



## SHORT COMMUNICATION

FIELD PERFORMANCE OF *ALNUS CORDATA* LOISEL  
(ITALIAN ALDER) INOCULATED WITH *FRANKIA*  
AND VA-MYCORRHIZAL STRAINS IN MINE-SPOIL  
AFFORESTATION PLOTSERICA LUMINI,<sup>1</sup>\* MARCO BOSCO,<sup>1</sup>† GIGLIOLA PUPPI,<sup>2</sup> RAFFAELLA ISOPI,<sup>2</sup>  
MAURO FRATTEGANI,<sup>3</sup> ENRICO BURESTI<sup>3</sup> and FRANCO FAVILLI<sup>1</sup><sup>1</sup>Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Firenze, Piazzale delle Cascine 27, 50144 Florence, <sup>2</sup>Dipartimento di Biologia Vegetale, Università 'La Sapienza', Largo Cristina di Svezia 24, 00165 Rome and <sup>3</sup>Istituto Sperimentale per la Selvicoltura, Viale S. Margherita 80, 52100 Arezzo, Italy

(Accepted 5 November 1993)

## INTRODUCTION

The difficulty in establishing vegetation on mine spoils, where mycorrhizal and actinorhizal potential is poor or lacking (Danielson, 1985), can be overcome by the use of planting stock which have already formed microbial root associations (Wheeler *et al.*, 1991).

Surface mining of lignite banks in Santa Barbara (AR, central Italy) results in the production of large quantities of mine-spoil soils. In this area, mixed stands of *Alnus cordata*, *Elaeagnus* spp and broad-leaf timber trees have been successfully used since 1980 for revegetation purposes on >200 ha of mine spoils (Buresti, 1990). However, unknown *Frankia* populations were introduced with nursery-soil infected plants.

The use of container-grown seedlings on reclamation sites presents an ideal opportunity for introducing selected *Frankia* and VAM symbionts, shown to improve seedling performance following outplanting. In 1991, different combinations of genetically-characterized *Frankia* spp and *Glomus* spp strains were inoculated on *A. cordata* seedlings in the nursery. A field trial was then made to determine if inoculated *A. cordata* plants would outperform uninoculated ones in soils lacking *Frankia* and mycorrhizal inocula, and if inoculation benefits would persist for a long time.

## MATERIALS AND METHODS

The study site presented sub-acid (pH 5–6.5) silty clay soils consisting of 30% clay, 40% silt, 29%

sand and characterized by maintenance of available soil water during dry seasons (Buresti, 1990). Local climate is typically sub-mediterranean with 940 mm of average annual precipitation.

Seeds of alder (*A. cordata* Loisel.) were sown in 750 ml plastic pots filled with a steam-sterilized non-fertilized soil mixture (peat moss:lignite mine spoil, 1:1; pH 5). Fungal strains, *Glomus mosseae* (LMSS) and *G. fasciculatum* (LFSC) were isolated from sand dunes by Puppi *et al.* (1986) and maintained in pot-cultures. The inoculum consisted of a mixture of infected white clover roots and sand, containing respectively 4.5 spores g<sup>-1</sup> of *G. mosseae* or 8.8 spores g<sup>-1</sup> of *G. fasciculatum*. Fungal inoculum (7 g) was placed in each pot at sowing. *Frankia* strain UFI 01010104 (AcI4), isolated from *A. cordata* (Margheri *et al.*, 1983), was grown in static conditions for 2 months at 28°C (Margheri *et al.*, 1989). Cultured *Frankia* inoculum was prepared by centrifuging and washing the mycelium with N-free Hoagland's solution (Hoagland and Arnon, 1950) before homogenization. 6 weeks old seedlings were inoculated by applying 2 ml of cell suspension near the root collar.

The experimental design consisted of six groups of 150 pots each, treated as follows: uninoculated control, AcI4, LMSS, LFSC, AcI4 + LMSS, AcI4 + LFSC.

The seedlings were grown in a greenhouse under natural day length and light intensity (summer–autumn) for 5 months and regularly watered with tap water. Cross-contamination among treatments was prevented by keeping the six groups separated by a distance of 50 cm on greenhouse benches.

Prior to outplanting, 10 pots per treatment were sampled. Shoot height (H) and collar diameter (D) of the tallest seedling of each pot were recorded,

\*Author for correspondence.

†Collaborator via fellowship under O.E.C.D. Project on Biological Resource Management.

and collar diameter squared  $\times$  total height ( $D^2H$ ), a suitable surrogate measure of aboveground biomass (Ruehle *et al.*, 1984), was calculated. Percent mycorrhizal infection (gridline intersect method, Giovannetti and Mosse, 1980), and actinorhizal nodule establishment were also estimated. N and P leaf concentrations were determined using standard laboratory procedures: N assay by the Kjeldhal method and P by the Vanadomolybdophosphoric Acid Colorimetric method.

Alders were planted in mid-January 1992 on mine-spoil banks in plots of 40 plants per treatment, mixed in a  $3 \times 3$  m square planting design with commercially-valuable trees (*Quercus robur*, *Fraxinus oxypylla* and *Prunus avium*). Survival in the field was measured after 2 months and the dead plants replaced. N and P contents were assayed at the end of the growing season. One year after outplanting, shoot height and collar diameters were measured on 25 alders randomly sampled from each plot. A limited sampling was also made to assess mycorrhizal and actinorhizal status, 7 months later. Data were statistically analyzed with the GLM-ANOVA subroutine of NCSS statistical package (Hintze, 1992).

#### RESULTS AND DISCUSSION

The inoculation of selected *Frankia* and VAM symbionts along with sterilized container potting media produced a high degree of nodulation (ranged from 89 to 93%) and mycorrhizal infection (30% on average) on *A. cordata* seedlings. No infection occurred in uninoculated alders. Moreover, the use of the soil mix (lignite mine spoil:peat moss), instead of soil-less mixes, was demonstrated to be a proper choice for early seedling growth.

At the outplanting, aboveground biomass, as  $D^2H$  (Fig. 1), was significantly increased by *Frankia* inoculation ( $P = 0.016$ ). The trend of aboveground biomass was roughly comparable to that of N concentration in leaves: on average, 2.00% in *Frankia*-inoculated plants, and 0.86% in non-*Frankia* inoculated plants. The growth effect might be therefore attributed to increased N availability.

Plant survival after the first winter in the field was good for all the treatments (83% on average), very likely due to the similarity of texture and moisture retention characteristics between potting mix used and planting site soil. One year after outplanting, the differences in biomass between inoculated and uninoculated alders were much more consistent than those revealed prior to outplanting (Fig. 2). *Frankia* inoculation showed a remarkable effect on aboveground biomass ( $P < 0.0001$ ), but also a mycorrhizal effect ( $P = 0.004$ ) could be detected. In particular, double-inoculated plants showed the highest  $D^2H$  biomass index, while single-mycorrhizal ones showed only a small advantage over control plants. Foliage N concentration appeared to be more comparable among the treatments (2.6% on average). Although

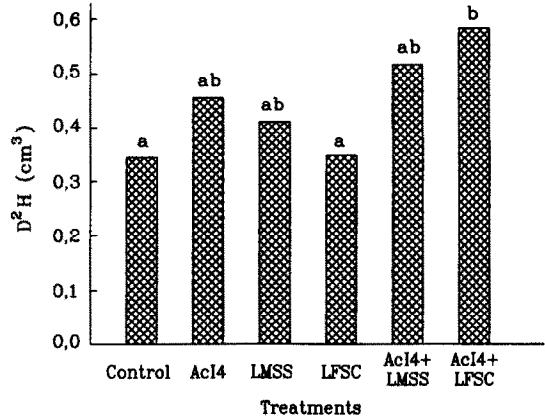


Fig. 1. Aboveground biomass ( $D^2H$ ,  $cm^3$ ) at outplanting. Values are the means of 10 replicates. Means followed by same letter are not significantly different (Fisher's LSD;  $P = 0.05$ ).

N was not a limiting factor in the soil, N uptake was limited and growth was reduced in non-actinorhizal or inefficiently VAM-infected alders. P concentration in soil was even less limiting ( $200 \mu g P_2O_5 g^{-1}$ , pH 5.7), and P concentration in leaves showed a 'dilution' effect, namely, a lower concentration in the more developed plants (on average 0.10%, but 0.12% in LFSC and 0.15% in control plants).

This trend could be explained by the general stressed status of the control plants (leaf chlorosis, limited growth) which could have determined unbalanced uptake of any nutrients. In fact, unnodulated seedlings can take up nutrients other than N in excess amounts but only when N is supplied symbiotically can they make use of them for growth (Wheeler *et al.*, 1991).

It has been reported that the efficiency of symbiont combinations, selected under controlled conditions

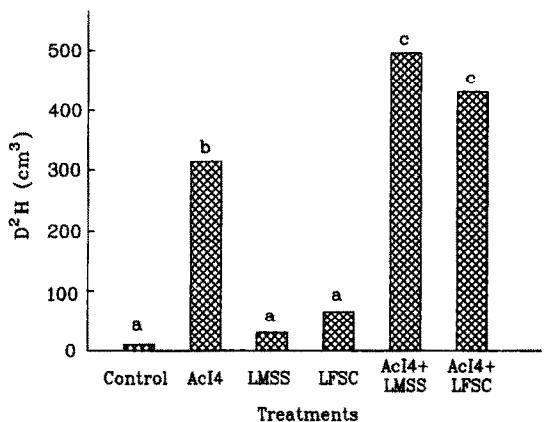


Fig. 2. Aboveground biomass ( $D^2H$ ,  $cm^3$ ) after 1 yr of outplanting in mine spoils. Values are the means of 25 replicates. Means followed by same letter are not significantly different (Fisher's LSD;  $P = 0.05$ ).

(Chatarpaul *et al.*, 1989; Russo, 1989; Fragga-Beddiar and Le Tacon, 1990), might not be confirmed in field applications (Hooker and Wheeler, 1986; Sheppard *et al.*, 1988). In contrast our results over the first year of outplanting showed that the association of *Frankia* Acl4 and *Glomus* spp was the most effective choice for mine spoils. We offer, therefore, some different explanations to account for such a discrepancy. First, the use of a local provenance of alder seeds as well as local sources of microsymbionts could have matched the local climate conditions. It is also possible that characteristics of our study site were more favorable to plant development than those used in previous attempts. Finally, the lack of competition by indigenous microsymbionts could have elevated the effect of inoculated strains.

The relative richness of assimilable P, in our field conditions, seemed to give more advantage to *Frankia* treatments than mycorrhizal treatments. Further investigations are in progress in order to clarify the relative contributions of mycorrhizal fungi and frankiae to successful plantation in disturbed environments and to establish the potentiality of other alder species and microbial sources.

*Acknowledgements*—ENEL (Italian National Electricity Board) is gratefully acknowledged for providing the experimental planting site and aiding in plot establishment. This research was supported in part by (Italian National Research Council) CNR, Comitato Nazionale Scienze Agrarie).

#### REFERENCES

- Buresti E. (1990) Gli impianti forestali delle discariche dell'ENEL, miniera di S. Barbara nel Valdarno. ENEL.
- Chatarpaul L., Chakravarty P. and Subramanian P. (1989) Studies in tetrapartite symbioses. *Plant and Soil* **118**, 145–150.
- Danielson R. M. (1985) Mycorrhizae and reclamation of stressed terrestrial environment. In *Soil Reclamation Processes: Microbiological Analyses and Application* (Tate R. L. and Klein D. A., Eds), pp. 173–201. Dekker, New York.
- Fragga-Beddiar A. and Le Tacon F. (1990) Interaction between a VA mycorrhizal fungus and *Frankia* associated with *Alnus glutinosa*. *Symbiosis* **9**, 247–258.
- Giovannetti M. and Mosse B. (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist* **84**, 489–500.
- Hintze J. L. (1992) *Number Cruncher Statistical System*. Kaysville, Utah.
- Hoagland D. R. and D. I. Arnon. (1950) *The Water-culture Method for Growing Plants Without Soil*. California Agricultural Experimental Station Circular No. 347. University of California, Berkeley.
- Hooker J. E. and Wheeler C. T. (1986) The effectivity of *Frankia* for nodulation and nitrogen fixation in *Alnus rubra* and *A. glutinosa*. *Physiologia Plantarum* **70**, 333–341.
- Margheri M. C., Tredici M. R. and Florenzano G. (1983) Isolamento e coltura dell'endofita del genere *Frankia* da noduli radicali di *Alnus cordata* in Italia. *Annali di Microbiologia ed Enzimologia* **33**, 137–148.
- Margheri M. C., Bosco M., Vagnoli L. and Favilli F. (1989) Compatibility of *Frankia* strains belonging to different host-specificity groups with *Alnus glutinosa*, *A. incana* and *A. cordata*. *Annali di Microbiologia ed Enzimologia* **39**, 247–255.
- Puppi G., Chiapperi F., Tabacchini P. Sanvito A. and Carpigo F. (1986) Endogonaceae del litorale tirrenico. *Micologia Italiana* **15**, 7–14.
- Ruehle J. L., Marx D. H. and Muse H. D. (1984) Calculated non-destructive indices of growth response for young pine seedlings. *Forest Science* **30**, 469–474.
- Russo R. O. (1989) Evaluating alder-endophyte (*Alnus acuminata*–*Frankia*–*Mycorrhizae*) interaction. *Plant and Soil* **118**, 151–155.
- Sheppard L. J., Hooker J. E., Wheeler C. T. and Smith R. I. (1988) Glasshouse evaluation of the growth of *Alnus rubra* and *Alnus glutinosa* on peat and acid brown earth soils when inoculated with four sources of *Frankia*. *Plant and Soil* **110**, 187–198.
- Wheeler C. T., Hollingsworth M. K., Hooker J. E., McNeill J. D., Mason W. L., Moffat A. J. and Sheppard L. J. (1991) The effect of inoculation with either cultured *Frankia* or crushed nodules on nodulation and growth of *Alnus rubra* and *Alnus glutinosa* seedlings in forest nurseries. *Forest Ecology and Management* **43**, 153–166.