988).

The important role of AMF in nutrient uptake and translocation and the fact that AMF are a major component of soil microbial biomass (Hamel et al. 1991) promotes study of their extraradical hyphae. Published methods to quantify AMF hyphae include extraction and direct measurement of extraradical hyphae from soil using the membrane filter technique (Abbott et al. 1984), the chitin assay (Bethlenfalvay et al. 1982; Bethlenfalvay and Ames 1987), and the quantification of specific phospholipids or fatty acids (Olsson et al. 1995). However, the reliability of these techniques for measuring the extraradical hyphae of AMF is questionable as it is difficult to distinguish AMF hyphae from many non-symbiotic fungal hyphae (Sylvia 1992). The chitin assay also measures chitin from soil insects, other fungi or other organisms (Sylvia 1992) and lipids occur

in older infections and mature arbuscules but not in younger parts of the hyphae and arbuscules (Cox and Sanders 1975).

The growth cabinet experiment reported here was conducted to evaluate to what extent fungal hyphae extracted from soil under mycorrhizal plants may be non-mycorrhizal and to determine the abundance of AMF extraradical hyphae relative to those of other soil fungi.

Materials and methods

Experimental design

A sandy loam soil was collected from a corn field, passed through a 2-mm sieve, and placed in 15-cm pots. The soil had a pH of 6.2 (in water) and contained 2.56% organic carbon, 57% sand, 25% silt and Mehlich III extractable nutrient levels of 82 mg P, 81 mg K, 1365 mg Ca, 91 mg Mg, 2.5 mg Zn, and 1.1 mg Cu per g of soil. Each of the pots received 1.1 kg of soil.

The design of the experiment was a randomized complete block with five replicates and five soil treatments: (1) soil planted to corn (*Zea mays* L.), (2) soil planted to barley (*Hordeum vulgare* L.), (3) soil planted to canola (*Brassica napus* L.), (4) soil amended with 6000 kg ha^{-1} of corn residues (stover) and (5) soil supporting no plants and receiving no amendments (control). Corn supports higher levels of AMF colonization than barley, while canola is a non-host plant for AMF.

Corn residues were added at the normal field rate in an attempt to stimulate microbial growth in the absence of the plant. The residues were collected from the previous-year corn field, ground and passed through a 500-mm sieve. The material was soaked in deionized water for 5 days prior to mixing with the soil.

All pots were inoculated with mycorrhizal corn root fragments from corn plants grown in pots for 4 weeks in a growth cabinet. Roots were collected and chopped to a length of 1.5–2 cm. The roots were colonized to 17% root length by mycorrhizal fungi. Fresh roots (3 g) were thoroughly mixed with the soil in each pot just before planting. Corn roots were also added to residue-amended and to control pots.

Germinated seeds of corn, barley or canola (6 per pot) were planted and thinned to 4 plants per pot after 5 days. The experimental pots, including the pots without plants, were maintained in a growth cabinet for 5 weeks under a photoperiod of 15 h and day/night temperatures of 25 °C/16 °C. Soil moisture was adjusted to field capacity every other day. A solution of NH_4NO_3 and KNO_3 was added to each pot at the rate equivalent to 100 kg N $ha^{-1}$and 50 kg K_2O ha^{-1} at the beginning of the experiment.

Hyphal extraction and measurement

At harvest, the membrane filter technique modified from Abbott et al. (1984) was used to extract extraradical hyphae from the soil of each experimental unit. Four soil cores (1.5 × 8 cm) were taken randomly with a sampler from each of the pots to make a composite sample. The soil from each composite sample was thoroughly homogenized by mixing and two 5 g subsamples were taken for each total hyphal length and metabolically active hyphal length determination, no plant roots were removed from the subsamples. Subsamples were placed in a blender with 300 ml deionized water and blended for 30-60 s to homogenize the soil suspension. The blender was a simple drink mixer and blending was done at the lowest speed available. Preliminary studies showed that a 15-20 s blending was no different in resulting hyphal length to that of a 30–60 s blending, however, a 1–2 s blending gave significantly lower values in our experimental soil. The blended suspension was poured through a 250 μm sieve and washed by applying high-pressure water. The residue was collected on a 40 μm sieve, transferred to a 40-ml water-filled beaker and shaken for 5 s to resuspend the recovered mycelium. This suspension, containing the hyphae, was then decanted onto a filter and filtered under vacuum. Each subsample was extracted three times and the measurements were combined; this had been shown in preliminary testing to give adequate hyphal extraction and the lowest variation.

The hyphae were stained by flooding the filter with acid fuchsin (0.2%, in equal volumes of lactic acid, glycerol and water) for several minutes before determination of total hyphal length by microscopic examination. The excess stain was removed by rinsing the hyphae and filter paper with deionized water and vacuum-filtration. The recovered hyphae were measured by the modified grid-line intersect method (Tennant 1975) on a grid drawn on a small petri dish (4 cm^2 area with 2-mm squares) randomly placed over the filter. At least 50 microscopic fields were observed per filter. A dissecting microscope was used to observe hyphae at ×50 magnification. The hyphae recovered from the other subsamples were stained by flooding the filters with a solution composed of equal volumes of iodonitrotetrazolium (INT) (1 mg ml^{-1}), NADH (3 mg ml^{-1}) and 0.2 M Tris buffer pH 7.4 (Sylvia 1988) to reveal metabolically active hyphae. The filters were incubated for 12-16 h at room temperature. The lengths of these metabolically active hyphae were measured as above. Though this method for the extraction of extraradical hyphae was not absolutely quantitative, it did allow relative comparisons between the treatments.

Plant root preservation and percentage root colonization

Corn, barley and canola root systems were separated from the soil on a 850 μm sieve under running water. Random samples of washed roots were collected, cut into pieces of 1–1.5 cm length, placed in tissue-embedding capsules and kept in a formalin-acetic-acid-alcohol solution (Phillips and Hayman 1970). Root samples were autoclaved in 10% KOH for 15 min, rinsed well with deionized water and stained with acid fuchsin (0.02%) in lactoglycerol (Brundrett 1994). The percentage mycorrhizal root colonization was measured by the grid-line intersect method (Giovannetti and Mosse 1980). A dissecting microscope was used to observed hyphae at ×40–50 magnification.

Statistical analysis

Statistical analyses of the data were performed using the general linear model procedure in the Statistical Analysis System (SAS Institute 1988). Analysis of variance was carried out on the abundance of hyphae in the soil under mycorrhizal and non-mycorrhizal plants, as well as in amended and non-amended soil. Regression analysis was performed using root colonization as the dependent variable to examine any trends in the relationship between root colonization and total hyphal abundance.

Results

Within 5 weeks, root colonization by indigenous AMF had reached 32% and 27% in corn and barley respectively, but in canola only a few points of infection and no internal structures were observed (Fig. 1). Under corn, the abundances of total and viable hyphae were 92 cm cm^{-3} and 83 cm cm^{-3}, respectively, and under barley the values were 66.6 cm cm^{-3} and 50.2 cm cm^{-3}, respectively. The abundances of both total and

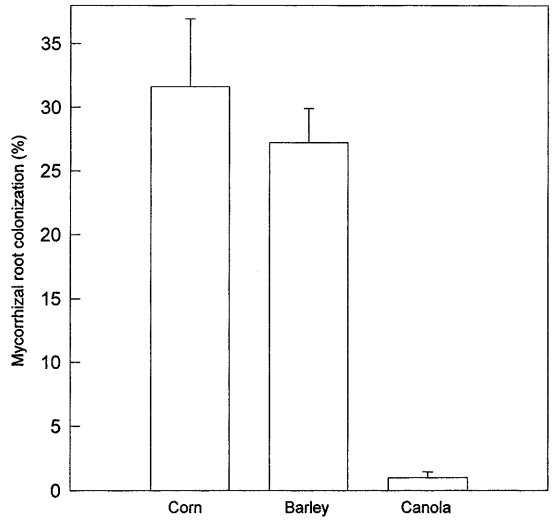

Fig. 1 Percentage root colonization of corn, barley and canola. *Error bars* represent SEM

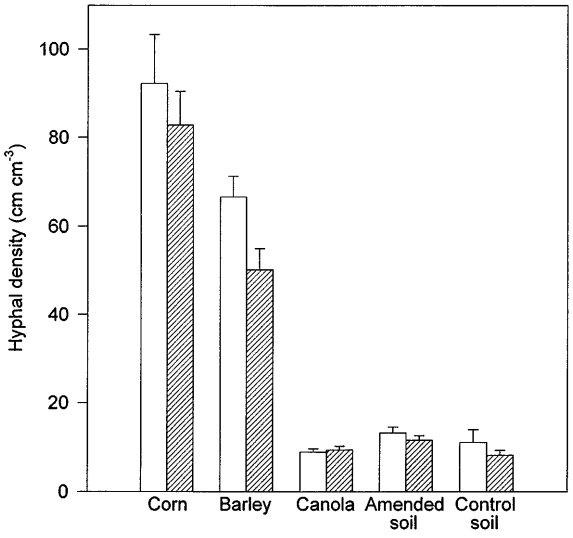

Fig. 2 Density of total (□) and metabolically active (▨) hyphae in soil under mycorrhizal and non-mycorrhizal plants or in soil without plants and amended or not with corn residues. *Error bars* represent SEM

metabolically active hyphae were very low under canola, in the soil receiving corn residues and in the control soil (Fig. 2), and there was no significant ($P < 0.05$) difference in hyphal abundance between these three treatments. There was little difference between total hyphal abundance and the abundance of metabolically active hyphae in the soil of the control treatments (Fig. 2). This suggests that few, if any, of the non-viable hyphae measured were the remains of mycorrhizal hyphae from corn plants growing in the field from where the soil was taken, most of these old hyphae had been degraded by the time of sampling. Regression analysis showed strong positive relationship ($R^2 = 0.85$, $P < 0.001$) between the percentage of root colonization and the abundance of hyphae (Fig. 3).

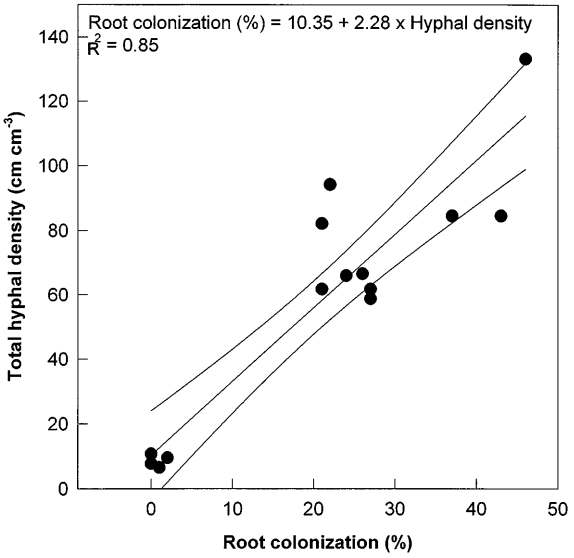

Fig. 3 Relationship between the percentage root colonization and the density of soil hyphae in soil under corn, barley and canola (95% confidence limit)

Discussion

Non-mycorrhizal plants can stimulate the growth of fungi through rhizosphere effects (Bowen and Rovira 1991; Veirheilig et al. 1995) but they do not allow the development of AMF, which are biotrophic (Morton 1990). Some authors have reported that the Brassicaceae, in contrast, can inhibit AM fungal growth (Glenn et al. 1988; Schreiner and Koide 1993). Our results, however, showed that canola neither inhibited nor stimulated AM fungal growth when compared with our plantless controls.

The addition of plant residues to soil is not expected to stimulate AMF but can increase the proliferation of saprophytic fungi (Broder and Wagner 1988). However, in our experiment the addition of corn residues to soil had little effect on hyphal proliferation; the addition of the equivalent of 6000 kg ha^{-1} of corn residues to our pots did not increase the amount of soil hyphae over the plantless, non-amended control.

Over fivefold more hyphae were associated with mycorrhizal plants than with canola and other controls. These results correspond to the observation of Bécard and Piché (1990), who measured rapid hyphal proliferation on a host-root cultures but not on a nonhost root culture, and suggested the prevalence of mycorrhizal hyphae in soil under mycorrhizal plants.

Root colonization in corn, barley and canola was well related with the abundance of hyphae in soil. From this observation, we suggest that mycorrhizal fungi, when present, are major contributors to soil hyphae. If

we assume that the hyphae observed in the canola, amended or control soils were non-mycorrhizal, and that non-mycorrhizal hyphae in soil under corn and barley were equally abundant, then AMF hyphae made up over 83% of the hyphae measured in the soil of the mycorrhizal treatments.

Our observations provide evidence that the presence of a mycorrhizal plant is by far the major determinant to hyphal proliferation in soil. Although our findings of increased fungal hyphae under mycorrhizal corn and barley do not directly prove that these hyphae are AMF, the fact that neither a non-mycorrhizal plant nor the addition of corn residues had an effect on the observed hyphae supports the hypothesis that we measured mostly AMF hyphae in soils in which mycorrhizal plants were growing. Our results agree with those of authors using the chitin assay, who reported the prevalence of mycorrhizal biomass in soil growing mycorrhizal plants (Bethlenfalvay and Ames 1987). Unfortunately, until DNA analysis or immunological techniques are developed to unequivocally recognize the mycorrhizal character of soil hyphae, the study of the extraradical mycelium will remain based on indirect evidence only.

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