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Inoculation of *Alnus cordata* with Selected Microsymbionts: Effects of *Frankia* and *Glomus* spp. on Seedling Growth and Development

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Abstract

Positive effects of dual inoculation with arbuscular mycorrhizal (AM) fungi and *Frankia* on *Alnus cordata* Loisel. are reported. Such beneficial effects may vary according to the combination of plant species, *Frankia* and mycorrhizal strains. Efficiency of two different AM strains, *Glomus fasciculatum* (LFSC) and *Glomus mosseae* (LMSS), originally obtained from Italian sand dunes, and a *Frankia* strain, UFI 01010104 (AcI4), alone and in combination, in promoting growth of *Alnus cordata* seedlings in a steam-sterilized non-fertilized soil mixture (peat moss:lignite mine spoil, 1:1; pH 5) was evaluated. The seedlings were grown in a greenhouse under natural day-length and light intensity, and watered as needed. After five months of growth, both fungal strains tested colonized alder seedlings. Co-inoculation of *Frankia* with mycorrhizal strains increased nodule dry weight, significantly with LFSC. *Frankia* significantly increased mycorrhizal infection by LMSS, while reducing LFSC percent infection; mycorrhizal root length was, however, not affected. No significant differences were observed in

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the mycorrhizal efficiency of the two fungal strains in promoting plant growth. Mycorrhizal inoculation had the largest effects on root architecture, increasing root length and root branching.

Keywords: *Glomus*, *Frankia*, *Alnus*, tripartite symbiosis

1. Introduction

Rhizospheric microorganisms may affect the uptake of nutrients and water by plant roots; significant interactions may also occur between these organisms, and, in turn, affect the plants. Among the microorganisms closely associated with plant roots, arbuscular mycorrhizal (AM) fungi play a major role in the productivity and healthiness of agricultural and forest ecosystems, supplying the host plant with inorganic nutrients in exchange for carbohydrates (Harley and Smith, 1983). Mycorrhizae may also improve photosynthetic rate, water uptake (Abbott and Robson, 1984; Nelsen, 1987; Smith and Gianinazzi-Pearson, 1988; Puppi and Bras, 1990), and disease resistance (Caron, 1989).

AM fungi may interact with other soil microorganisms either directly, antagonistically or mutualistically, or indirectly, by affecting host plant physiology and root exudation in the rhizosphere (Linderman, 1988, 1992; Garbaye, 1991; Rovira, 1959). The most important interaction is probably that between mycorrhizae and N_2-fixing organisms. Several studies in recent years have investigated the positive interactions between arbuscular mycorrhizal fungi and *Rhizobium* as well as other diazotrophic bacteria on leguminous and non-leguminous plants (Barea et al., 1992; Kennedy and Tchan, 1992). However, the effect of arbuscular mycorrhizal fungi on nitrogen fixing bacteria associated with non-leguminous plants is far less documented than the effects on *Rhizobium*-legume symbioses (Cervantes and Rodriguez-Barrueco, 1992). Especially worth of attention is the case of the actinomycete *Frankia*. In temperate and cool climates actinorhizal plants seem to fill the ecological niche occupied by woody legumes in the tropics (Dawson, 1990).

Recent studies have reported positive interactions of arbuscular mycorrhizal fungi and *Frankia* on several species of the genus *Alnus*, very important trees for exploitation of marginal lands in temperate regions. The increase in plant and nodule dry weight, and in nitrogenase activity may be mediated by incr eased phosphorus availability (Russo, 1989). Mycorrhizae, however, may be more effective than phosphorus addition (Fraga-Beddiar and Le Tacon, 1990). More recently, Jha et al. (1993) confirmed that *Frankia* and *G. mosseae* in combination on *Alnus nepalensis* caused an increase in nodule dry weight and nitrogenase activity over a range of phosphorus treatments. *Frankia*, on the other hand, increased mycorrhizal colonization. The beneficial effects may

vary according to the combination of plant species, *Frankia* and mycorrhizal strain.

The aim of the present work was to evaluate the growth effects of two Italian AM strains and of an Italian *Frankia* strain, alone and in combination with the two AM strains, on *Alnus cordata* seedlings inoculated in a nursery and to determine the best co-association of symbionts for the host plant and the best intersymbiont compatibility. Part of the nursery-inoculated plants were then used for a field trial, on a mine-spoil soil depleted of native *Frankia* and AM microorganisms, to evaluate if, and in what way, the effects observed in the nursery of single and dual inoculation where maintained under field conditions (Lumini et al., 1994).

2. Materials and Methods

Alnus cordata Loisel. seeds were sown into 750 ml plastic pots filled with a steam-sterilized non-fertilized soil mixture (peat moss:lignite mine spoil, 1:1; pH 5), 2–4 seeds per pot. Fungal strains, *Glomus fasciculatum* (LFCS) and *Glomus mosseae* (LMSS), were originally obtained from sand dunes (Puppi et al., 1986), and maintained in pot-cultures on *Trifolium repens* cv. Huja. The inoculum consisted of a mixture of infected white clover roots and sand, containing 4.5 spores g^{-1} of *G. mosseae* or 8.8 spores g^{-1} of *G. fasciculatum*, respectively. Seven grams of fungal inoculum were placed in each pot at the sowing. *Frankia* strain UFI 01010104 (AcI4), previously isolated from *Alnus cordata* (Margheri et al., 1983), was grown under static conditions for two months at 28°C on BAP medium (Murry et al., 1984) supplemented with Tween 80 (0.5 g l^{-1}). The inoculum was prepared by centrifuging and washing the mycelium with a N-free Hoagland solution (Hoagland and Arnon, 1950), followed by homogenization. Six-weeks old seedlings were inoculated by dropping 2 ml of cell suspension near the root collar.

Seven groups of 150 pots each were treated as follows: uninoculated Control, *Frankia* ACI4, *Glomus mosseae* LMSS, *Glomus fasciculatum* LFSC, *Frankia* AcI4 plus *G. mosseae* LMSS, *Frankia* AcI4 plus *G. fasciculatum* LFSC. Cross contamination among treatments was prevented by keeping the seven groups separate by a distance of 50 cm on greenhouse benches.

The seedlings were grown in a greenhouse under natural day-length and light intensity (summer-autumn), watered as needed and no fertilizer added. After five months of growth, 10 pots per treatment were randomly chosen, and the best plant per pot examined. The parameters observed were: shoot height, shoot diameter, shoot dry weight, tap root length, root dry weight (dry weights

were estimated after oven-drying at 70°C for 72 hr). From these data, total dry weight and root:shoot ratio were also estimated.

Roots of AM-inoculated plants were stained according to Koske and Gemma (1989) and root length, linear frequency of root tips and percent mycorrhizal infection were estimated by the gridline intersect method (Giovannetti and Mosse, 1980). From these data, specific root length (as root length per weight unit) and root branching index were calculated. Total root length was estimated from specific root length and root dry weight data. For *Frankia*-inoculated seedlings, number of nodules, number of nodule lobes, and nodules dry weight were also determined. Hand-cut sections 30–50 μm thick stained with cotton blue in lactic acid were examined by light microscopy.

Concentration of nutrients in leaf samples was determined using standard laboratory procedures: N by Kjeldhal method and P by Vanadomolybdophosphoric Acid Colorimetric method. LSD of data was evaluated by Fisher's test at 0.05 P level with NCSS statistical package (Hintze, 1992). Factor effects were estimated by GLM ANOVA and significant correlations with the Multiple Regression subroutine of the same package.

3. Results

Microbial infection and intersymbiotic interactions (Table 1)

Table 1. Mean and standard error of: nodule dry weight (NOD), mycorrhizal percent infection (% M), estimated mycorrhizal (mycRL) and total root length (RL), specific root length (SRL) and root branching, as tips/cm (Rb/cm). In the same column different letters indicate significant differences (p=0.05, n=5 for root architectural parameters of Control and AcI4, otherwise n=10).

	NOD (mg)	% M	mycRL (m)	RL (m)	SRL (cm/mg)	Rb/cm
Control				2.7 ± 0.5 a	0.7 ± 0.1 a	0.11 ± 0.01 ab
AcI4	12.7 ± 1.9 a			4.2 ± 1.4 ab	1.3 ± 0.2 ab	0.08 ± 0.01 a
LMSS		19.1 ± 2.8 a	1.4 ± 0.6 a	6.3 ± 2.2 ab	1.9 ± 0.3 bc	0.20 ± 0.01 d
LMSS + AcI4	21.4 ± 4.2 ab	40.5 ± 2.1 c	2.4 ± 0.2 ab	6.2 ± 0.6 ab	2.5 ± 0.2 cd	0.20 ± 0.01 d
LFSC		32.0 ± 2.8 b	3.6 ± 1.1 b	10.3 ± 2.7 bc	3.1 ± 0.4 d	0.12 ± 0.02 bc
LFSC + AcI4	21.8 ± 4.4 b	24.3 ± 1.6 a	3.1 ± 0.4 ab	13.2 ± 2.0 c	2.3 ± 0.4 bcd	0.16 ± 0.01 c
ANOVA Table (probability values corresponding to F statistics):						
Mycorrhiza	0.5	n.s.	0.03	0.0006	0.0001	0.0000
Frankia	—	0.007	n.s.	n.s.	n.s.	n.s.
Interaction	—	0.0000	n.s.	n.s.	0.04	0.08

No infection was detected in uninoculated plants. Both fungal strains infected alder seedlings. Co-inoculation of AcI4 with mycorrhizal strains increased nodule alder seedlings. Co-inoculation of AcI4 with mycorrhizal strains increased nodule dry weight, significantly with LFSC. AcI4 inoculation significantly improved root colonization by LMSS, while the percent of infection by LFSC was lower. Nevertheless, mycorrhizal root length was higher in LFSC-inoculated plants, due to greater root extension.

Root architecture (Table 1)

Mycorrhizal inoculation had the largest effects on root architecture, increasing specific root length, root branching, and estimated total root length. The latter parameter was specially increased by LFSC, i.e., mycorrhizal root length was actually greater in LFSC than in LMSS-inoculated plants. LMSS enhanced root branching.

Growth effects and nutrient concentration (Table 2)

The main effects of AcI4 inoculation were observed on aboveground biomass, as increased shoot dry weight and diameter. Shoot dry weight was in positive correlation with nodule dry weight. Since the trend of aboveground biomass was roughly comparable to that of N concentration in leaves, the improved growth may reasonably be attributed to increased N availability.

Table 2. Mean and standard error of: shoot height (SH), shoot diameter (SD), shoot dry weight (SDW), and root:shoot ratio (R/S), and percent nitrogen and phosphorus content. In the same column, different letters indicate significant differences (p=0.05, n=10).

	SH (mm)	SD (mm)	SDW (g)	R/S	N	P
Control	86.2 ± 10.5 a	1.9 ± 0.2 a	0.10 ± 0.02 a	4.0 ± 0.6 c	1.06	0.09
AcI4	97.0 ± 3.2 ab	2.1 ± 0.2 ab	0.15 ± 0.02 ab	2.1 ± 0.3 ab	1.92	0.09
LMSS	94.8 ± 7.0 ab	2.0 ± 0.1 ab	0.14 ± 0.03 ab	2.9 ± 0.6 abc	1.05	0.11
LMSS + AcI4	86.4 ± 4.1 a	2.4 ± 0.1 b	0.16 ± 0.02 b	1.7 ± 0.2 a	2.37	0.08
LFSC	81.4 ± 4.2 a	2.0 ± 0.1 ab	0.10 ± 0.02 ab	3.3 ± 0.8 bc	1.02	0.12
LFSC + AcI4	107.7 ± 7.1 b	2.2 ± 0.2 ab	0.21 ± 0.01 c	3.0 ± 0.4 abc	2.12	0.10
ANOVA Table (probability values corresponding to F statistics):						
Mycorrhiza	n.s	n.s.	n.s.	n.s.	—	—
Frankia	0.08	0.02	0.0004	0.006	—	—
Interaction	0.04	n.s.	0.07	n.s.	—	—

Allocation to roots, expressed as root:shoot ratio, was, on the contrary, significantly reduced by AcI4 inoculation. Mycorrhizal inoculation had no effects on aboveground growth parameters. *Frankia* effects on shoot height and dry weight were significantly larger in LFSC-inoculated plants than in the Control or LMSS-inoculated plants.

4. Discussion

Frankia inoculation was confirmed as a key factor to improve growth of alder seedlings, as suggested by the correlation between nodule and shoot dry weights. These results confirm the findings of Chatarpaul et al. (1989), Fraga-Beddiar and Le Tacon (1990) and Jha et al. (1993). The reduced allocation to roots, observed in *Frankia*-inoculated plants, may also be attributed to increased availability of N.

Inoculation with arbuscular-mycorrhizal fungi appears to be more important in shaping root architecture, both in terms of root elongation and branching, than in promoting plant growth. According to Fitter (1987), a highly branched, absorbing root system limits the volume of soil explored, but optimizes nutrients' transport and cost efficiency, while thinner root systems (higher SRL) increase the volume of soil explored at minimum cost. Mycorrhizae are known to increase root branching, and expected to reduce specific root length (Fitter, 1987), since they increase the volume of soil explored through hyphal extension. Moreover, mycorrhizal dependency is roughly related to root diameter (Baylis, 1975), and a larger root cortex offers more tissue for colonization.

In the present experiment, root branching was significantly increased by LMSS, while LFSC increased it only in combination with *Frankia*; specific root length, on the contrary, was increased by both fungal strains, especially by LFSC. Such effects seem not to be linked to the fungal species, since in previous experiments, LFSC was more effective than LMSS in promoting root branching of clover plants under poor nutrient conditions, and both strains reduced specific root length (Puppi et al., 1987, and unpublished data). Recently Atkinson et al. (1994), reviewing the impact of mycorrhizal colonization on root architecture, observed that the direction and magnitude of the effects of AM fungi on root topology clearly are variable, and too few species have been tested. It may therefore be possible that the same fungal species has different effects on different species, or for a given plant species, according to its physiological status or environmental conditions (Tisserant et al., 1991 in: Atkinson et al., 1994).

In the present experiment, no large differences were observed in the mycorrhizal efficiency of the two fungal strains. Atkinson et al. (1994) suggested that

more intensively branched root systems are expected to be more dynamic, that is, they may have a higher turnover rate. Longer, less ramified root systems could, on the contrary, allocate more resources for roots surviving as wood roots. The biomass values recorded one year after the outplants of mycorrhizal plants (Lumini et al., 1994) showed better growth of LFSC-inoculated plants in comparison to the LMSS-inoculated ones, suggesting an advantage of the more extended root system. An increase in plasticity in mycorrhizal roots, as a response to soil conditions, has also been suggested (Puppi and Bragaloni, 1990). Such a mechanism could be an important point for survival and adaptation to environmental conditions in mycorrhizal plants.

The positive interaction between *Frankia* and arbuscular mycorrhizae may have the same basis as suggested for the case of *Rhizobium* and arbuscular mycorrhizae (Barea et al., 1992). That is the ability of mycorrhizal fungi to take up P from the soil and increase the phosphorus supply to nodules through plant translocation (Kawai and Yamamoto, 1986). On the other hand, our results on P contents do not confirm an increased translocation of this element to the host plant in mycorrhizal-only or dual inoculated seedlings, and, as already reported, mycorrhizae may be more effective than phosphorus addition (Fraga-Beddiar and Le Tacon, 1990). Other mechanisms could therefore be involved in the interaction between *Frankia* and arbuscular mycorrhizae.

Further investigations are required to distinguish between the relative contributions of mycorrhizal fungi and *Frankia* to plant growth in the field, and the most useful parameters to predict successful performances in the field of nursery-inoculated plants.

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