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Interactions between a VA Mycorrhizal Fungus and *Frankia* Associated with Alder (*Alnus glutinosa* (L.) Gaetn.)*

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Abstract

In this paper we examine differences in growth, mycorrhizal infection, nodulation and nitrogen fixation of young seedlings of *Alnus glutinosa* inoculated or non inoculated with *Glomus fasciculatum* or *Frankia*, at two levels of phosphorus in the soil.

At low level of phosphorus in the soil and without VA mycorrhizal infection, nitrogen fixation was very poor.

Nitrogen fixation per plant was stimulated by the addition of 50 ppm P to the soil. Phosphorus fertilization increased the effectiveness of nitrogen fixation by increasing the mean weight per nodule, the number of nodules per plant and the nitrogen fixation per mg of nodule tissue.

VA mycorrhizal infection did not affect the efficiency of nitrogen fixation per mg of nodule tissue, as phosphorus fertilization did. But, compared to phosphorus fertilization, VA mycorrhizal infection multiplied the number of nodules two fold and their mean weight three fold. This increased nitrogen fixation per plant by three hundred per cent.

VA mycorrhizas improve nitrogen fixation more by undetermined mechanisms than by providing phosphorus to the host. These unknown mechanisms contribute to an increase of the number of *Frankia* infection points on Alder roots, and to an increase, after infection, of nodule tissue.

Introduction

Mycorrhizal fungi can stimulate or inhibit each other as well as other soil microorganisms. These interactions are of great importance for plant growth.

The most important of synergistic effect is probably the interaction between mycorrhizas and *Rhizobia*. This subject has been quite extensively reviewed by Daft

*Reviewed

(1978), Mosse (1977), Munns and Mosse (1980), and also in a range of research papers.

VA mycorrhizas markedly improve nodulation and nitrogen fixation by helping to provide the rather high phosphorus requirements for the fixation process. They possibly have some other, as yet undertermined secondary effect, leading to better nitrogen fixation. In return the improved plant-growth due to a better nitrogen supply offers more roots for mycorrhizal colonization. Does a similar interaction exist for actinomycetes (*Frankia*) living in nodules of Alder? Until now, it was not known wether mycorrhizal infection had similar effects on the symbiotic assocation *Alnus-Frankia.*

Alder is associated with a nitrogen fixing actinomycete, *Frankia.* The nodules are perennial coralloid structures in which *Frankia* grows and fixes dinitrogen symbiotically (Akkermans 1982). The main part of the nitrogen fixed is transported to the roots, shoots and leaves of the host plant (Dommergues and Krupa 1978). Alder is also associated with VA mycorrhizal fungi (Diagne and Le Tacon 1982) and ectomycorrhizal fungi (Trappe 1979).

These fungi improve the growth of their hosts by several mechanisms, among which improved phosphorus nutrition is the most important (Mosse 1973).

The aim of the present paper is to study the possible interactions between VA mycorrhizal fungi and *Frankia.* We have examined differences in growth, mycorrhizal infection, nodulation and nitrogen fixation of four month old seedlings of *Alnus glutinosa* inoculated with *Frankia,* and inoculated or non inoculated with *Glomus fasciculatum* at two levels of phosphorus in the soil.

Material and Methods

Seeds of a single provenance of *Alnus glutinosa* (East of France) were used to minimize genotypic variation. Seeds were surface sterilized in 30% hydrogen peroxide for 20 minutes, and aseptically placed on to a glucose-yeast-peptone agar (Rovira 1959) in Petri dishes. After 4 days, seeds showing no microbial contamination were germinated on wet filter paper in Petri dishes.

Sterile one month old seedling were sown on the surface of 2 litre pots containing a previously steam fumigated soil. It was the A1 horizon of a forest brown calcic soil with a pH of 6.1.

Table 1. Analyses of the A1 horizon of the forest brown calcic soil used in the experiment (phosphorus was extracted with NaOH M/10 and H2 S04 M/250).

Organic matter %	Total C %	Total N %	C/N	pH in water	Exchangeable cations in m.e./100g				P_2O_5 ‰	A %	Lf %	Lg %	Sf %	Sg %
					Ca	K	Mg	T						
18.1	9.04	0.58	15.6	6.1	44.2	1.28	0.68	46.0	0.08	49.2	18.9	6.3	2.2	0.8

All the seedlings in all the treatments were inoculated just after transplanting with a water suspension prepared from a pure culture of *Frankia*, (Lalonde 1979). We have used a Finnish *Frankia* strain isolated from grey Alder (*Alnus incana*) (strain AJRX isolated in 1983 by Assi Weber, University of Helsinki, Finland).

Half of the *Frankia* inoculated seedlings were also inoculated with a VA mycorrhizal fungus *Glomus fasciculatum*. Mycorrhizal treatment consisted of adding onion root fragments previously infected with G. *fasciculatum* to the steamed soil.

The inoculum was placed 50 mm below the seeds. The fungus was the strain E3 of *Glomus fasciculatum* ((Thaxter), Gerd. and Trappe) isolated by Barbara Mosse at Rothamsted, Great Britain.

Each treatment was divided in two by addition or no addition of 50 ppm soluble P as Ca orthophospate. In a previous experiment we have found that in this soil the addition of 50 ppm of soluble P led to the optimum growth and to the optimum P nutrition of Alder without mycorrhizal infection. The following treatments were established:

F	no phosphorus, seedlings inoculated with *Frankia*
F + P	50 ppm of phosphorus, seedlings inoculated with *Frankia*
F + G	no phosphorus, seedlings inoculated with *Frankia* and *Glomus fasciculatum*.
F + G + P	50 ppm of phosphorus, seedlings inoculated with *Frankia* and *Glomus fasciculatum*.

The experimental design was a randomized complete block with 9 replicates. There was one seedling per pot. Combinations of mycorrhizas and P fertilization, either present or absent, were arranged in a 2×2 factorial treatment design.

The potted plants were grown in an air-conditioned glass-house with the following conditions: light period 16 h, day/night temperature: 20°C/16°C. The pots were watered to field capacity regularly with sterile water.

The seedlings were harvested after four months. At harvest the plants were removed from the pots and the soil was washed gently off the roots. The following parameters were measured:

- nitrogenase activity;
- fresh and dry weight of roots and shoots;
- percentage of mycorrhizal infection;
- number of nodules per plant;
- fresh weight of nodules per plant.

The total root system of each plant was incubated in flasks of 560 ml during one hour at 20°C. Air was withdrawn and replaced by the same volume of C_2H_2, so as to give 10% of C_2H_2 in the gas phase.

Gas samples of 0.5 ml were taken after one hour. The amount of C_2H_2 was immediately determined by gas chromatography (GIRDEL 3000). The C_2H_4 formed was calculated by comparison with a standard gas mixture of C_2H_4 in air (Kawai and Yamamoto 1986).

Root samples for determining *Glomus fasciculatum* infection were obtained by slicing a thin section down the entire length of the intact root systems. Root segments, approximately 2–3 cm long, were stained with acid fuchsin (solution at 0,02% in lactic acid, glycerol and water 875/62/63 V/V).

Stained roots were spread in a Petri dish, and selected at random by a grid intersect method. A microscopic infection count was taken on 100 root segments.

Results and Discussion

Effects on dry weight of the shoots and roots per plant (Figs. 1 and 2, Table 2).

The growth of Alder seedlings was very poor without phosphorus and when inoculated with *Frankia* alone. The addition of 50 ppm of soluble phosphorus significantly increased the dry weight of shoots and roots. VA mycorrhizal inoculation dramatically increased Alder growth and more than phosphorus fertilization. The addition of phosphorus had no significant effect when the seedlings were mycorrhizal.

Effects on VA mycorrhizal infection (Table 2).

All the non-inoculated seedlings were devoid of VA mycorrhizas, and all the inoculated seedlings had VA mycorrhizas. The addition of soluble phosphorus to the soil significantly decreased the percentage of the mycorrhizal infection.

Effects on number of nodules per plant (Fig. 4, Table 2).

There were very few nodules in the treatment with *Frankia* alone. Phosphorus fertilization markely increased the number of nodules.

VA mycorrhizal inoculation had a striking effect and increased the number of nodules more than phosphorus fertilization. Phosphorus addition had no effect when the seedlings were mycorrhizal.

Effects on fresh weight of nodules per plant and on the mean fresh weight per nodule (Fig. 4, Table 2).

On the treatment with *Frankia* alone the fresh weight of nodules was very weak (3.88 mg of nodules per plant). The addition of phosphorus increased it significantly (119.3mg of nodules per plant). The VA mycorrhizal infection had an extraordinary effect without phosphorus addition (677.4 mg of nodules per plant). The addition of phosphorus still slightly increased the fresh weight of nodules per plant (879.6 mg), but not significantly.

The addition of phosphorus alone multiplied the mean fresh weight of nodules three fold, while VA mycorrhizal infection alone multiplied it ten fold.

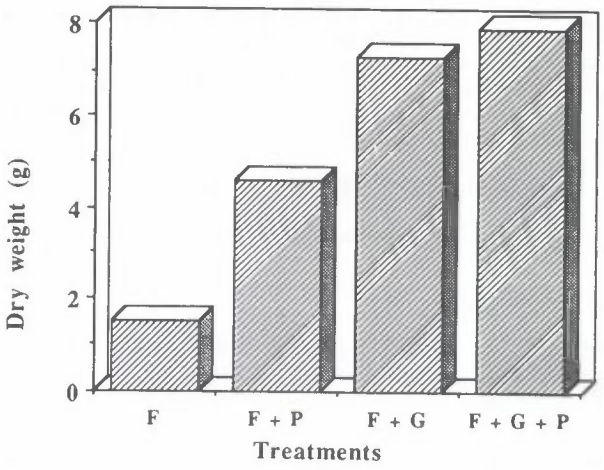

Figure 1. Dry weight of the shoots per plant.

F	–	F+G	**
F	–	F+P	**
F+P	–	F+G	**
F+P	–	F+P+G	**
F+G	–	F+P+G	n.s.

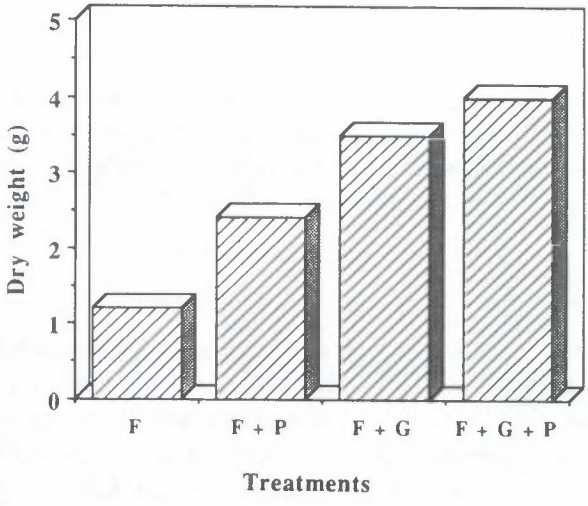

Figure 2. Dry weight of the root system per plant.

F	–	F+G	**
F	–	F+P	**
F+P	–	F+G	**
F+P	–	F+P+G	**
F+G	–	F+P+G	n.s.

Table 2. Effects of treatments on dry weight of the shoot and root system per plant, on VAM infection, on nodulation and nitrogenase activity.

Treatments	F	F+P	F+G	F+G+P
Dry weight of the shoots per plant (g)	1.5	4.6	7.3	7.9
Dry weight of the root system per plant (g)	1.2	2.4	3.5	4.0
VAM infection per plant in per cent	0	0	58	33
Number of nodules per plant	1.8	15.9	30.5	30.9
Fresh weight of nodules per plant (mg)	3.88	119.3	677.4	879.6
Mean fresh weight per nodule (mg)	2.18	7.5	22.2	28.4
Nitrogenase activity per plant in nanomoles of C2H4 per hour per plant	33	2416	6153	7329
Nitrogenase activity per mg of fresh nodule tissue in nanomoles of C2H4 per hour	8.52	20.25	9.08	8.33

From Figs 3 and 4, and from table 2, it can be concluded that VA mycorrhizal infection, compared to phosphorus addition alone, increased the number of nodules per plant and much more the mean weight per nodule. But the question is: did VA mycorrhizal infection increase nitrogen fixation?

Effects on nitrogenase activity per plant (Fig. 5, Table 2).

The nitrogenase activity per plant was very weak in the treatment F with *Frankia* alone (33 nanomoles of C_2H_4 per hour). The addition of phosphorus (treatment F + P) considerably increased the nitrogenase activity per plant (2416 nanomoles of C_2H_4 per hour). Compared to phosphorus fertilization (treatment F + P), VA mycorrhizal infection (treatment F + G) doubled the nitrogenase activity per plant. The addition of phosphorus (treatment F + G + P) still slightly increased it, but not significantly.

Effects on nitrogenase activity per mg of fresh nodule tissue (specific nitrogenase activity) (Fig. 6, Table 2).

In treatment F the nitrogenase activity was 8.52 nanomoles of C_2H_4 per mg of fresh

nodule tissue and per hour. Phosphorus addition alone (treatment F + P) increased significantly the nitrogenase activity (20.25 nanomoles of C_2H_4). Phosphorus addition coupled with mycorrhizal infection (treatment F + G + P) did not increase it. It is difficult to explain this absence of interaction between phosphorus nutrition and VA mycorrhizal infection on nitrogen fixation per mg of fresh nodule tissue.

To the question, did VA mycorrhizal infection increase nitrogen fixation? We can answer yes if we speak in terms of nitrogenase activity per plant, and no if we speak in terms of specific nitrogenase activity.

Conclusion

Nitrogen fixation per plant was stimulated by the addition of 50 ppm of P to the soil, only in the non mycorrhizal treatment. Improved phosphorus nutrition by phosphorus fertilization modified the effectiveness of nitrogen fixation in three ways:

- first by increasing the number of nodules
- secondly by increasing the mean weight per nodule
- thirdly by increasing the specific nitrogenase activity.

Compared to phosphorus fertilization alone, VA mycorrhizal infection improved nitrogen fixation per plant much more. But VA mycorrhizal infection modified only two components of nitrogen fixation effectiveness:

- the number of nodules per plant
- the mean weight of nodules.

VA mycorrhizal infection did not affect the nitrogenase activity per mg of nodule tissue, as phosphorus fertilization did alone.

But compared to phosphorus fertilization alone, VA mycorrhizal infection multiplied the number of nodules per plant by two and the mean weight per nodule by three. This increased the nitrogen fixation per plant by three even though mycorrhizal infection did not stimulate the specific nitrogenase activity.

The synergistic effect between *Frankia* and VA mycorrhizas is similar to the interaction observed by different authors between *Rhizobia* and VA mycorrhizas.

The interaction between *Rhizobia* and VA mycorrhizas is generally attributed to the ability of mycorrhizas to take up P from the soil and to increase the phosphorus supply to nodules through plant translocation (Kawai and Yamamoto 1986). We have also to consider that mycorrhizas could assist the uptake of micro nutrients eg. molybden, which is necessary for nitrogen fixation.

Other mechanisms, which are probably non nutritional, could also be involved in the synergestic effect, that we have observed between *Frankia* and VA mycorrhizas. These unknown mechanisms contribute to increase the number of *Frankia* infection points on Alder roots, and to increase, after infection, the growth of nodule tissues.

The increase of *Frankia* infection points by VA mycorrhizal infection could be a rhizosphere effect, i.e. effect on saprophytic growth of Frankia or effect on *Frankia*

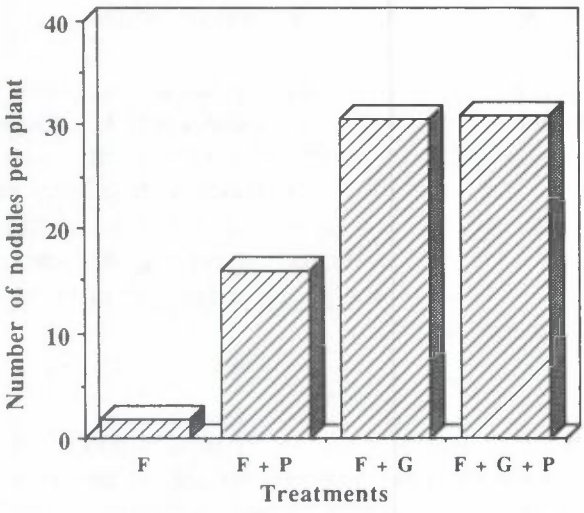

Figure 3. Number of nodules per plant.

F	–	F+G	**
F	–	F+P	**
F+P	–	F+G	**
F+P	–	F+P+G	**
F+G	–	F+P+G	n.s.

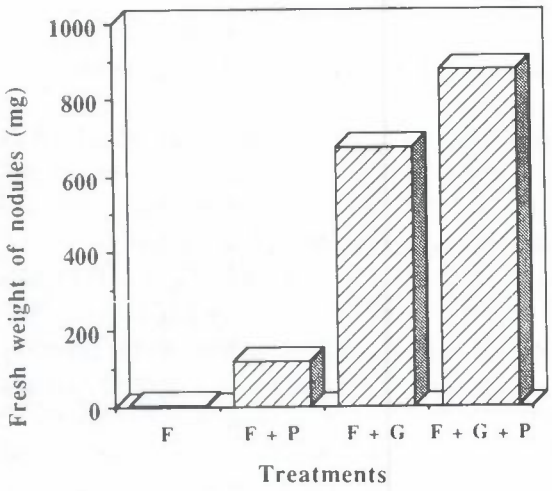

Figure 4. Fresh weight of nodules per plant.

F	–	F+G	**
F	–	F+P	**
F+P	–	F+G	**
F+P	–	F+P+G	**
F+G	–	F+P+G	n.s.

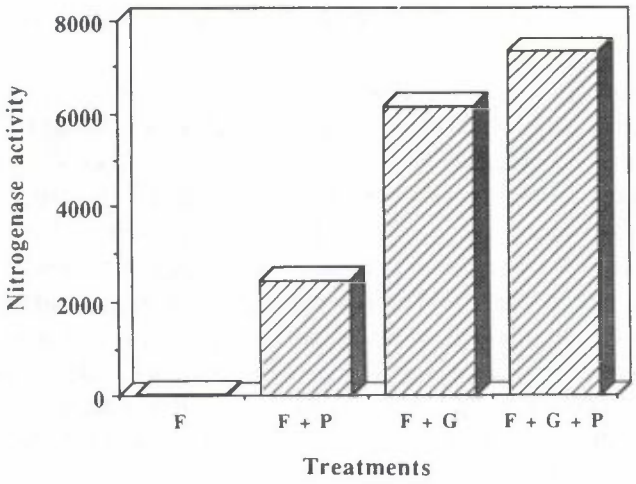

Figure 5. Nitrogenase activity per plant in nanomoles of C_2H_4 per hour.

F	–	F+G	**
F	–	F+P	**
F+P	–	F+G	**
F+P	–	F+P+G	**
F+G	–	F+P+G	n.s.

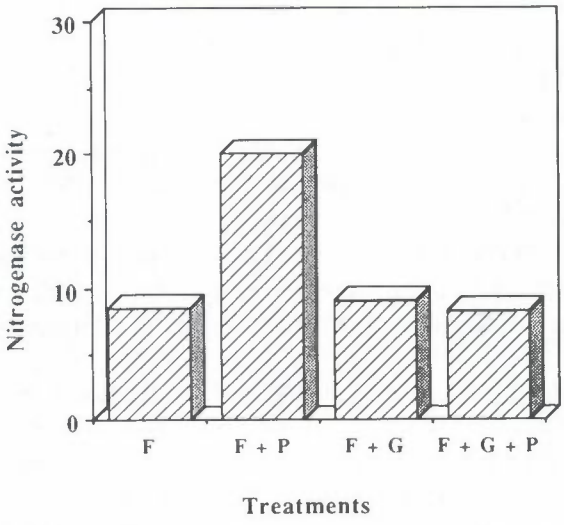

Figure 6. Nitrogenase activity per mg of fresh nodule tissue in nanomoles of C_2H_4 per hour.

F+P	–	F			**
F+P	–	F+G			**
F+P	–	F+P+G			**
F –	F+G	–	F+P+G		n.s.

spore germination through root exudation which is modified by mycorrhizal infection and mineral nutrition (Rovira 1959, Harris et al., 1985). Meyer and Linderman (1986) have shown that *Glomus fasciculatum* infection influences the population of actinomycetes in the rhizosphere. More recently Pacovsky (1989) found that the colonization of sorghum roots by *Glomus etunicatum* enhanced the establishment and persistence of *Azospirillum brasilense* in the endorhizosphere of sorghum. According Pacovsky, who excludes the possibility that host nutritional status was responsible for the elevated *Azospirillum* counts in mycorrhizal roots, penetration of cortical cells by VAM fungi could provide a route of entry for *Azospirillum* into the endorhizosphere. Similar mechanisms may explain the synergetic effect between *Glomus fasciculatum* colonization and *Frankia* infection. The increased growth of nodule tissue could be hormonal effect (Edress, Davis and Burger 1984).

This interaction between VAM and *Frankia* stimulated Alder growth and should encourage the pratical use of double inoculation of Alder in the nursery.

REFERENCES

Akkermans, A.D.L. 1982. Biology of actinorhizal plants and their application in forestry: in: Proceedings of the Second National Symposium on Biological Nitrogen fixation. Helsinki, June 8-10-1982.

Daft, M.J. 1978. Nitrogen fixation in nodulated and mycorrhizal crop plants. *Ann. Appl. Biol*, **88:** 461–465.

Diagne, O., and Le Tacon, F. 1982. Interaction fertilisation-mycorhization nodulation chez *Alnus incana* in Les mycorhizes = Biologie et utilisation. Ed. INRA, Les Colloques de l'INRA, n° **13:** 199–206.

Dommergues, Y.R. and Krupa, S.V. 1978. Interactions between non-pathogenic soil microorganisms and plants. Developments in Agricultural and managed-forest ecology. Elsevier Scientific Publishing Company, Amsterdam, Oxford, New York 335–371.

Edress, M.H., Davis, R.M., and Burger D.W. 1984. Influence of mycorrhizal fungi on cytokinin production in sour orange, *J. Amer. Soc. Hort. Sci.* **109:** 587–590.

Harris, D., Pacovsky, R.S. and Paul, E.A. 1985. Carbon economy of soybean – *Rhizobium-Glomus* associations. *New Phytol.* **101:** 417–440.

Kawai, Y. and Yamamoto, Y. 1986. Increase in the formation and nitrogen fixation of soybean nodules by vesicular-arbuscular mycorrhiza. *Plant Cell Physiol.* **27**(3): 339–405.

Lalonde, M. 1979. Immunological and ultrastructural demonstration of nodulation of the European *Alnus glutinosa* host plant by actinomycetal isolate from the north American *Comptonia peregrina* (L.) coult. root nodule. *Bot. Gaz.*, **140**, 35–43.

Meyer, J.K., and Linderman, R.G. 1986. Selective influence on populations of rhizosphere and rhizoplane bacteria on actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. *Soil Biol. Biochem*, **18:** 191–196.

Mosse B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. *Annual Review of Phytopathology*. Vol. 11, 1973, pp. 171–196.

Mosse B. 1977. The role of mycorrhiza in legume nutrition on marginal soils. in: Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture (J.M. Vincent, A.S. Whitney and J. Bose, eds), pp. 175–292. University of Hawaii, College of Tropical Agriculture Miscellaneous Publication 145.

Munns, D.N. and Mosse, B. 1980. Mineral nutrition of legume crops. in: Advances in Legume Sciences (R.J. Summerfield and A.H. Bunting, eds), pp. 115–125.

Pacovsky, R.S. 1989. Diazotrophy establishment and maintenance in the *Sorghum-Glomus-Azospirillum* association. *Can. J. Microbiol.* **35:** 977–981.

Rovira, A.D. 1959. Root excretions in relation to the rhizosphere effect. IV influence of plant species, age of plant, light temperature and calcium nutrition on exudation *Plant and soil*, **11**, 53–64.

Trappe, J.M. 1979. Mycorrhiza-nodule-host interrelationships in symbiotic nitrogen fixation: A quest in need of Questers. in: Symbiotic nitrogen fixation in the management of temperate forests. Proceedings of Workshop held April 2-5-1979, pp. 267–286. Ed.: J.C. Gordon, C.T. Wheeler, D.A. Perry.