



Original article

Pseudotsuga menziesii invasion in native forests of Patagonia, Argentina: What about mycorrhizas?



María Eugenia Salgado Salomón^{a,c,*}, Carolina Barroetaveña^{a,b,c,1,2},
Mario Rajchenberg^{a,b,c,1,2}

^aIngeniería Forestal Universidad Nacional de la Patagonia S.J. Bosco, Sarmiento 849, Esquel, Chubut, Argentina

^bCentro Forestal CIEFAP, CC 14, 9200 Esquel, Chubut, Argentina

^cConsejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Article history:

Received 24 May 2012

Accepted 31 January 2013

Available online 19 March 2013

Keywords:

Ectomycorrhizas

Endomycorrhizas

DSE

Invasive species

Nothofagus forests

P. menziesii

Soil features

ABSTRACT

Pseudotsuga menziesii is one of the most widely planted conifers in the Patagonian Andes of Argentina, with invading characteristics that are widely reported. Nevertheless, little is known about the role of its obligate mycorrhizal associations in limiting or fostering the establishment of invading seedlings. We studied the richness and abundance of endo- (AM) and ectomycorrhizae (EM) present in *P. menziesii* seedlings growing in six *Nothofagus* forests invaded by *P. menziesii* seedlings (*Nothofagus* + *P. menziesii*) matrices. One transect along the maximum effective recruitment distance (ERA) was established at each site in order to wrench seedlings and sample soils. *P. menziesii* showed effective associations with a wide range of mycorrhizal symbionts: AM (ranging between 13.21 and 37.11%), EM (ranging between 79.91 and 89.14%) and Dark Septate Endophytes (DSE). Seedlings' mycorrhization percentages were always high, suggesting a good nursery effect provided by neighboring plantations. Mycorrhizal abundance (AM % and EM%), EM morphotypes richness and evenness showed significant differences between sites, indicating that *P. menziesii* displays a high plasticity being capable to select the more convenient mycorrhizal arrangement at each invaded site.

© 2013 Elsevier Masson SAS. All rights reserved.

1. Introduction

Pseudotsuga menziesii (Mirb.) Blanco is one of the most widely planted exotic Pinaceae in NW Patagonia, Argentina, valued for its timber quality and growth rate (Andenmatten et al., 2002). This activity has been encouraged by state financial support in order to create a productive timber development in the region (Laclau et al., 1999). Plantations have been located along a latitudinal gradient from Neuquén to Chubut provinces, preferably at sites with native forest boundaries, with sufficient rainfall and protected from heavy frost. This situation has begun to generate negative impacts on native vegetation by displacement or invasion of native forests given the invasive characteristics of *P. menziesii* (Orellana and Raffaele, 2010; Richardson et al., 2008; Sarasola et al., 2006).

Plant invasions threaten biodiversity conservation and incur large economic costs (Akter et al., 2011; Binimelis et al., 2007; Mack et al., 2000; Pimentel et al., 2005); however, why particular invasions succeed and others fail is often not well understood. It is well known that these processes are strongly influenced by environmental site conditions such as soil and climatic variables (Davies et al., 2000), that soil biota may promote invasion (Callaway et al., 2004; Klironomos, 2002; Mangla et al., 2008) and that facilitation by beneficial soil microbes such as mycorrhizas may directly control biological invasions (Richardson et al., 2000; Simberloff and Von Holle, 1999; Horton and van der Heijden, 2008).

Mycorrhizal fungi are known to play a major role in nutrient transfer and allocation (Ibijbjen et al., 1996; Hoffland et al., 2004; Landeweert et al., 2001; Simard et al., 1997a, 1997b; Smith and Read, 2008). Nevertheless, environmental factors such as temperature, soil moisture and nutrients content modulate mycorrhizal distribution (Brundrett, 1991). Key factors affecting the potential benefit of mycorrhizas in particular sites are the supply of soil Na, P and N (Abbott and Robson, 1991; Grove et al., 1991); it has been shown that excessive Na, P or N levels in soil inhibit mycorrhizal formation and restrict the activity of most mycorrhizal fungi (Juniper and Abbott, 1993; Malajczuk et al., 1990). The influence of

* Corresponding author. Ingeniería Forestal Universidad Nacional de la Patagonia S.J. Bosco, Sarmiento 849, Esquel, Chubut, Argentina. Tel./fax: +54 2945 450820.

E-mail addresses: mesalgadosalomon@gmail.com (M.E. Salgado Salomón), cbarroetavena@ciefap.org.ar (C. Barroetaveña), mrajchenberg@ciefap.org.ar (M. Rajchenberg).

¹ Tel./fax: +54 2945 450820.

² Tel./fax: +54 2945 453948.

soil water and temperature on mycorrhizal fungi is variable; it may affect fungal host-species combination, fungal spores germination, photosynthesis rate and development of the host plant (Entry et al., 2002).

Recent researches carried out in Patagonia (Argentina) have shown high abundance of ecto- (EM) and ectendo- (ECM) mycorrhizas in *P. menziesii* nurseries and planted seedlings (Barroetaveña, 2004; Barroetaveña and Rajchenberg, 2003; Barroetaveña et al., 2007) confirming their essential role in the establishment and growth of this species (Trappe and Strand, 1969; Wright, 1971). The presence of endomycorrhizas (AM) has also been reported by Cázares and Smith (1996) and Cázares and Trappe (1993) in native forests of the species, but they have not been studied in introduced *P. menziesii*. Though there is no evidence that AM/EM succession occurs, it is possible that AM fungi colonize cortical cells before EM fungi (Cázares and Smith, 1996). Because *Nothofagus* is an ectomycorrhizal genus (Singer and Morello, 1960), generalists AM fungi could be provided by the understory vegetation (Fontenla et al., 1998) and used by *P. menziesii* seedlings when invading native forests from Patagonia. Nevertheless, no studies have inquired into the role of native forest soils neighboring *P. menziesii* plantations as providers of their obligate mutualistic associations (mycorrhizas).

The aim of this work was to evaluate the mycorrhizal status of invasive *P. menziesii* seedlings growing in *Nothofagus* + *P. menziesii* matrices, to analyze the relationships between mycorrhizal status with soil and climatic variables, and to discuss the possible roles of mycorrhizas in the invasion process by *P. menziesii*.

2. Materials and methods

2.1. Sampling sites

The study was conducted at six sites with *Nothofagus* forests neighboring *P. menziesii* plantations showing invasion of the latter native forests of the former. The area is located at NW Patagonia (Argentina) in the deciduous forest District, Sub-Antarctic Province, Sub-Antarctic Domain (Cabrera and Willink, 1980). It presents perennial forests dominated by species of *Nothofagus dombeyi* (Mirb.) Oerst., *N. obliqua* (Mirb.) Oerst. or *N. nervosa* (Phil.) Krasser, accompanied mainly by *Luma apicula* (DC.) Burret, *Chusquea culeou* E. Desv., *Berberis* sp. and *Schinus patagonicus* (Phil.) I. M. Johnston. ex Cabrera. For details on sampling sites features see Table S1 (supplementary data online).

2.2. Seedling and soil sampling

A transect was established at each site, along the maximum effective recruitment distance (ERA) (Sarasola et al., 2006). At each site 25 invasive *P. menziesii* seedlings between 2 and 6 years old were selected, wrenched and kept in plastic bags. Additionally, soil samples were obtained and homogenized in order to obtain a composite soil sample per site.

Soil samples were air-dried and sieved (2 mm). Features of the ≤ 2 mm fraction were analyzed as follows: Soil pH (SpH, Bailey, 1943), Total nitrogen content (TN, Kjeldhal method, Bremner, 1960; Bremner and Mulvaney, 1982), Organic matter content (OM, Davies, 1974), exchangeable cations including Ca (CaC), Mg, K and Na (Schollenber and Simon, 1945), Electrical conductivity (EC, Blakemore et al., 1987), and Available phosphorous (P, Bray and Kurtz, 1945). The annual rainfall and mean temperature at each site were obtained from the database of the Argentinean National Weather Service (Servicio Meteorológico Nacional, 2010).

Seedlings measurements included stem height (H, from apex to collar), stem collar diameter (CD, measured at ground level), seedling root system length (RL, sum of principal and lateral roots lengths).

2.3. Mycorrhizal status evaluation

AM and EM colonization percentages for each seedling were estimated following Brundrett et al. (1996), using the complete root system. Ectomycorrhizal colonization percentage (%EM) was estimated as:

$$\%EM = (\text{number of EM tips} / \text{total tips of the root system}) \times 100$$

Endomycorrhizal colonization percentage (%AM) was estimated by the grid intersect method (Brundrett et al., 1996), expressed as:

$$\%AM = (\text{number mycorrhizal intersects} / \text{total intersects}) \times 100$$

EM morphotypes were characterized, determined and classified according to Goodman et al. (1996), and the reference works by Barroetaveña et al. (2006, 2007), Agerer (1994, 2001) and Agerer and Rambold (2004–2010). Clearing and staining of roots to evaluate arbuscular mycorrhizas followed Cázares and Smith (1996) and Cázares and Trappe (1993).

EM richness was calculated as the number of different morphotypes per seedling. Morphotype richness (S) was calculated as the number of different morphotypes found at each site. EM morphotypes were used to estimate Shannon's diversity index (H' , Shannon and Weaver, 1949). Evenness index (E) was calculated following Pielou (1969):

$$E = H' / \ln(S)$$

The number of morphotypes equally abundant per site (SH) was estimated following Pla (2006):

$$SH = e^{H'}$$

2.4. Statistical analysis

AM and EM colonization percentages, and EM richness between sites were not normally distributed and variances were not homogeneous (Shapiro Wilk and Levene tests, respectively) (Everitt, 2005). Arcsine data transformation was performed. Differences in AM%, EM% and richness between sites were analyzed by using the generalized linear mixed models (GLMM) applying the restricted maximum likelihood estimation method with subsequent comparison with DGC test (exclusive groups formation test) (Di Rienzo et al., 2002) in R for R-DCOM in InfoStat (Di Rienzo et al., 2010).

Comparisons of EM richness, S, H' , E and SH among sites were analyzed with Kruskal Wallis test.

To further analyze the relationships between site variables and mycorrhizal status, a Spearman correlation test (r) was performed, including mean temperature (MT), annual rainfall (PP), soil parameters (SpH, TN, OM, CaC, Mg, K, Na, EC and P) and EM%, AM%, H' , E, SH, and S.

All analyses were performed at 0.05 significance level with the statistical package InfoStat for Windows, version 2011 (Di Rienzo et al., 2011).

3. Results

3.1. EM status

EM colonization percentage was very high at all sites, ranging between 79.91 and 89.14% (Fig. 1). However, there were significant differences between two groups of sites ($df = 5$; $F = 4.79$;

$p = 0.0005$, DGC test), with Corcovado, Est. Newmeyer and Victoria Island presenting significant lower values, than Est. Quechuquina, Foyel and ENFORSA.

3.2. AM status

Values of AM% ranged between 13.21 and 37.11% (Fig. 1). A general analysis showed that the study sites presented significant differences in AM% with sites forming three groups ($df = 5$; $F = 9.62$; $p < 0.0001$, DGC test), one including Corcovado with the highest AM% value, another with Est. Quechuquina with the lowest AM% value, and the other group with intermediate values formed by the remaining four sites (Fig. 1).

During AM analysis, a constant presence of Dark Septate Endophytes (DSE) was detected in seedlings from all sites.

3.3. EM morphotypes richness

Individual seedling richness ranged between 2.9 and 4.5 morphotypes per seedling (Fig. 1) and showed that there were significant differences between two groups of sites ($df = 5$; $F = 6.99$; $p < 0.0001$, DGC test) Corcovado and Est. Newmeyer with higher values and Est. Quechuquina, ENFORSA, Foyel and Victoria Island with lower values (Fig. 1).

Morphotype richness ranged between 5 and 12 different morphotypes per site, and showed significant differences between sites ($H = 1390.96$; $n = 250$; $p < 0.0001$, Kruskal Wallis test), Corcovado and Est. Newmeyer with the highest values, Foyel with the lowest value, while Victoria Island, ENFORSA and Est. Quechuquina formed an intermediate group (Figs. 2 and 3).

Shannon's diversity index showed significant differences between sites ($p < 0.0001$, Kruskal Wallis test) as did Evenness ($H = 1347.9$; $n = 250$; $p < 0.0001$, Kruskal Wallis test) (Fig. 3); while in Est. Newmeyer and Foyel EM morphotypes were more evenly distributed, Corcovado showed fewer but dominant morphotypes, and ENFORSA, Victoria Island and Est. Quechuquina formed a single intermediated group. The number of equally abundant morphotypes ranged from 3 to 5 morphotypes per site (Fig. 3).

3.4. Relationships between mycorrhizal status with soil and climatic variables

Soil analysis showed that PP, MT, SpH, OM, CaC, EC and TN differed between sites from a biological point of view based on reference values for the region (La Manna et al., in press) and were included in the correlation analysis; see Table S1 (supplementary data online) for details on soil features. Other variables such as P (varying from 6 to 7 mg/kg) and Na (varying from 138 to 230 ppm) were always low, K (varying from 168.13 to 402.73 ppm) and Mg (varying from 638.4 to 912 ppm) were always high, while soil texture was always sandy loam; therefore, none of them were included in the correlation analysis.

Spearman correlation analysis was consistent with the results shown by the GLMM. EM colonization percentage was weakly correlated with mean temperature ($r = 0.21$, $p = 0.02$, Spearman correlation analysis, Table 1) and soil pH ($r = -0.28$, $p = 0.0026$, Spearman correlation analysis, Table 1). AM colonization percentage was correlated more strongly with annual precipitation ($r = -0.47$, $p < 0.0001$, Spearman correlation analysis, Table 1) and N content ($r = 0.34$, $p = 0.0002$, Spearman correlation analysis, Table 1), and richness and Evenness were correlated with Ca content ($r = -0.81$, $p = 0.05$ and $r = -0.89$, $p = 0.05$, respectively, Spearman correlation analysis, Table 1).

4. Discussion

The occurrence of AM and EM in the same individual has been reported for many tree genera such as *Acacia*, *Alnus*, *Casuarina*, *Cupressus*, *Eucalyptus*, *Juniperus*, *Pinus*, *Populus*, *Salix*, *Tilia* and *Ulmus* (Becerra et al., 2005; Cázares and Smith, 1996; Cázares and Trappe, 1993; dos Santos et al., 2001; Horton et al., 1998; Molina et al., 1992; Smith et al., 1998; Wagg et al., 2008). In this work we report the co-occurrence of AM, EM and DSE in the same individuals of *P. menziesii* for the first time.

Collier and Bidartondo (2009) found a 'sit-and-wait' strategy for the mycorrhizae they studied, stating that trees invade first and later do mycorrhizas. Results of this work indicate that *P. menziesii*

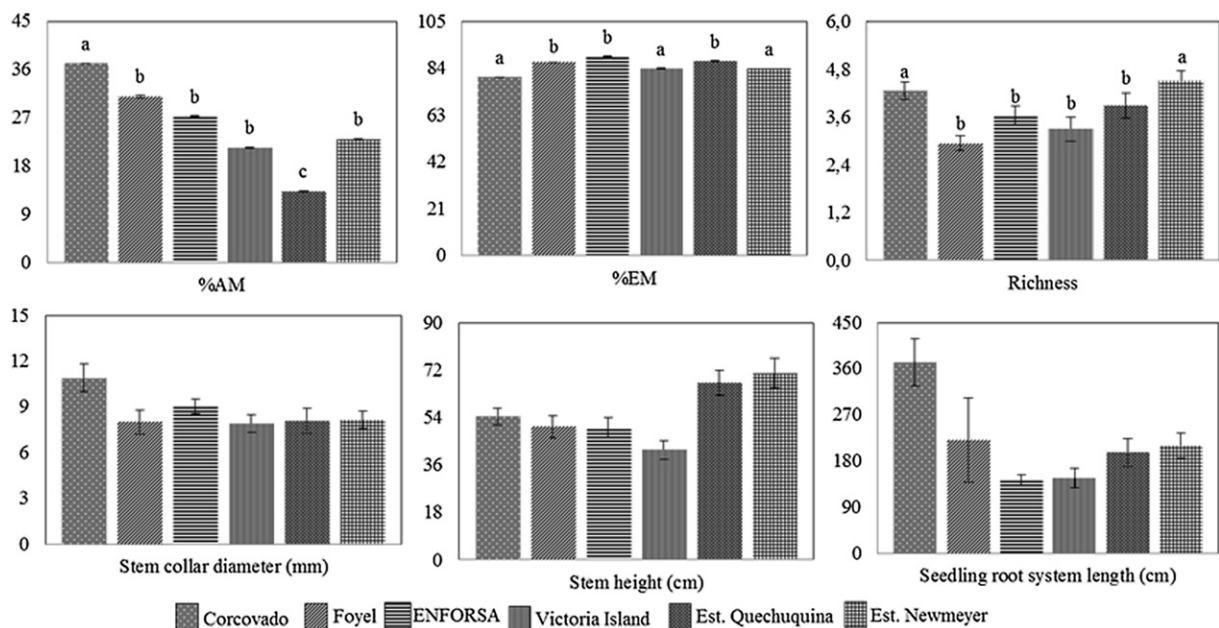


Fig. 1. Mean AM% and EM%, EM richness (expressed as number of different morphotypes per seedling) and seedlings features of *P. menziesii* found at each site. *Different letters means significantly differences between sites ($p = 0.05$).

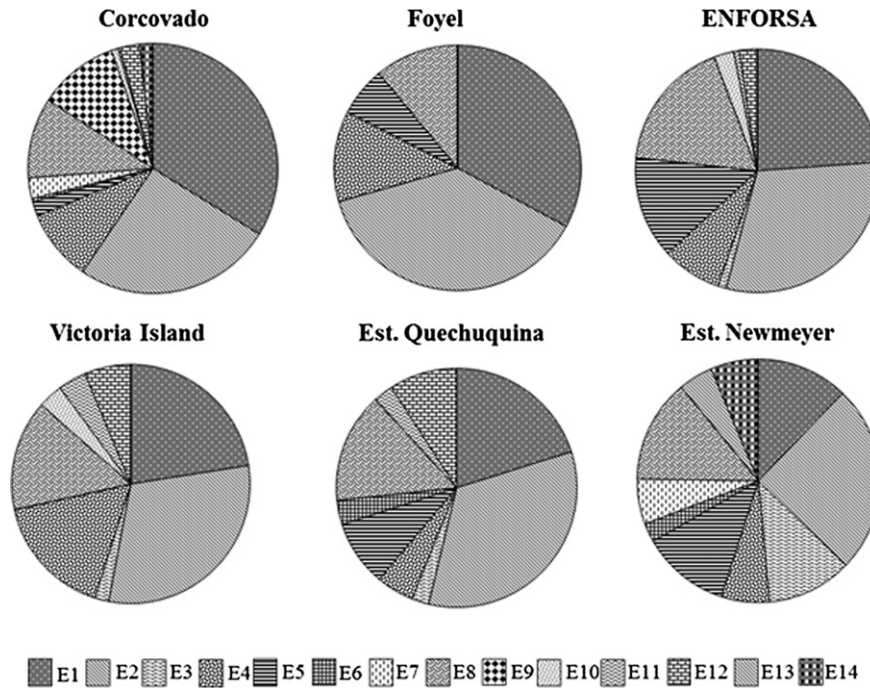


Fig. 2. Colonization rates of *P. menziesii* seedlings with different EM morphotypes (En).

forms AM and EM associations while invading, as it presents high abundance of both AM and EM associations in invading seedlings since 1 year old. These results also suggest that neighboring plantations provide a good nursery effect, at least regarding EM species, as it was demonstrated for *Pinus ponderosa* Dougl. ex Laws. plantations in Patagonia (Salgado Salomón, 2007; Salgado Salomón et al., 2011). Most of the EM species reported for *P. menziesii* plantations and nurseries from Patagonia are exotic for the region (Barroetaveña et al., 2006, 2007), with only two taxa with cosmopolitan distributions [*Thelephora terrestris* Ehrh and *Hebeloma*

mesophaeum (Pers.) Quél.] shared with *Nothofagus* forests (Singer, 1969).

Zhang et al. (2010) have hypothesized that ubiquity and presumed low level of host specificity of AM fungi determine that they play a minor role in mediating invasion of exotic plants. Nevertheless, it should be pointed out that such ubiquity and low specificity guarantee a functional mutualistic association to many kinds of plants including invading ones. The high levels of AM colonization found in invasive *P. menziesii* in this study support this idea, although their identity and the specific contribution to seedling

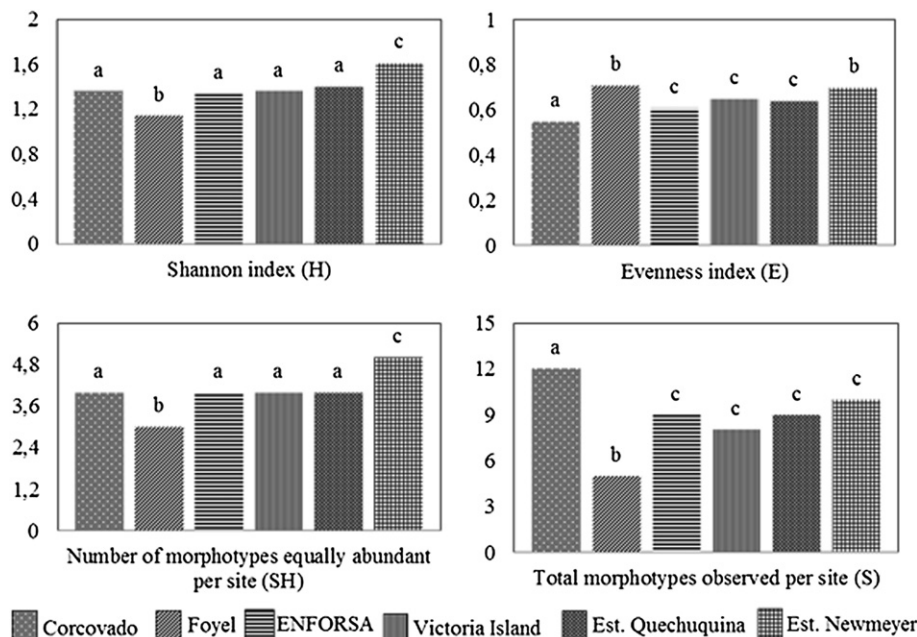


Fig. 3. Shannon index, Total observed morphotypes and evenness at each site *Different letters means significantly differences between sites ($p = 0.05$).

Table 1

Spearman correlation's coefficient between AM y EM colonization, Shannon index, evenness index, morphotype richness and environmental features.

	EM%	AM%	PP ^a	TM ^b	pHS ^c	OM ^d	CaC ^e	EC ^f	TN ^g	S _H ^h	S ⁱ	E ^j	H ^k
EM%	1	0.33	0.28	0.02	2.60E-03	0.92	0.31	0.07	0.55	0.31	0.04	0.34	0.1
AM%	-0.09	1	8.30E-08	8.50E-04	8.50E-04	3.10E-04	0.04	0.02	1.80E-04	0.57	3.40E-03	3.40E-03	0.1
PP	0.1	-0.47	1	1.00E-12	1.60E-09	1.00E-12	1.70E-03	1.50E-10	1.00E-12	3.10E-06	3.50E-03	2.90E-09	1.00E-12
TM	0.21	-0.31	0.64	1	1.10E-08	4.30E-03	0.95	1.00E-12	0.14	5.80E-05	0.44	7.40E-05	1.30E-06
pHS	-0.28	0.31	-0.53	-0.5	1	0.02	0.36	1.40E-12	0.13	1.10E-05	0.5	0.36	0.03
MO	1.00E-02	0.33	-0.61	-0.26	-0.21	1	1.00E-05	0.82	1.00E-12	2.20E-03	2.60E-09	1.00E-12	0.04
Ca	0.09	-0.19	0.29	0.01	0.09	-0.4	1	0.05	8.40E-05	0.01	1.00E-12	1.00E-12	1.20E-03
CE	0.17	-0.23	0.55	0.94	-0.6	0.02	-0.18	1	0.33	1.00E-12	2.20E-04	0.14	3.60E-11
CN	0.06	0.34	-0.68	-0.14	-0.14	0.96	-0.36	0.09	1	0.11	5.90E-08	1.90E-09	8.10E-04
SH	-0.1	-0.05	0.42	0.37	-0.4	0.28	-0.26	0.62	0.15	1	0	0.32	1.00E-12
S	-1.90E-01	0.27	-0.27	0.07	0.06	0.52	-0.81	0.34	0.48	0.62	1	0	4.60E-10
E	0.09	-0.27	0.52	0.36	0.09	-0.61	0.9	0.14	-0.52	-0.09	-0.66	1	0.97
H	-0.16	-0.15	0.62	0.43	-0.2	-0.19	-0.3	0.57	-0.31	0.85	0.54	-3.00E-03	1

^a PP: annual rainfall (mm).^b MT: mean temperature (°C).^c SpH: soil pH.^d OM: organic matter (%).^e CaC: soil calcium content (ppm).^f EC: electrical conductivity (ds).^g TN: total soil nitrogen content (%).^h S_H: Number of morphotype equally abundant per site (Shannon Index).ⁱ S: Total morphotype observed per site.^j E: Evenness index.^k H: Shannon index. Spearman correlation' coefficients are below the main diagonal, *p* values are above the main diagonal. Significant coefficients and *p* values of the variables are shaded.

establishment have to be determined. On the other hand, EM fungi are disproportionately represented in some families such as Pinaceae and Fagaceae in temperate and boreal forests (Baxter and Dighton, 2005), and *P. menziesii* is estimated to associate with 514 species of EM fungi over its entire range (Barroetaveña et al., 2007). The variation in fungal species associated to a single plant species portends a wide range of mycorrhizal functional attributes. In this study we have observed different mycorrhizal arrangements (AM and EM) in seedlings from different sites. Even with similar morphotype richness, sites differed in 'morphotypes selected' by plants, which accounted for the variety of possibilities that *P. menziesii* seems to have when invading *Nothofagus* forests. Moreover, almost all sites showed co-dominance of 4–5 morphotypes, but these were never the same (see Fig. 2).

The beneficial contributions of AM colonization increase with increasing plant moisture deficit (Entry et al., 2002), therefore the symbiosis is encouraged under these conditions. AM symbiosis may alleviate plant water deficit by several mechanisms, including increasing water uptake due to hyphae extraction of soil water (Auge et al., 1992; Davies et al., 1992), altering hormonal levels causing changes in stomatal conductance (Drüge and Schönbeck, 1992), increasing turgor by lowering leaf osmotic potential (Davies et al., 1993), improving nutrition of the host (Johnson and Hummel, 1985) and improving plant recovery after drought through maintenance of the soil-root continuum (Reid and Bowen, 1979; Sweatt and Davies, 1984). This could explain the significant (though weak), negative correlation between AM colonization percentages with annual rainfall found in this study. Evenness and richness were weakly and negatively correlated with Ca soil content, and a possible explanation is that Ca ions have been reported as antimicrobial compounds (Nout, 1992) and Mayor and Henkel (2006) found Ca concentration was higher when EM fungi were not present.

DSE have been reported for many conifers, mainly *Pinus* spp. (Cázares and Trappe, 1993; Grünig et al., 2008; Peterson et al., 2008; Usuki and Narisawa, 2007; Wagg et al., 2008) and in various environments (Mandyam and Jumpponen, 2005), but not for *P. menziesii*. There is little information regarding DSE functioning. Jumpponen (2001) summarizes the evidence for and

against DSE being a mycorrhizal association. An increase in shoot P was observed in tree species inoculated with DSE (Haselwandter and Read, 1982; Jumpponen et al., 2002), and it is possible that DSE fungi provide invading plants, such as *P. menziesii*, with access to more recalcitrant pools of C, N and P in litter and other detritus (Peterson et al., 2004). But other authors support the idea that DSE would be facultative biotrophic fungi because they are commonly found in naturally senescent roots (Caldwell et al., 2000; Jumpponen and Trappe, 1998; Robertson, 1954). In any case, the presence of microsclerotia that ensure the reproduction of these fungi in the ecosystem (Jumpponen and Trappe, 1998; Yu et al., 2001), as found in the *P. menziesii* seedlings in our study, indicates DSE were functional and active.

5. Conclusions

P. menziesii invasive seedlings are plastic regarding its mycorrhizal associations, able to form AM, EM and DSE in a single seedling. Proportions of the three associations, including different EM-morphotypes vary according to site conditions. Annual rainfall, soil pH, mean temperature and Ca were associated to the different mutualistic arrangements formed at each site. *P. menziesii* co-invasives with its symbionts and is successful in the establishment period of invasion during the first 5 years old.

Author contributions

Conceived and designed the experiments: MR, CB and MESS. Performed the experiments: MESS. Analyzed the data: MESS. Wrote the paper: MESS, CB and MR.

Acknowledgments

We are grateful to owners and plantations keepers for allowing us to work in their properties. Funding from CONICET through PIP 80101000 project, from FONCyT through PICT 2007-00493 and from Universidad Nacional de la Patagonia SJ Bosco through PI-750 are warmly acknowledged. We are grateful to Asociación Parques Nacionales, Patagonian Delegation for allowing the work in Nahuel

Huapi and Lanín National Parks. Lic. Andrés de Errasti, technician Juan Monges and Ing. Damián Sotto were most helpful during field work. Our sincere thanks to Dr. Laura Pla for her invaluable assistance with statistical analysis. Two anonymous reviewers and Dr. J. Shyloff are warmly thanked for constructive criticism and improvement of the ms. MESS is fellow and CB and MR are researchers for the National Research Council of Argentina (CONICET).

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.actao.2013.01.018>.

References

- Abbott, L.K., Robson, A.D., 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agriculture, Ecosystems and Environment* 35, 121–150.
- Agerer, R. (Ed.), 1994. *Colours Atlas of Ectomycorrhizae*. Einhorn-Verlag.
- Agerer, R., 2001. Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11, 107–114.
- Agerer, R., Rambold, G., 2004–2010. Deemy, an Information System for Characterization and Determination of Ectomycorrhizae. Available in: www.deemy.de.
- Akter, S., Kompas, T., Ward, M., 2011. Economic Consequences of Biological Invasions: The Impacts of Invasive Species Threats on Queensland's Bioregional Attributes. Environmental Economics Research Hub, Crawford School of Public Policy, The Australian National University. Environmental Economics Research Hub Research Reports 10105.
- Andenmatten, E., Gonda, H.E., Letorneau, F., Cortez, G.O., Bava, J.O., 2002. Proyección de la distribución diamétrica en plantaciones de pino ponderosa en la provincia de Neuquén. Informe final. Proyecto PIA 15/58. SAGPyA, Argentina, p. 29.
- Auge, R.M., Stodola, A.J.W., Brown, M.S., Bethlenfalvy, G.J., 1992. Stomata² response of mycorrhizal cowpea and soybean to short-term osmotic stress. *New Phytologist* 120, 117–125.
- Bailey, W., 1943. Soil science 55,143. In: Jackson, M.L., 1970. *Análisis químico de suelos*. Ediciones Omega S.A. Barcelona.
- Barroetaveña, C., 2004. Estudio de las Ectomicorizas de plantines y plantaciones de pino ponderosa (*Pinus ponderosa* Doug. ex Laws.) y pino oregón (*Pseudotsuga menziesii* (Mirb.) Blanco) en la región Andino Patagónica. Tesis para optar al título de Doctor en Ciencias Biológicas, UN del Comahue, CRUB, San Carlos de Bariloche, Octubre de 2004. p. 225.
- Barroetaveña, C., Rajchenberg, M., 2003. Las micorrizas y la producción de plántulas de *Pseudotsuga menziesii* (Mirb.) Franco en la Patagonia, Argentina. *Bosque* 24, 3–15.
- Barroetaveña, C., Cázares, E., Rajchenberg, M., 2007. Ectomycorrhizal fungi associated with ponderosa pine and Douglas-fir: a comparison of species richness in native western North American forests and Patagonian Plantations from Argentina. *Mycorrhiza* 17, 355–373.
- Barroetaveña, C., Rajchenberg, M., Cázares, E., 2006. Mycorrhizal fungi of *Pseudotsuga menziesii* an introduced tree species in Central Patagonia (Argentina). *Nova Hedwigia* 83, 53–66.
- Baxter, J.W., Dighton, J., 2005. Diversity-functioning relationships in ectomycorrhizal fungal community. In: Dighton, J. (Ed.), *The Fungal Community: Its Organization and Role in the Ecosystem*, third ed. CRC Press, USA, pp. 383–398.
- Becerra, A., Zak, M.R., Horton, T.R., Micolini, J., 2005. Ectomycorrhizal and arbuscular mycorrhizal colonization of *Alnus acuminata* from Calilegua National Park (Argentina). *Mycorrhiza* 15, 525–531. <http://dx.doi.org/10.1007/s00572-005-0360-7>.
- Binimelis, R., Born, W., Monterroso, I., Rodríguez-Labajos, B., 2007. Socio-economic impact and assessment of biological invasions. *Ecological Studies Biological Invasions* 6, 331–347.
- Blakemore, L.C., Searle, P.L., Daly, B.K., 1987. *Methods for Chemical Analysis of Soils*. Lower Hutt, NZ. In: Soil Bur. Sci. Rep NZ, p. 80.
- Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic and available forms of phosphorus in soils. *Soil Science* 59, 39–45.
- Bremner, J.M., 1960. Determination of Nitrogen in soil by the Kjeldahl method. *Journal of Agricultural Science* 55, 1–23.
- Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen total. In: Page, et al. (Eds.), *Methods of Soil Analysis*, second ed. Part 2 Agronomy 9 Am. Soc. of Agron., Inc., Madison, Wis, pp. 595–624.
- Brundrett, M.C., 1991. Mycorrhizas in natural ecosystems. In: *Advances in Ecological Research*, Vol. 21. Academic Press, London, pp. 171–313.
- Brundrett, M., Bougher, N., Grove, T., Malajczuk, N., 1996. Working with Mycorrhizas in Forestry and Agriculture. Monograph 32. Australian Center for International Agricultural Research, Canberra, Australia, p. 374.
- Cabrera, A.L., Willink, A., 1980. Biogeografía de América Latina. Monografía 13. In: Serie de Biología. The General Secretariat of the Organization of American States, Washington, DC, p. 122.
- Caldwell, B.A., Jumpponen, A., Trappe, J.M., 2000. Utilization of major detrital substrates by dark-septate root endophytes. *Mycologia* 92, 230–232.
- Callaway, R.M., Thelen, G.C., Rodriguez, A., Holben, W.E., 2004. Soil biota and exotic plant invasion. *Nature* 427, 731–733.
- Cázares, E., Trappe, J.M., 1993. Vesicular endophytes in roots of Pinaceae. *Mycorrhiza* 2, 153–156.
- Cázares, E., Smith, J.E., 1996. Ocurrente of vesicular-arbuscular mycorrhizae in *Pseudotsuga menziesii* and *Tsuga heterophylla* seedlings grown in Oregon coast range soil. *Mycorrhiza* 6, 65–67.
- Collier, F.A., Bidartondo, M.I., 2009. Waiting for fungi: the ectomycorrhizal invasion of lowland heathlands. *Journal of Ecology* 97, 950–963. <http://dx.doi.org/10.1111/j.1365-2745.2009.01544.x>.
- Davies, A.D., Grime, D.J.P., Thompson, K., 2000. Fluctuating resources in plant communities: a general theory of invisibility. *Journal of Ecology* 88, 528–534.
- Davies, B.E., 1974. Loss-on ignition as an estimate of soil organic matter. *Soil Sci. Proc.* 38, 150.
- Davies Jr., F.T., Potter, J.R., Linderman, R.G., 1992. Mycorrhiza and repeated drought exposure affect drought resistance and extra-radical hyphae development of pepper plants independently of plant size and nutrient content. *Journal of Plant Physiology* 139, 289–294.
- Davies Jr., F.T., Potter, J.R., Linderman, R.G., 1993. Drought resistance of mycorrhizal pepper plants independent of leaf P concentration—response in gas exchange and water relations. *Physiologia Plantarum* 87, 45–53.
- Di Rienzo, J.A., Guzman, A.W., Casanoves, F., 2002. A multiple comparisons method based on the distribution of the root node distance of a binary tree obtained by average linkage of the matrix of euclidean distances between treatment means. *JABES* 7 (2), 129–142.
- Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W., 2011. *InfoStat Versión 2011*. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL: <http://www.infostat.com.ar>.
- Di Rienzo, J.A., Macchiavelli, R.E., Casanoves, F., 2010. *Modelos Mixtos en InfoStat*. Manual del Usuario. Editorial Brujas, Córdoba, Argentina.
- dos Santos, V.L., Muchovej, R.M., Borges, A.C., Neves, J.C.L., Kasuya, M.C.M., 2001. Vesicular-arbuscular-/ecto-mycorrhiza Succession in seedlings of *Eucalyptus* spp. *Brazilian Journal of Microbiology*. ISSN: 1517-8382 32. ISSN: 1517-8382, 81–86.
- Drüge, U., Schönbeck, F., 1992. Effect of vesicular-arbuscular mycorrhizal infection on transpiration, photosynthesis and growth of flax (*Linum usitatissimum* L.) in relation to cytokinin levels. *Journal of Plant Physiology* 141, 40–48.
- Entry, J.A., Rygielwicz, P.T., Watrud, L.S., Donnelly, P.K., 2002. Arbuscular mycorrhizal response to adverse soil conditions. In: Sharma, A.K., Johri, B.N. (Eds.), *Arbuscular Mycorrhizae: Interactions in Plants, Rhizosphere and Soils*. Science Publishers, Inc, Enfield (NH), Plymouth, USA, pp. 135–159.
- Everitt, B.S., 2005. *An R and S-PLUS Companion to Multivariate Analysis*. British Library Cataloguing in Publication Data. © Springer-Verlag London Limited, USA.
- Fontenla, S., Godoy, R., Rosso, P., Havrylenko, M., 1998. Root associations in Austrocedrus forests and seasonal dynamics of arbuscular mycorrhizas. *Mycorrhiza* 8, 29–33.
- Goodman, D.M. (Ed.), 1996. *Concise Descriptions of North American Ectomycorrhizae*. Mycologue publications & Canada-B.C. Forest Res. Dev. Agreement. Canadian Forest Service, Victoria, Canada.
- Grove, T.S., Malajczuk, N., Burgess, T., Thomson, B., Hardy, G., 1991. Growth responses of plantation *Eucalyptus* to inoculation with selected ectomycorrhizal fungi. Durban, South Africa. In: Schönau, A.P.G. (Ed.), *Symposium on Intensive Forestry: The Role of Eucalyptus*, pp. 86–93.
- Grünig, C.R., Queloz, V., Sieber, T.N., Holdenrieder, O., 2008. Dark septate endophytes (DSE) of the *Phialocephala fortinii* s.l.-*Acephala applanata* species complex in tree roots: classification, population biology and ecology. *Botany* 86, 1355–1369.
- Haselwandter, K., Read, D.J., 1982. The significance of a root fungus association in two *Carex* species of high-alpine plant communities. *Oecologia* 53, 352–354.
- Hoffland, E., Kuypers, T.W., Wallender, H., 2004. The role of fungi in weathering. *Frontiers in Ecology and the Environment* 2, 258–264.
- Horton, T.R., Cázares, E., Bruns, T.D., 1998. Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. *Mycorrhiza* 8, 11–18.
- Horton, T.R., van der Heijden, M.G.A., 2008. The role of symbiosis in seedling establishment and survival. In: Leck, M.A. (Ed.), *Seedling Ecology and Evolution*. Cambridge University Press, pp. 189–213.
- Ibjibij, J., Urquaga, S., Ismaili, M., Alve, J.R., Boddey, R.M., 1996. Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, and nitrogen fixation of three varieties of common bean (*Phaseolus vulgaris*). *New Phytologist* 134, 353–360.
- Johnson, C.R., Hummel, R.L., 1985. Influence of mycorrhizae and drought stress on growth of *Poncirus x Citrus* seedlings. *Horticulture Science* 20, 754–755.
- Jumpponen, A., 2001. Dark septate endophytes — are they mycorrhizal? *Mycorrhiza* 11, 207–211.
- Jumpponen, A., Trappe, J.M., 1998. Dark septate endophytes: a review of facultative biotrophic root colonizing fungi. *New Phytol* 140, 295–310.
- Jumpponen, A., Trappe, J.M., Cázares, E., 2002. Occurrence of ectomycorrhizal fungi on the forefront of retreating Lyman Glacier (Washington, U.S.A.) in relation to time since deglaciation. *Mycorrhiza* 12, 43–49.
- Juniper, S., Abbott, L., 1993. Vesicular-arbuscular mycorrhizas and soil salinity. *Mycorrhiza* 4, 45–57.
- Klironomos, J.N., 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417, 67–70.

- La Manna, L., Buduba, C., Irisarri, J., Ferrari, J., Cremona, M.V., Los nutrientes del suelo en la Región Andino Patagónica: una aproximación a la interpretación de datos analíticos. *Patagonia Forestal* 17(3), in press.
- Laclau, P., Pozo, L.M., Huerta, G., Mazzuchelli, M., Andenmatten, E., Letourneau, F., 1999. Rentabilidad de la forestación con coníferas en Patagonia. Informe Final. Proyecto Forestal de Desarrollo/SAGPyA. Programa de Ámbito Nacional Forestal/INTA. Dirección General de Bosques, Fauna y Parques Provinciales, Neuquén, p. 119.
- Landeweert, R., Hoffland, E., Finlay, R., Kuyper, T.W., van Breemen, N., 2001. Linking plants to rocks: ectomycorrhizal fungi mobilise nutrients from minerals. *Trends in Ecology and Evolution* 16, 248–254.
- Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., Bazzaz, F.A., 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Journal of Applied Ecology* 10, 689–710.
- Malajczuk, N., Lapeyrie, F., Garbaye, J., 1990. Infectivity of pine and eucalypt isolates of *Pisolithus tinctorius* on roots of *Eucalyptus urophylla* in vitro. I. Mycorrhizal formation in model systems. *New Phytologist* 114, 627–631.
- Mandyam, K., Jumpponen, A., 2005. Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Studies in Mycology* 53, 173–189.
- Mangla, S., Inderjit, Callaway, R.M., 2008. Exotic invasive plant accumulates native soil pathogens which inhibit native plants. *Journal of Ecology* 96, 58–67.
- Mayor, J.R., Henkel, T.W., 2006. Do ectomycorrhizas alter leaf-litter decomposition in monodominant tropical forests of Guyana? *New Phytologist* 169, 579–588.
- Molina, R., Massicotte, H.B., Trappe, J.M., 1992. Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen, M.F. (Ed.), *Mycorrhizal Functioning: An Integrative Plant-fungal Process*. Chapman & Hall, New York, pp. 357–423.
- Nout, M.J.R., 1992. Ecological aspects of mixed-culture food fermentations. In: Carroll, G.C., Wickow, D.T. (Eds.), *The Fungal Community: Its Organization and Role in the Ecosystem*, second ed. Marce Dekker INC, New York, pp. 817–851.
- Orellana, I.A., Raffaele, E., 2010. The spread of the exotic conifer *Pseudotsuga menziesii* in *Austrocedrus chilensis* forests and shrublands in northwestern Patagonia, Argentina. *New Zealand Journal of Forestry Science* 40, 199–209.
- Peterson, R.L., Massicotte, H.B., Melville, L.H., 2004. *Mycorrhizas: Anatomy and Cell Biology*. NRC Research Press. National Research Council of Canada, Ottawa, Ontario, Canada.
- Peterson, L.R., Wagg, C., Pautler, M., 2008. Association between microfungus endophytes and roots: do structural features indicate function? *Botany* 86, 445–456.
- Pielou, E., 1969. *An Introduction to Mathematical Ecology*. Wiley Interscience, New York.
- Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52, 273–288, <http://dx.doi.org/10.1016/j.ecolecon.2004.07.013>.
- Pla, L., 2006. Biodiversidad: Inferencia basada en el índice de Shannon y la riqueza. *Interciencia* 3 (8), 583–590.
- Reid, C.P.P., Bowen, G.D., 1979. Effects of soil moisture on VA mycorrhiza formation and root development in *Medicago*. In: Harley, J.L., Russel, R.S. (Eds.), *The Soil-root Interface*. Academic Press, New York, pp. 211–220.
- Richardson, D.M., Allsopp, N., D'Antonio, C., Milton, S.J., Rejmánek, M., 2000. Plant Invasions – the role of mutualisms. *Biological Reviews* 75, 65–93.
- Richardson, D.M., van Wilgen, B.W., Nuñez, M.A., 2008. Alien conifer invasions in South America: short fuse burning? *Biol Invasions* 10, 573–577.
- Robertson, N.F., 1954. Studies on the mycorrhiza of *Pinus sylvestris*. I. Patterns of development of mycorrhizal roots and its significance for experimental studies. *New Phytologist* 53, 253–283.
- Salgado Salomón, M.E., 2007. Dispersión del inóculo micorrícico en plantaciones y en un vivero de pino ponderosa en Patagonia. Tesis para optar al título de Ingeniera Forestal, Universidad Nacional de la Patagonia “SJB”, Facultad de Ingeniería, Sede Esquel, Septiembre de 2007, vol. 75.
- Salgado Salomón, M.E., Barroetaveña, C., Rajchenberg, M., 2011. Do pine plantations provide mycorrhizal inoculum for seedlings establishment in grasslands from Patagonia, Argentina? *New Forests* 41, 191–205, <http://dx.doi.org/10.1007/s11056-010-9220-1>.
- Sarasola, M.M., Rusch, V.E., Schlichter, T.M., Ghera, C.M., 2006. Invasión de coníferas forestales en áreas de estepa y bosque de ciprés de la cordillera en la región Patagónica. *Ecología Austral* 16, 143–156.
- Schollenber, C.J., Simon, R.H., 1945. Determination of exchange capacity and exchangeable bases in soil—ammonium acetate method. *Soil Science* 59, 13–25.
- Servicio Meteorológico Nacional, 2010. <http://www.mineria.gov.ar/estudios/irn>. (accessed 30.08.10.)
- Shannon, C.E., Weaver, W., 1949. *The Mathematical Theory of Communication*. University of Illinois press, Urbana IL, EEUU.
- Simard, S.W., Jones, M.D., Durall, D.M., Perry, D.A., Myrold, D.D., Molina, R., 1997a. Reciprocal transfer of carbon isotopes between ectomycorrhizal *Betula papyrifera* and *Pseudotsuga menziesii*. *New Phytologist* 137, 529–542.
- Simard, S.W., Jones, M.D., Durall, D.M., Perry, D.A., Myrold, D.D., Molina, R., 1997b. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388, 579–582.
- Simberloff, D., Von Holle, B., 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1, 21–32.
- Singer, R., 1969. *Mycoflora Australis*. J. Cramer, Stuttgart, Germany, p. 405.
- Singer, R., Morello, J.H., 1960. Ecotrophic forests tree mycorrhizae and forest communities. *Ecology* 41, 549–551.
- Smith, J.E., Johnson, K.A., Cázares, E., 1998. Vesicular mycorrhizal colonization of seedlings of Pinaceae and Betulaceae after spore inoculation with *Glomus intraradices*. *Mycorrhiza* 7, 279–285.
- Smith, S.E., Read, D.J., 2008. *Mycorrhizal Symbiosis*, third ed. Academic Press, Cambridge, U.K.
- Sweatt, M.R., Davies Jr., F.T., 1984. Mycorrhizae, water relations, growth, and nutrient uptake of *Geranium* grown under moderately high phosphorus regimes. *Journal of American Society Horticulture Science* 109, 210–213.
- Trappe, J.M., Strand, R.F., 1969. Mycorrhizal deficiency in a Douglas fir Region Nursery. *Forest Science* 63, 381–389.
- Usuki, F., Narisawa, K., 2007. A mutualistic symbiosis between a dark septate endophytic fungus, *Heteroconium chaetospora*, and a non mycorrhizal plant, Chinese cabbage. *Mycologia* 99 (2), 175–184.
- Wagg, C., Pautler, M., Massicotte, H.B., Peterson, R.L., 2008. The co-occurrence of ectomycorrhizal, arbuscular mycorrhizal, and dark septate fungi in seedlings of four members of the Pinaceae. *Mycorrhiza* 18, 103–110, <http://dx.doi.org/10.1007/s00572-007-0157-y>.
- Wright, E., 1971. Mycorrhizae on Douglas Fir and Ponderosa Pine Seedlings. *Research Bulletin* 13, paper 670. Forest Research Lab. Oregon State University, Corvallis Oregon, USA, p. 36.
- Yu, T., Nassuth, A., Peterson, R.L., 2001. Characterization of the interaction between the dark septate fungus *Phialocephala fortinii* and *Asparagus officinalis* roots. *Canadian Journal of Microbiology* 47, 741–753.
- Zhang, Q., Yang, R., Tang, J., Yang, H., Hu, S., Chen, X., 2010. Positive feedback between mycorrhizal fungi and plants influences plant invasion success and resistance to invasion. *PLoS ONE* 5 (8), e12380, <http://dx.doi.org/10.1371/journal.pone.0012380>.