

Alnus acuminata in dual symbiosis with *Frankia* and two different ectomycorrhizal fungi (*Alpova austroalnicola* and *Alpova diplophloeus*) growing in soilless growth medium

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(Received January 11, 2008; Accepted August 20, 2008)

Abstract

In this study we investigated the capacity of Andean alder (*Alnus acuminata* Kunth), inoculated with *Frankia* and two ectomycorrhizal fungi (*Alpova austroalnicola* Domínguez and *Alpova diplophloeus* ([Zeller and Dodge] Trappe and Smith), for nodulation and growth in pots of a soilless medium that contained vermiculite or a mixture of ground basalt rock and vermiculite. The seedlings were inoculated with *Frankia* suspensions prepared from root nodules of *A. acuminata*, followed by inoculation with spores of either one of the two *Alpova* species. The seedlings were grown in a greenhouse for 12 months. The seedlings grown in the vermiculite-based growth medium containing large (1-3 mm) basalt particles and *Alpova austroalnicola* or medium-sized (0.5-1 mm) basalt particles and *A. diplophloeus* had the heaviest shoot and root nodule dry weights and abundant ectomycorrhizal colonization. Ectomycorrhizas formed by *A. acuminata* with *Alpova austroalnicola* is described here for the first time. Growth of *Alnus acuminata* inoculated with ectomycorrhizal fungi and *Frankia* in the soilless primary minerals indicates that Andean alder can alter resource supply by tapping an otherwise unavailable nutrient source.

Keywords: *Alpova*, *Alnus acuminata*, ectomycorrhiza, *Frankia*

1. Introduction

Alnus acuminata Kunth (Andean alder), a member of the Betulaceae, is distributed along the Andes from Venezuela to latitude 28°S in northwestern Argentina (Grau, 1985). Andean alder is mainly harvested for firewood, pulp, and timber. It is an important species recommended for management in land reclamation, watershed protection, agroforestry, and erosion control (National Academy of Sciences, 1984). *A. acuminata* is tolerant to infertile soils given its ability to form ectomycorrhizal (ECM), arbuscular mycorrhizal and actinorrhizal relationships (Cervantes and Rodríguez Barrueco, 1992).

It is through these highly efficient symbioses, in which microsymionts benefit from plant photosynthates, that actinorrhizal plants such as alders colonize poor substrates, enrich soil and initiate plant succession (Roy et al., 2007).

Mycorrhizal symbiosis is generally advantageous for plants growing in nutrient-poor soils (Smith and Read, 1997). Adequate ECM formation is especially critical for trees growing on poor soils or in environments where the seedlings must be established quickly in order to survive (Perry et al., 1987). ECM fungi are known to colonize mineral soils, and in many laboratory experiments it has been confirmed that ECM fungi stimulate dissolution of minerals. Organic acids produced by ECM fungi can probably accelerate rock solubilization from solid mineral substrates, enhancing nutrient (P, K, Ca and Mg) availability for uptake by plants (Watteau and Berthelin,

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1990; Paris et al., 1995). The abilities of different ECM fungi to mobilize inorganic nutrients might be species specific (Lapeyrie et al., 1991). Actinorhizal alders such as *Alnus tenuifolia* and *A. rubra* nodulated by *Frankia* solubilize basalt rocks, thereby releasing base-elements for uptake by plants and rapidly increasing soil organic matter (Li and Bormann, 2003; Yamanaka et al., 2003). Ectomycorrhizal fungi are expected to improve nodulation of actinorhizal plants, perhaps by solubilizing rock minerals, and thus improving nutrient acquisition by the plants (Yamanaka et al., 2003). These combined processes may be important for the survival and establishment of these plants in degraded, nutrient-limited soil ecosystems, thereby improving forest productivity (Bormann et al., 1998; Li and Strzelczyk, 2000).

From studies on ECM of alder species in North America, Europe and South America, it is known that ectomycorrhizal symbionts on *Alnus* spp. roots are dominant (Becerra et al., 2002, 2005a,b,c; Miller et al., 1991; Pritsch 1996; Pritsch et al., 1997). *Alnus acuminata* is associated with a number of ECM fungi belonging to the genera *Russula*, *Lactarius*, *Inocybe*, *Laccaria*, *Cortinarius*, *Gyrodon* and *Naucoria* (Singer and Morello, 1961; Raithelhuber, 1988; Moser, 2001).

Mycorrhizae of *Alpova diplophloeus* (Zeller and Dodge) Trappe and Smith and *Alnus* spp. (except *A. acuminata*) have been described in detail (Godbout and Fortin, 1983; Massicotte et al., 1986; Molina, 1979; Wiedmer and Senn-Irlet, 2001). *Alpova austroalnicola* Dominguez is a new fungal species of *Alpova* recently collected and described under *A. acuminata* in the Yunga district of Argentina (Nouhra et al., 2005). The mycorrhizae on *A. acuminata* formed with *Alpova austroalnicola* were never characterized.

The aims of the present study were: 1) determine the capacity of *Alnus acuminata* to nodulate and to growth in nutrient poor substrates when inoculated with *Frankia* and two ectomycorrhizal fungi under controlled conditions, and 2) characterize the ectomycorrhizae formed by *Alpova austroalnicola* and *A. diplophloeus* synthesized with *A. acuminata*.

2. Materials and Methods

Soil preparation

Basalt rocks were collected from La Calera district (31°21' 10.3"S, 64°21' 26.6"W), Cordoba, Argentina. The rocks were ground and wet sieved to a grain size of between 250 µm to 3 mm. In order to remove fine dust, the minerals were washed in 0.1 M HCl, followed by demineralized water for several days until the effluent reached the pH of the demineralized water. The treatments

were pots containing: 1) rocks of 1-3 mm in diameter for L (large size), 2) rocks 0[500 µm - 1 mm in diameter for M (medium size), 3) rocks of 250-500 µm in diameter for S (small size), and 4) sterilized vermiculite served as control. Vermiculite is a light, porous silicate mineral that serves as a base medium; it retains moisture and gives an aerated root environment. Fifty grams of each rock particle size were mixed with vermiculite (1:1) and placed into tubes (25 cm deep, 7 cm in diameter), the bottom of which was sealed with a 50 µm mesh nylon screen to prevent the particles of vermiculite and the ground basalt rock from falling out of the tube. The mixed rock-vermiculite substrates as well as pure vermiculite were sterilized by autoclaving for 1 h at 121°C. The chemical composition of basalt rock and vermiculite was analyzed using the Cameca SX50 electron microprobe and are shown in Table 1.

Seedlings

Seeds of *Alnus acuminata* were surface-sterilized in 30% H₂O₂ for 20 min and washed several times with sterilized distilled water. After sterilization, the seeds were placed on a moist filter paper in a petri-dish and exposed to light at room temperature until they germinated. The emerging seedlings were transplanted into the pots described above. Fresh nodules (clusters of modified lateral roots) with *Frankia* were harvested during faU from an *A. acuminata* forest in the Yungas, Argentina. The nodules were kept in a refrigerator for about one month before they were used as an inoculum. About 5 g of root nodules were gently crushed in a mortar and pestle. All treatments and controls were inoculated by pouring 1 ml of a well-fragmented inoculum suspension (approximately 30 mg fresh weight nodules) near the base of the seedlings.

Three weeks after *Frankia* inoculation, the seedlings were inoculated with spores of *Alpova austroalnicola* or *A. diplophloeus* depending on the treatments. The treatments received dual inoculation with *Frankia* and *A. austroalnicola* or *Frankia* and *A. diplophloeus*.

Table 1. Chemical composition of the basalt-rock and vermiculite (% of dry weight) used as substrates in the experiment.

Variable	Basalt-rock*	Vermiculite*
SiO ₂	72	33-36.5
Al ₂ O ₃	14	11-17
Fe ₂ O ₃	3	4-11
MgO	1	18-27
Na ₂ O	3	<0.5
K ₂ O	4	<0.1
CaO	1	<0.5
TiO ₂	0.3	<0.2
MnO	0.05	Not detected
P ₂ O ₅	0.16	Not detected

*Mean of 5 samples.

Table 2. Growth of *Alnus acuminata* inoculated with *Frankia* and *Alpova austroalnicola* and grown in soilless substrates.

Treatments	Height (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)	Number of nodule lobes / seedling	Nodule lobe dry weight (g)	ECM (%)
Control	1.57 ± 0.42d	0.013 ± 0.02c	17.80 ± 3.20b	0.031 ± 0.037b	3.57 ± 3.17c	5.2.10 ⁻⁴ ± 8.08 10 ⁻⁴ c	-
S	3.76 ± 2.04c	1.05 ± 0.864b	21.43 ± 1.43a	0.42 ± 0.33b	94.05 ± 52.43b	0.08 ± 0.03b	+/-
M	15.70 ± 1.41b	1.77 ± 0.82b	21.85 ± 0.85a	2.08 ± 0.86a	123.00 ± 41.14b	0.14 ± 0.08b	+/-
L	18.90 ± 2.49a	2.68 ± 0.41a	22.22 ± 1.12a	2.67 ± 0.61a	178.40 ± 26.41a	0.48 ± 0.08a	+++

Treatments: Control with vermiculite only; small rock particles (S, 250–500 µm); medium-sized rock particles (M, 500 µm – 1 mm); large rock particles (L, 1–3 mm). ECM measured as percentage of colonization on 100 roots (- 0%; +/- <10%; + 10–40%; ++ 40–75%; +++ 75–100%), nodulation measured as number of nodules lobes. Values in each column followed by different letters differ at $P < 0.05$ according to a Tukey test. Values are mean of 10 seedlings ± standard error for each treatment.

Table 3. Growth of *Alnus acuminata* inoculated with *Frankia* and *Alpova diplophloeus* and grown in soilless substrates.

Treatments	Height (cm)	Shoot dry weight (g)	Root length (cm)	Root dry weight (g)	Number of nodule lobes / seedling	Nodule lobe dry weight (g)	ECM (%)
Control	1.51 ± 0.29d	5.8.10 ⁻³ ± 8.9 10 ⁻⁴ d	12.48 ± 4.58b	0.011 ± 2.10 ⁻³ b	3.52 ± 1.84c	2.10 ⁻⁴ ± 1.9 10 ⁻⁴ c	-
S	7.1 ± 3.8c	1.15 ± 0.81c	22.41 ± 1.78a	1.95 ± 1.41a	142.4 ± 55.03b	0.35 ± 0.03b	+
M	17.4 ± 1.95a	3 ± 0.39a	22.53 ± 0.94a	2.50 ± 1.75a	283.2 ± 95.16a	0.59 ± 0.23a	++
L	13.24 ± 0.73b	2 ± 0.44b	22.96 ± 2.89a	2.1 ± 0.56a	194.00 ± 40.48b	0.40 ± 0.11b	+++

Treatments: Control with vermiculite only; small rock particles (S, 250–500 µm); medium-sized rock particles (M, 500 µm – 1 mm); large rock particles (L, 1–3 mm). ECM measured as percentage of colonization on 100 roots (- 0%; +/- <10%; + 10–40%; ++ 40–75%; +++ 75–100%), nodulation measured as number of nodules lobes. Values in each column followed by different letters differ at $P < 0.05$ according to a Tukey test. Values are mean of 10 seedling ± standard error for each treatment.

Sporocarps of *A. austroalnicola* were collected from an Andean alder forest in the Yungas (Nouhra et al., 2005); sporocarps of *A. diplophloeus* were collected from a red alder (*A. rubra* Bong.) forest near Florence, Oregon, USA. For each fungus, a spore suspension was prepared by homogenizing the sporocarps in distilled water in a Kenwood blender at a high-speed for about 3 min. One ml spore suspension containing 1×10^6 spores, determined by haematocytometry, was inoculated at the base of the seedlings.

Ten replicates were prepared for each treatment. Seedlings were grown in a greenhouse for 12 months with a 24/16°C (day/night) regime under a 16/8 h photoperiod, and watered every day. Water content of the growth substrates was kept constant by addition of water. No fertilization was provided to the seedlings. The pots from different treatments were systematically rotated to different bench positions once a week to minimize differences due to the location in the greenhouse.

Data collection

The formation of ECM was observed with a stereomicroscope. Description of the ECM followed the methodology of Agerer (1991, 1999), for describing color, mantle layers, branching pattern, emanating hyphae,

characteristics of rhizomorphs and cystidia. Mantle views were examined and photographed with a Zeiss AxioPhot light microscope at 200–1,000x magnification. Characterization of the Hartig net followed the nomenclature by Godbout and Fortin (1983). The quantification of the ECM colonization was estimated visually and characterized using five classes of mycorrhizal root colonization: - (0%); +/- (<10%); + (10–40%); ++ (40–75%); +++ (75–100%)(Schenk, 1982).

Seedling growth was measured as shoot height, root length (main root and first lateral roots) and as shoot dry weight and root dry weight after drying at 50°C to a constant weight. The capacity for nodulation was measured as number of nodule lobes and dry weights of nodule lobes after drying as above.

Data analyses

The variables shoot height, root length, number of nodule lobes and dry weights of shoot, roots and nodule lobes were analyzed by two-way analysis of variance and contrast test (Tukey, $P < 0.05$) to determine the significance of mean differences between treatments of basalt-rock and inoculation. Statistical analyses were performed with Infostat (2001).

3. Results

Growth

Alnus acuminata seedlings with dual *Frankia-Alpova austroalnicola* or *Frankia-A. diplophloeus* inoculation in vermiculite-basalt growth medium had significantly higher shoot height, dry weights of shoot and nodule lobes, and number of nodule lobes than the seedlings in vermiculite only (fig. 1A, B; Tables 2 and 3). With *Frankia* and *A. austroalnicola* inoculations, the seedlings cultivated in vermiculite mixed with large basalt particles had significantly higher shoot height, shoot dry weight, number of nodule lobes, and nodule lobe dry weight than in the treatments with small or medium-sized basalt particles (Fig. 1A, Table 2). However, *A. acuminata* seedlings inoculated with *Frankia* and *Alpova diplophloeus* and grown in the medium-sized basalt particles mixed with vermiculite produced significantly higher shoot dry weight, number of nodule lobes, and nodule lobes dry weight than in the treatments with small or large basalt particles (Fig. 1B, Table 3).

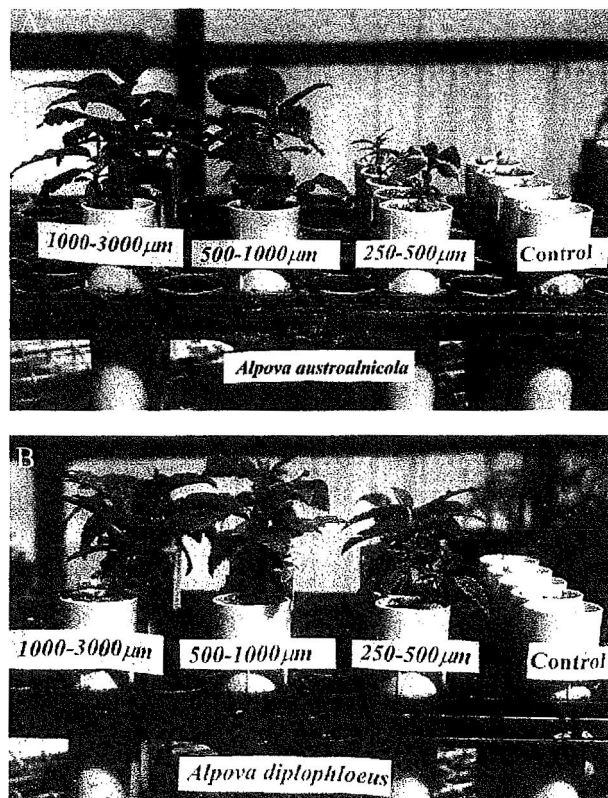


Figure 1. A: *Alnus acuminata*-*Frankia-Alpova austroalnicola* plants in different basalt-rock treatments. B: *Alnus acuminata*-*Frankia-Alpova diplophloeus* plants in different basalt-rock treatments. The numbers indicate particle size of basalt in mixture with vermiculite. Control denote vermiculite only.

A very low nodulation was observed in controls grown in vermiculite only as compared to all other treatments. This could be due to available phosphorus released from breakdown of basalt, by not from vermiculite (Table 1), by Andean alder.

Ectomycorrhizal formation

Ectomycorrhizal formation by *Alpova austroalnicola* and *A. diplophloeus* on *Alnus acuminata* were observed in all treatments except in the control. Abundant ECM in the mixture of large basalt particles and vermiculite were observed with both fungi (Tables 2 and 3; Fig. 2A and 3A). No other ECM types were seen on the plants.

Description of the morphotypes

Alpova austroalnicola-*Alnus acuminata*. Monopodial pinnate to irregular pinnate (fig. 2A), root tips straight, bent to tortuous, up to 10 mm long and 0.3-0.6 mm in diameter, yellowish brown to brown. Root tips are acute to cylindrical. Emanating hyphae: abundant, irregularly sinuous, membranaceously yellow to brown, branched, thin walled, 2-5 µm in diameter, regularly septate, clamped, surface rough with crystals; hyphal ends simple or inflated (Fig. 2B). Hyphae with simple anastomoses without septa (Agerer 1991, Type A). Rhizomorphs: hyaline to pale yellow, straight, up to 20 µm in diameter (Fig. 2C). Mycorrhiza mantle (plane view): mantle usually continuous over the root apex. Outer mantle layer plectenchymatous (Fig. 2D), with bundles of hyphae growing in parallel and forming a distinct ring-like pattern (Agerer and Rambold, 1998); surface rough, with few mineral particles and isodiametric to elliptic crystals; hyphae membranaceously yellowish, thin-walled, septa always with clamps, hyphae forming terminally globular inflations, 3-6 µm in diameter. Middle mantle layer plectenchymatous (Fig. 2E), hyphae arranged without pattern, 2-4 µm in diameter, cell walls thin. Inner mantle layer plectenchymatous (Fig. 2F), parallel hyphae running in parallel to root axis, 2-4 µm in diameter, clamps not observed. Mycorrhiza (cross section); mantle 50-90 µm thick (Fig. 2G), differentiated into a plectenchymatous outer layer of tangentially arranged hyphae 2-7 µm in diameter, a middle plectenchymatous layer of globose cells 2-5 µm in diameter, and a plectenchymatous inner layer of globose cells 1.2-5 µm in diameter; paraepidermal Hartig net, hyphae lined up in one row between epidermal cells 2-8 µm in diameter. Voucher specimen: ectomycorrhizas under *A. acuminata*, in herbarium A. Becerra AB 21 (CORD).

Alpova diplophloeus-*Alnus acuminata*. Monopodia] pinnate (Fig. 3A), root tips straight, bent to tortuous, up to 15 mm long and 0.4-0.9 mm in diameter, yellowish brown to brown; older mycorrhizae brown to dark brown. Root tips are cylindrical. Emanating hyphae: abundant,

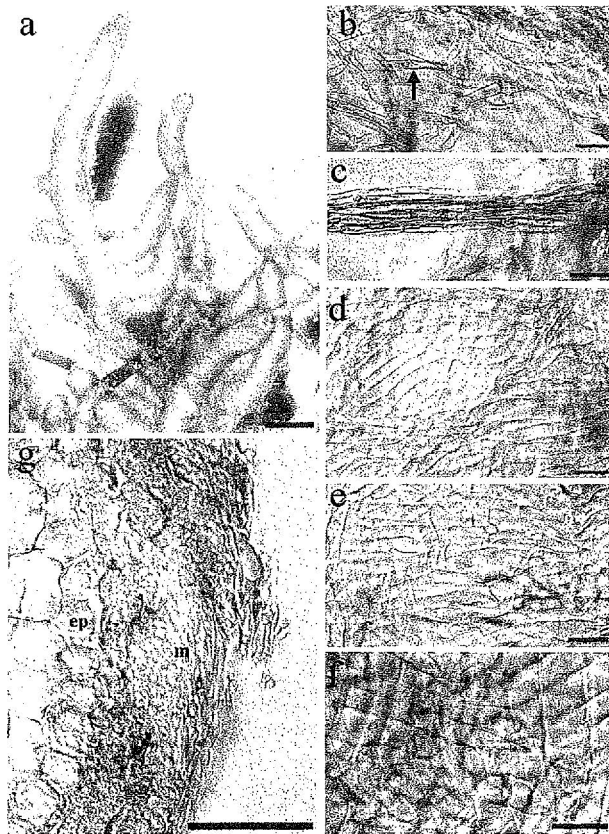


Figure 2. a: Light micrographs of *Alnus acuminata* ectomycorrhizas formed with *Alpova austroalnicola* in 1-3 mm sized basalt particles mixed with vermiculite. Bar = 5 mm. b: Emanating hyphae with inflated ends (white arrow) and crystals (black arrow). Bar = 10 µm. c: Rhizomorphs, Bar = 10 µm. d: Outer mantle layer plectenchymatous. Bar = 10 µm. e: Middle mantle layer plectenchymatous, Bar = 10 µm. f: Inner mantle layer plectenchymatous, Bar = 10 µm. g: Cross section showing the plectenchymatous mantle layers (m) and epidermal cells (ep). Bar = 50 µm.

irregularly sinuous, membranaceously yellow to brown, branched, thin walled, 2-6 µm in diameter, regularly septate, clamped, surface rough with crystals (Fig. 3B), hyphal ends simple or inflated. (Fig. 3C). Hyphae with simple anastomoses without septa (Agerer, 1991, Type A). Rhizomorphs: abundant, pale yellow, straight, upto 60 µm in diameter (Fig. 3D). Mycorrhiza mantle (plane view): mantle usually continuous over the root apex. Plectenchymatous outer mantle layer (Fig. 3E), membranaceously yellowish; surface rough, with few mineral particles and isodiametric to elliptic crystals; with bundles of hyphae growing in parallel and forming a distinct ring-like pattern (Agerer and Rambold, 1998), thin-walled, Clamped septa, hyphae forming terminally globular inflations, 3-9 µm in diameter. Middle mantle layer

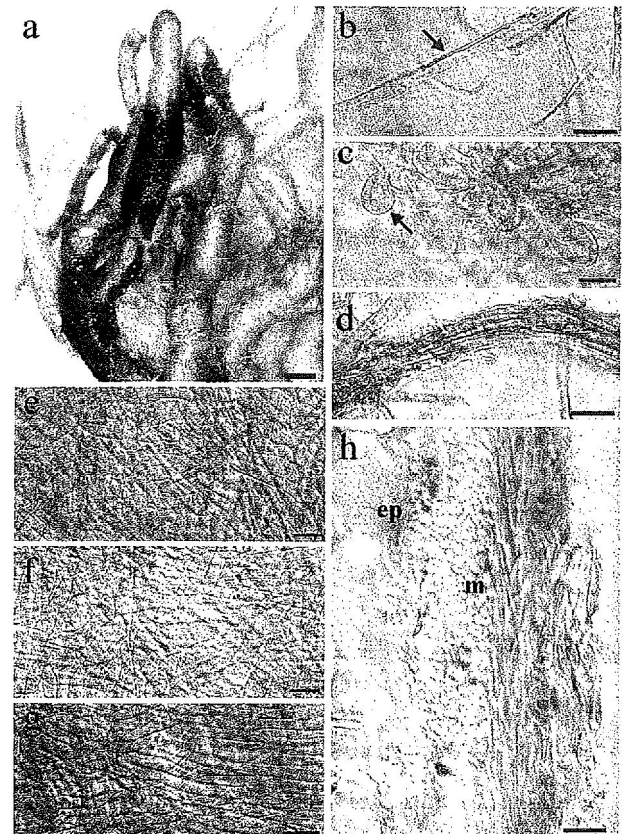


Figure 3. a: Light micrographs of *Alnus acuminata* ectomycorrhizas formed with *Alpova diplophloeus* in 1-3 mm sized basalt particles mixed with vermiculite. Bar = 5 mm. b: Emanating hyphae with crystals (arrow). Bar = 10 µm. c: Emanating hyphae with inflated ends (arrow). Bar = 10 µm. d: Rhizomorphs. Bar = 10 µm. e: Outer mantle layer plectenchymatous. Bar = 10 µm. f: Middle mantle layer plectenchymatous, Bar = 10 µm. g: Inner mantle layer plectenchymatous, Bar = 10 µm. h: Cross section showing the plectenchymatous mantle layers (m) and epidermal cells (ep). Bar = 10 µm.

plectenchymatous (Fig. 3F), hyphae arranged without pattern, 2-5 µm in diameter, cell walls thin. Inner mantle layer plectenchymatous (Fig. 3G), parallel hyphae running in parallel to root axis, 2-4 µm in diameter, clamps not observed. Mycorrhiza (cross section); mantle 40-90 µm thick (Fig. 3H), differentiated into a plectenchymatous outer layer of tangentially arranged hyphae 2-10 µm in diameter, a middle plectenchymatous layer of globose cells 2-7 µm in diameter, and a plectenchymatous inner layer of globose cells 2-7 µm in diameter; paraepidermal Hartig net, hyphae lined up in one row between epidermal cells 2-8 µm in diameter. Voucher specimen: ectomycorrhizas under *A. acuminata*, in herbarium A. Becerra AB 22 (CORD).

4. Discussion

The present study showed that *Alnus acuminata* grew, nodulated and formed ectomycorrhizas in soilless growth medium of vermiculite and ground basalt rocks. *Alnus acuminata* seedlings grew better in pots with large or medium-sized basalt particles than in pots with small particles. On the other hand, seedling growth of other *Alnus* species was significantly better in pots containing small particles size (0.2-0.6 mm) of basalt rock (Li and Bormann, 2003; Yamanaka et al., 2003). The potting mixtures used in the present study was basalt rock and vermiculite while Li and Bormann (2003) and Yamanaka et al. (2003) used basalt rock and perlite. Vermiculite is essentially a member of the phyllosilicate group of minerals, resembling mica in appearance. Mycorrhizal fungi or mycorrhizas are silicate-solubilizers, involved in nutrient cycling from relatively insoluble minerals (Leyval et al., 1990; Wätzel and Berthelin, 1990; Paris et al., 1995, 1996; Wallander et al., 2006; Balogh-Brunstad et al., 2008) due to their ability to release siderophores, oxalic acid and other non-volatile organic acids, which could enhance nutrient availability through solubilization and chelation of exchangeable or stable mineral resources. In this study, *Alnus acuminata* mycorrhizal plants were able to grow in a basalt rock-vermiculite mixture. The nutrients released from both primary minerals in pots of basalt-vermiculite mixture would enhance more seedling growth than the seedlings grown in pots with vermiculite alone. The vermiculite effects could shift preference of *A. acuminata* to large or medium basalt particles sizes, inducing a rapid and deep depletion of K in basalt and releasing other nutrients (Hinsinger et al., 1992).

Ectomycorrhizal formation by *Alpova austroalnicola* or *A. diplophloeus* were abundant in the large basalt particle treatment when inoculated together with *Frankia* and resulted in larger and healthier *Alnus acuminata* plants (Tables 2 and 3). This result is in accordance with the findings by Yamanaka et al. (2003).

In natural environments *Alnus acuminata* can reach a height of 25 m (average 10-15 m) with a stem diameter at breast height of 45 cm. Its annual height growth averages 1 m (Aceilolaza, 1995). Meanwhile, under laboratory conditions and in soilless substrates, *A. acuminata* seedlings grew up to 20 cm in a year. This is in agreement with other reports (Balza Viloria, 1959; Rojas et al., 1978).

The results of more nodule lobes in the bigger seedlings are in accordance with the findings by Rojas et al. (2001, 2002) in *A. rubra* and *Ceanothus velutinus* Dougl. which showed a positive correlation between seedling biomass and nodule biomass. One possible explanation could be the presence of phosphorus in the basalt-rock substrates, although in low amounts (Crawley 1). It has been shown that severe phosphate deficiency markedly impairs both host plant growth and symbiotic nitrogen fixation (Israel, 1987).

In this study, an increase of nodule lobes was observed in taller seedlings (which also showed longer and heavier roots). Another possible explanation for the results obtained is simply that N₂ fixation in the root nodules could be the only N source and that nodulation and plant growth are interrelated.

Alnus acuminata formed ectomycorrhizas with both *Alpova austroalnicola* and *A. diplophloeus*. In general, the *A. austroalnicola* ectomycorrhiza showed similar features to that of *A. diplophloeus*. The most conspicuous differences between the two were the mycorrhizal system and the differentiated rhizomorphs. In this study, the mantle and emanating hyphae of both ectomycorrhizae contained crystals. In contrast, Wiedmer and Senn-Irlett (2001) did not observe crystals in the mantle and emanating hyphae of *A. diplophloeus* ectomycorrhizae in *Alnus viridis*, *Alpova austroalnicola* and *A. diplophloeus* have earlier been found to be associated with *Alnus acuminata* in the Costa Rica and Yunga forests (Halling and Mueller, 2004; Nouhra et al., 2005) while the *Alpova austroalnicola*-*Alnus acuminata* mycorrhizae are characterized for the first time in this study. As far as we know, *Alpova diplophloeus*-*Alnus acuminata* ectomycorrhizae have not been described (De Roman et al., 2005) until this study. Their morphological characteristics were relatively similar to the ectomycorrhizae on *A. acuminata* and other *Alnus* species formed with *A. diplophloeus* (Brunner et al., 1990; Nouhra et al., 2003; Wiedmer and Senn-Irlett, 2001).

The results presented in this study showed that actinorhizal *Alnus acuminata* is able to grow in primary mineral substrates, indicating its capacity to breakdown primary minerals such as basalt rocks, thereby releasing nutrients available for uptake by the plant. It thus is able to survive and establish on highly degraded, nutrient-poor soil ecosystems (Li and Strzelczyk, 2000; Yamanaka et al., 2003). Its association with symbiotic rhizosphere microbes such as *Frankia* and mycorrhizal fungi can therefore have significant impact on belowground terrestrial processes involved in mobility and cycling of nutrients in nutrient-limited forest ecosystems. The presence of actinorhizal and mycorrhizal plants such as *A. acuminata* can, therefore, have significant impacts on soil fertility and productivity of forest ecosystems.

Acknowledgments

Technical assistance in the basalt rock analyses was provided by Geologist Fernanda Poklepovic. We are very grateful to Dr. Huss-Danell and two anonymous reviewers for the constructive suggestions on our manuscript. This research was supported by a doctoral position from CONICET to E.M., and the Deutsche Forschungsgemeinschaft (DFG), Germany to I. L. A.B. is researcher of CONICET.

REFERENCES

- Aceirolaza, P.G. 1995. Estructura y Dinamica de bosques de aliso (*Alnus acuminata* HBK subsp. *acuminata*) de la Provincia de Tucuman. PhD Thesis. Fac. de Ciencias Naturales e Instituto Miguel Lillo, National University of Tucuman, Argentina. 286 pp.
- Agerer, R. 1991. Characterization of ectomycorrhiza. In: *Techniques for the Study of Mycorrhiza. Methods in Microbiology*. Norris, J.R., Read, D.J., and Varma, A.K., eds. Academic, London, vol. 23, pp. 25-73.
- Agerer, R. 1999. Anatomical characteristics of identified ectomycorrhizas: an attempt towards a natural classification. In: *Mycorrhiza. Structure, Function, Molecular Biology and Biotechnology*. Vanna, A.X. and Hock, B., eds, 2nd edition. Springer, Berlin, Heidelberg, New York, pp. 633-682.
- Agerer, R. and Rambold, G. 1998. DEEMY, a DELTA-based information system for characterization and Determination of Ectomycorrhizae Version 1.1. Section Mycology, Institute for Systematic Botany, University of Munich.
- Balogh-Brunstad, Z., Keller, C.K., Dickinson, J.T., Stevens, F., Li, C.Y., and Bormann, B.T. 2008. Biotite weathering and nutrient uptake by ectomycorrhizal fungus, *Suillus tomentosus*, in liquid culture experiments. *Geochimica Cosmochimica Acta* 72: 2601-2618.
- Balza Viloria, M. 1959. Estudio sobre el aliso y su regeneración natural en el Valle del Alto Chama. Facultad de Ciencias Forestales, University of Los Andes. Venezuela.
- Becerra, A., Daniele, G., Dominguez, L., Nouhra, E., and Horton, T. 2002. Ectomycorrhizae between *Alnus acuminata* HBK. and *Naucoria escharoides* (Fr.:Fr.) Kummer from Argentina. *Mycorrhiza* 12: 61-66.
- Becerra, A., Nouhra, E., Daniele, G., Dominguez, L., and McKay, D. 2005a. Ectomycorrhizas of *Cortinarius helodes* and *Gyrodon monticola* with *Alnus acuminata* from Argentina. *Mycorrhiza* 15: 7-15.
- Becerra, A., Pritsch, K., Arrigo, N., Palma, M., and Bartoloni, N. 2005b. Ectomycorrhizal colonization of *Alnus acuminata* Kunth in northwestern Argentina in relation to season and soil parameters. *Annals of Forest Science* 62: 325-332.
- Becerra, A., Beenken, L., Pritsch, K., Daniele, G., Schloter, M., and Agerer, R. 2005c. Anatomical and molecular characterization of *Lactarius aff. omphaliformis*, *Russula alnijorullensis* and *Cortinarius tucumanensis* ectomycorrhizae on *Alnus acuminata*. *Mycologia* 97: 1047-1057.
- Bormann, B.T., Wang, D., Bormann, F.H., Benoit, G., April, R., and Snyder, M.C. 1998. Rapid, plant induced weathering in an aggrading experimental ecosystem. *Biogeochemistry* 43: 129-155.
- Brunner, I.L., Brunner, F., and Miller, O.K. 1990. Ectomycorrhizal synthesis with Alaskan *Alnus tenuifolia*. *Canadian Journal of Botany* 68: 761-767.
- Cervantes, E. and Rodriguez Barrueco, C. 1992. Relationships between the mycorrhizal and actinorhizal symbioses in non-legumes. In: *Methods in Microbiology*. Norris, J.R., Read, D.J., and Varma, A.K., eds. Academic Press, London, vol. 24, pp. 411-432.
- De Roman, M., Claveria, V. and De Miguel, A.M. 2005. A revision of the descriptions of ectomycorrhizas published since 1961. *Mycological Research* 109: 1063-1104.
- Godbout, C. and Fortin, I.A. 1983. Morphological features of synthesized ectomycorrhizae of *Alnus crispa* and *Alnus rugosa*. *New Phytologist* 94: 249-262.
- Grau, A. 1985. La expansión del aliso del cerro (*Alnus acuminata* HBK. subsp. *acuminata*) en el noroeste de Argentina. *Lilloa* 36: 237-247.
- Halling, R.E. and Mueller, G.M. 2004. *Common Mushrooms of the Talamanca Mountains, Costa Rica*. The New York Botanical Garden Press.
- Hinsinger, P., Jaillard, B. and Dufey, J.E. 1992. Rapid weathering of a trioctahedral mica by roots of ryegrass, *Soil Science Society of America Journal* 56: 977-982.
- Infostat. 2001. Programa de análisis estadísticos, Catedra de Estadística, Facultad de Ciencias Agropecuarias, National University of Cordoba.
- Israel, D.W. 1987. Investigation of the role of phosphorus in symbiotic dinitrogen fixation. *Plant Physiology* 84: 835-840.
- Lapeyrie, F., Ranger, J., and Vairelles, D. 1991. Phosphate-solubilizing activity of ectomycorrhizal fungi *in vitro*. *Canadian Journal of Botany* 69: 342-346.
- Leyval, C., Laheurte, P., Belgy, G., and Berthelin, J. 1990. Weathering of micas in the rhizospheres of maize, pine and beech seedlings influenced by mycorrhizal and bacterial inoculation. *Symbiosis* 9: 105-109.
- Li, C.Y. and Strzelczyk, E. 2000. Belowground microbial processes underpin forest productivity. *Phytan* 40: 129-134.
- Li, C.Y. and Bormann, B.T. 2003. Weathering of basalt rock by *Ted alder* (*Alnus rubra* Bong.) inoculated with *Frankia* and ectomycorrhizal fungus *Alpova diplophloeus*. 103rd General Meeting of the American Society for Microbiology, May 18-22, Washington, DC. p. 426.
- Massicotte, R.B., Peterson, R.T., Ackerley, C.A., and Piche, Y. 1986. Structure and ontogeny of *Alnus crispa* + *Alpova diplophloeus* ectomycorrhizae. *Canadian Journal of Botany* 64: 177-192.
- Miller, S.L., Koo, C.D., and Molina, R. 1991. Characterization of *Ted alder* ectomycorrhizae: a preface to monitoring below ground ecological responses. *Canadian Journal of Botany* 69: 516-531.
- Molina, R. 1979. Pure culture synthesis and host specificity of *Ted alder* ectomycorrhizae. *Canadian Journal of Botany* 57: 1223-1228.
- Moser, M. 2001. Some aspects of *Cortinarius* associated with *Alnus*. *Journées Européennes du Cortinaire* 3: 47-101.
- National Academy of Sciences. 1984. Bspecies para leña; arboles y arbustos para la producción de energía. CATIE, Turrialba, Costa Rica, pp. 343.
- Nouhra, E., Dominguez, L., Becerra, A., and Mangeaud, A. 2003. Clonización micorrízica y actinorrízica en plantines de *Alnus acuminata* (Betulaceae) cultivados en suelos nativos de *Alnus rubra*. *Boletín de la Sociedad Argentina de Botánica* 38: 199-206.
- Nouhra, E., Dominguez, L., Becerra, A., and Trappe, J. 2005. Morphology, molecular analysis and some ecological aspects of the hypogeous fungi, *Alpova austroalnicola* sp. nov. *Mycologia* 97: 598-604.
- Paris, F., Bonnaud, P., Ranger, J., and Lapeyrie, F. 1995. *In vitro* weathering of phlogopite by ectomycorrhizal fungi. I. Effect of TC⁺ and Mg²⁺ deficiency on phyllosilicate evolution. *Plant and Soil* 177: 191-201.
- Paris, F., Botton, S., and Lapeyrie, F. 1996. *In vitro* weathering of phlogopite by ectomycorrhizal fungi. II. Effect of K⁺ and Mg²⁺ deficiency and N sources on accumulation of oxalate and W. *Plant and Soil* 179: 141-150.
- Perry, D.A., Molina, R., and Amaranthus, M.P. 1987. Mycorrhizae, mycorrhizospheres, and reforestation: current

- knowledge and research needs. *Canadian Journal of Forest Research* 17: 929-940.
- Pritsch, K. 1996. Untersuchungen zur Diversität und Ökologie von Mykorrhizen der Schwarzerle [*Alnus glutinosa* (L.) Gaertn.]. PhD Thesis, University of Tübingen, Germany.
- Pritsch, K., Boyle, E., Munch, J.C., and Buscot, F. 1997. Characterization and identification of black alder ectomycorrhizas by PCR-RFLP analyses of the rDNA internal transcribed spacer (ITS). *New Phytologist* 137: 357-369.
- Raithelhuber, I. 1988. *Flora Mycologica Argentina*. Hongos II. MYCOSUT, ed. pp. 1-287.
- Rojas, H., Macía, F., and Lastra, I.A. 1978. Monografía del *Alnus jorullensis* H.B.K. *Colombia Forestal*: 5-22.
- Rojas, N.S., U. C.Y., Perry, D.A., and Ganio, L.M. 2001. *Frankia* and nodulation of red alder and snowbrush grown on soils from Douglas-fir forests in the H.J. Andrews experimental forest of Oregon. *Applied Soil Ecology* 17: 141-149.
- Rojas, N.S., U. C.Y., Perry, D.A., and Ganio, L.M. 2002. Interactions among soil biology, nutrition, and performance of actinorhizal plant species in the H.J. Andrews experimental forest of Oregon. *Applied Soil Ecology* 19: 13-26.
- Roy, S., Khalsa, D.P., and Greer, C.W. 2007. Combining alders, *Frankia* and mycorrhizae for the revegetation and remediation of contaminated ecosystems. *Canadian Journal of Botany* 85: 237-251.
- Schenk, N.C. 1982. *Methods and Principles of Mycorrhizal Research*. The American Phytopathological Society, St. Paul.
- Singer, R. and Morello, J.H. 1961. Ectotrophic forest tree mycorrhizae and forest communities. *Ecology* 41: 549-551.
- Smith, S.E. and Read, D.J. 1997. *Mycorrhizal Symbiosis*, 2nd Edition, Academic Press, London.
- Wallander, H., Hagerberg, D., and Aberg, G. 2006. Uptake of ⁸⁷Sr from microclimate and biotite by ectomycorrhizal fungi in a Norway spruce forest. *Soil Biology and Biochemistry* 38: 2487-2490.
- Wattcau, F. and Berthelin, J. 1990. Iron solubilization by mycorrhizal fungi producing siderophores. *Symbiosis* 9: 59-67.
- Wiedmer, E. and Senn-Irlet, B. 2001. *Alpova diplophloeus* (Zeller & Dodge) Trappe & A. H. Smith + *Alnus viridis* (Chaix) DC. *Descriptions of Ectomycorrhizae* 5: 1-8.
- Yamanaka, T., Li, C.Y., Bormann, B.T., and Okabe, H. 2003. Tripartite associations in an alder: effects of *Frankia* and *Alpova diplophloeus* on the growth, nitrogen fixation and mineral acquisition of *Alnus tenuifolia*. *Plant and Soil* 254: 179-186.