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Studies in tetrapartite symbioses

I. Role of ecto- and endomycorrhizal fungi and Frankia *on the growth performance of* Alnus incana

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Key words: *Alnus incana*, *Frankia*, *Glomus fasciculatus*, multiple symbiosis, *Paxillus involutus*

Abstract

Alnus incana seedlings were successfully inoculated with an endomycorrhizal fungus (*Glomus fasciculatus*), an ectomycorrhizal fungus (*Paxillus involutus*) and an isolate of *Frankia* (ACN1) simultaneously. The effects of the inoculation treatments on the growth performance of the seedlings were evaluated under controlled conditions.

The overall growth performance of the seedlings inoculated with the three organisms was better than those inoculated with *Frankia*, *G. fasciculatus* and *P. involutus* individually or with *Frankia* + *G. fasciculatus* and *Frankia* + *P. involutus* combinations. The highest growth performance and mycorrhizal infection occurred when the seedlings were inoculated simultaneously with *Frankia* + *G. fasciculatus* + *P. involutus*.

Introduction

Actinorhizal plants, originating from diverse geographical locations and habitats, have a wide range of potential use in forestry. They are amenable for use in the improvement and exploitation of marginal/disturbed lands, enhancement of growth of associated vegetation, production of timber for various purposes, erosion control and as fuelwood (Dawson, 1986; Torrey, 1978). The genus Alnus, is by far the most intensively studied one and species of this genus are reported to be largely responsible for high levels of soil nitrogen accretion, worldwide (Tarrant and Trappe, 1971). Grey alder (*Alnus incana*) is an important actinorhizal species in cool, temperate regions and is a promising short-rotation crop for energy forests in such climates. They have been widely used for the reclamation of peat bogs in Finland (Akkermans and Van Dijk, 1981; Granhall, 1982; Mikola *et al.*, 1983).

The roots of some actinorhizal plant species have been found to support ectomycorrhizae or VA mycorrhizae in addition to nitrogen-fixing nodules, simultaneously (Hall *et al.*, 1979; Rose, 1980; Rose and Youngberg, 1981). The presence of VA hyphae in nodular tissues of alders (Daft, 1983) and an ectomycorrhizal sheath around young nodules of *Alnus crispa* (Godbout and Fortin, 1983), indicates that the exclusion of nodular and mycorrhizal endophytes does not occur in actinorhizal plants. However, the presence of both ecto- and endomycorrhizae on the same root system has rarely been reported. Such an occurrence was observed on non-actinorhizal plants by Lapeyrie and Chilvers (1985) and Chilvers *et al.* (1987) on *Eucalyptus* spp. and by Read *et al.* (1977) on *Helianthemum chamecistus*. There was an overlapping succession, with an early peak of endomycorrhizae followed by a later build-up of ectomycorrhizae. A similar ecto- and endomycorrhizal succession has also been found on two actinorhizal plants, *Casuarina equisetifolia* (Ba *et al.*, 1987), and *Alnus glutinosa* (Beddiar, 1984).

In a survey of alders growing in a field demonstration area of the Petawawa National Forestry Institute, Chalk River, Ontario, Canada, we have found that nodulated alders (*Alnus incana* (L.) Moench, *A. japonica* and *A. glutinosa* (L.) Gaertn.) were infected by both ecto- and endomycorrhizal (VAM) fungi within the same root system. This suggests that multiple infection is a general phenomenon in this genus. However, no attempt has yet been made to study the effect of these multiple associations on the growth performance of such plants. This study reports the effect of an endomycorrhizal fungus (*Glomus fasciculatus* [Thaxter] Gerd.), an ectomycorrhizal fungus (*Paxillus involutus* [Batsch. ex. Fr.]), and a strain of Frankia (ACN1) on the growth performance of *Alnus incana* (L.) Moench seedlings under controlled conditions.

Materials and methods

Seeds of *Alnus incana* were surface sterilized with 30% H_2O_2 for 10 minutes, washed several times with sterile distilled water and then sown in Spencer-Lemaire containers (1.9 × 1.9 × 10.1 cm, length × width × height) in 22 × 37 cm tray containing sterile peat:vermiculite (3:1, w/w) with 25% of the recommended dose of slow-release fertilizer (Nutricote, Micromax, dolomitic lime, and gypsum). Two weeks later, seedlings of uniform size were transplanted into 10-cm clay pots (one seedling/pot) containing similar types of root substrate and fertilizer as described above. Two and a half week old seedlings of *A. incana* were inoculated with an endomycorrhizal fungus, *Glomus fasciculatus* (VAM) an ectomycorrhizal fungus, *Paxillus involutus* and a strain of *Frankia* (ACN1) in combination and alone. VAM inoculation was achieved by placing a band of soil containing 500 clamydospores of *Glomus fasciculatus* per gram of soil at a depth of 5 cm below the surface of the pots. The seedlings were then placed so that the roots were about 2.5 cm above the inoculum. Five ml suspensions of mycelial/hyphal preparations of *Paxillus involutus* and/or *Frankia* were injected into the rooting zone of the seedlings. The control group of seedlings received no inoculum. There were eight replicates per treatment. The seedlings were kept in a growth chamber at 16 h day light at 25°C:20°C (Day:Night) and watered on alternate days. The seedlings were harvested and evaluated 10 weeks after inoculation. Incidence of VAM and ectomycorrhizae in the above root systems were assessed in two ways: (1) as root length infected, using grid-intersect method (Giovannetti and Mosse, 1980), and (2) as numbers of roots hosting each type of mycorrhizal fungus within 1 cm of the root apex. Briefly, VAM estimation was done as follows: The roots were cleared with tap water, cut into 1 cm sections, and boiled with 10% KOH for 10 min. Following treatment with 5% H_2O_2 the sections were stained with cotton blue. The stained sections were mounted in lacto-phenol and examined at 250 × and 400 × for presence of VAM. A root was considered infected if it showed hyphae, vesicles or arbuscles or any combinations of these structures which are characteristic of VAM infections. For ectomycorrhizal estimation, the roots were carefully washed to remove adhering soil particles. The total number of short roots were determined for each seedling and the percentage of ectomycorrhizal roots calculated. A root was considered to be ectomycorrhizal if it showed mantle or hartig net or both. In cases of doubt thin sections of short roots were examined microscopically after staining with cotton blue.

Incidence of nodule infected by *Frankia* were determined under stereo microscope. In addition, shoot and root length and their dry weight, nodule dry weight, and total dry weight were determined for individual seedlings.

The data were subjected to analysis of variance (Steel and Torrie 1960). The individual means were compared using Duncan's New Multiple Range Test using SAS software (SAS Inst. Inc. 1985).

Results and discussion

Under controlled conditions, we have successfully inoculated axenically grown seedlings of *Alnus incana* with an ectomycorrhizal fungus, *Paxillus involutus*, an endomycorrhizal fungus (VAM), *Glomus fasciculatus* and a *Frankia* strain ACN1 simultaneously. Figure 1 shows the ectomycorrhizal roots and *Frankia* nodules on the triple inoculated root system. Figure 2 illustrates the presence of VAM on both field grown Alnus and on the same roots shown in Figure 1. As seen in Figure 3, the tetra-partite association resulted in visibly larger and healthier seedlings when compared to controls.

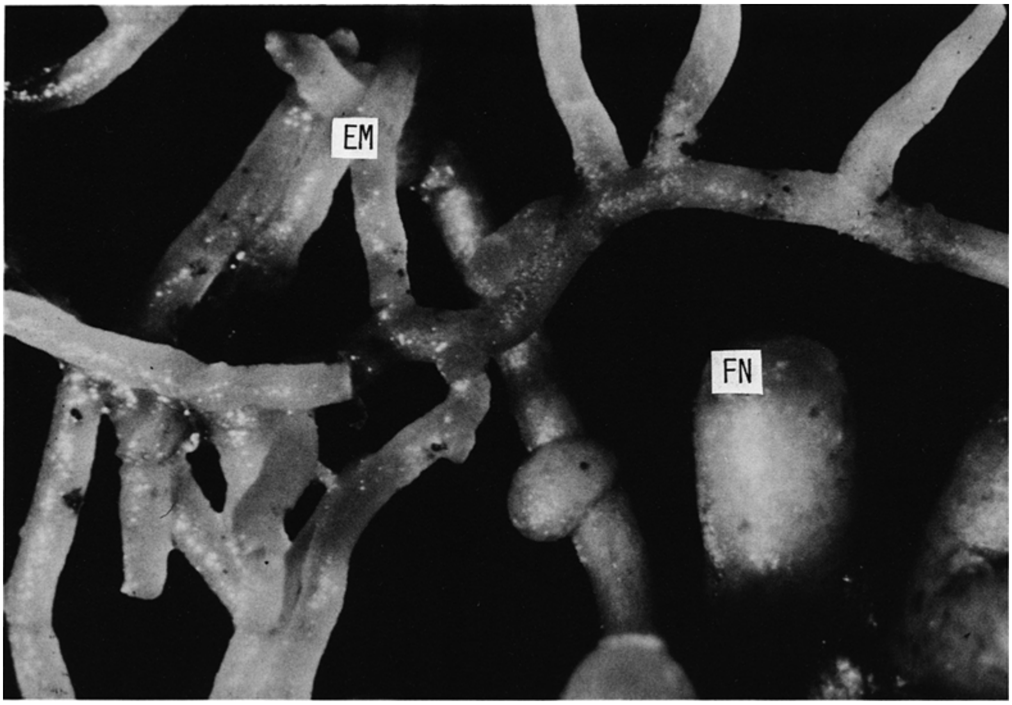

Fig. 1. Root system of triple inoculated *Alnus incana* showing ectomycorrhizal roots (EM) and *Frankia* nodules (FN).

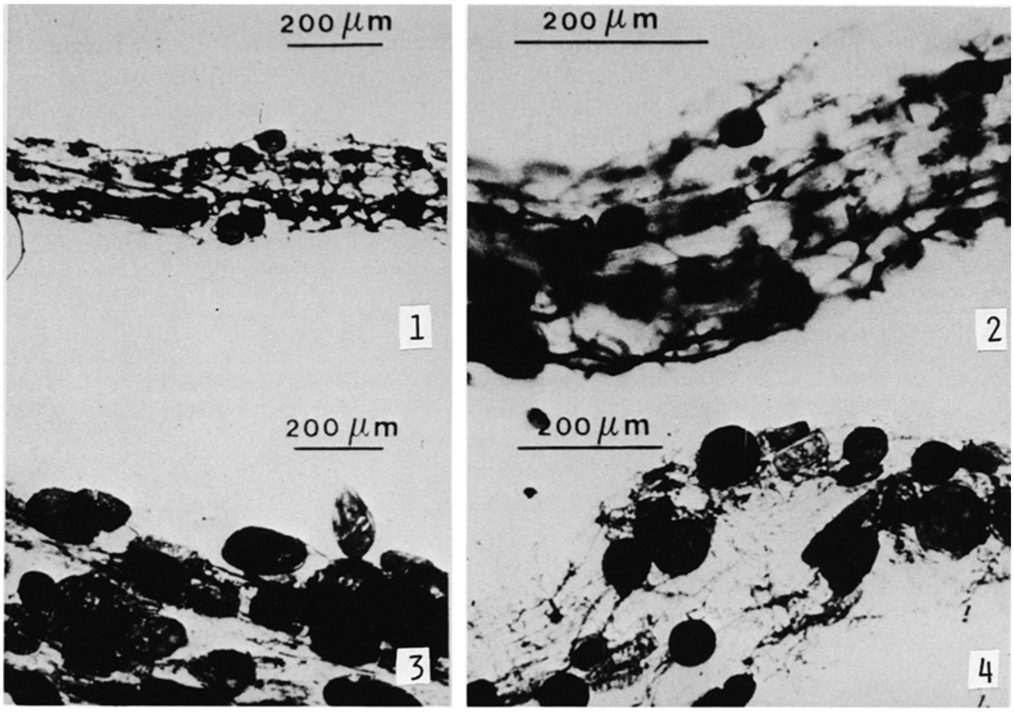

Fig. 2. VAM on roots of 2 yr-old *Alnus japonica* (1), *Alnus glutinosa* (2), *Alnus incana* (3) growing in the field, and on *Alnus incana* seedling (4) inoculated with *Glomus fasciculatus* along with *Frankia* and *Paxillus involutus*.

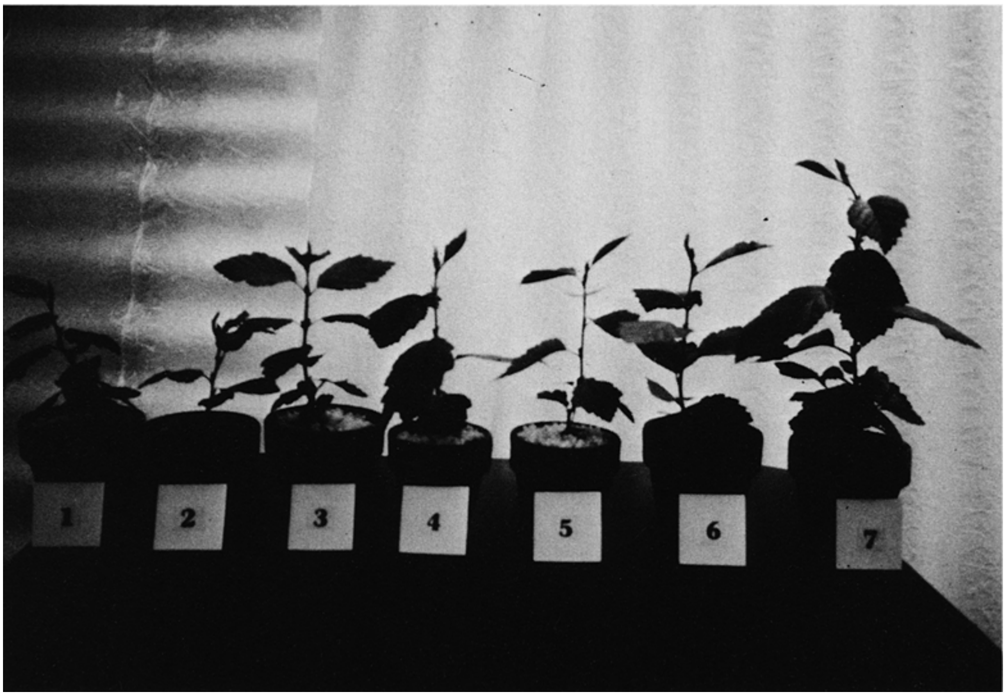

Fig. 3. Alnus incana seedlings after different inoculation treatments. 1 = *Frankia*, 2 = Control, 3 = *Glomus fasciculatus*, 4 = *Paxillus involutus*, 5 = *Frankia* + *G. fasciculatus*, 6 = *Frankia* + *P. involutus*, 7 = *Frankia* + *G. fasciculatus* + *P. involutus*.

Table 1 compares the overall growth performance and mycorrhizal infection of *A. incana* inoculated and non-inoculated seedlings. Shoot and root length, and total dry weight, were significantly higher when the seedlings were inoculated in combination with mycorrhizal fungi and *Frankia* than single inoculation. Nodule dry weight also significantly increased when *Frankia* and mycorrhizal fungi were inoculated together than *Frankia* alone. The total dry weight was highest when the seedlings were inoculated with all the three symbionts (*G. fasciculatus*, *P. involutus* and *Frankia*) together

Table 1. Effect of ectomycorrhizae (*P. involutus*), endomycorrhizae (*G. fasciculatus*), and *Frankia* on seedling growth and mycorrhizal infection of *A. incana*

Treatment	Growth parameters					
	Shoot height (cm)	Root length (cm)	Shoot:root	Nodule dry wt. (mg)	Total dry wt. (g)	Mycorrhizal infection (%)
Control	10a	15a	1.4	0	1.72a	0
G. fasciculatus	12ab	15a	1.6	0	2.10a	42.5a
P. involutus	12ab	16ab	1.7	0	2.23ab	55.3b
Frankia	13bc	20b	1.3	8.3a	2.41b	0
G. fasciculatus + *Frankia*	15c	30c	1.1	9.5ab	3.6c	58.0b
P. involutus + *Frankia*	18d	30c	1.3	12.0bc	4.01d	69.6c
G. fasciculatus + *P. involutus* + *Frankia*	20d	31c	1.3	12.5c	5.11e	65.0c (endo) 85.0d (ecto)

Values are the means of 8 replicates. Means followed by same letter in columns for a parameter are not significantly ($P = 0.05$) different from each other.

than single or dual (*G. fasciculatus* + *Frankia* or *P. involutus* + *Frankia* inoculation. Both ecto- and endomycorrhizal infection increased significantly when the seedlings were grown together with *Frankia*. The similar phenomenon was observed by Diem and Gauthier (1982). They found that inoculation with *G. mossae* increased nodulation and growth of *Casuarina equisetifolia*. The growth of *A. incana* was optimal, without N or P supplies, when inoculated by both *Frankia* and*G. mossae* (Diagne and Le Tacon, 1982). Mejstrik and Benecke (1969) found that ectomycorrhized and nodulated roots of *A. viridis* absorb phosphorus five times more quickly than did nodulated roots alone. The benefits of tripartite symbioses (*Frankia* + *P. involutus* + *A. glutinosa*) was also discussed by Chatarpaul *et al.*, (1988). The multiple symbioses of alders is a likely strategy that allows host tree to grow in extreme environmental conditions. Both ecto and endomycorrhizal plants are known for their ability to absorb more nutrients and water, producing growth regulators, resist root pathogens, and survive in adverse soil conditions such as low pH and high soil temperature (Harley and Smith, 1983). The formation of root nodules by *Frankia* is considered essential for the survival and establishment of actinorhizal plants in nitrogen-poor sites such as sandy and gravelly soils, raw mineral soils, and wet soils. Their role in fixing nitrogen in forest crops and agricultural plants are well known (Dawson, 1986).

Our results suggest that the presence of a multiple symbioses (ectomycorrhizae + endomycorrhizae + *Frankia*) could have added benefits to the actinorhizal plants. There is also an indication that ecto- and endomycorrhizae cohabit adult trees and are not merely successional. Additional work with the multiple association will be necessary to test the hypothesis of synergism or antagonism between the mycorrhizal and actinorhizal system. As a continuation, further detailed studies on the nitrogenase activity and nutrient uptake of this multiple symbiosis of *A. incana* are currently in progress.

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