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Original article

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

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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.

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 began 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 (Ibijbijen 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

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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. Johnst. 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 $\leq$2 mm fraction were analyzed as follows: Soil pH (SpH, Bailey, 1943), Total nitrogen content (TN, Kjeldhal method, Bremmer,1960; Bremmer 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 Shanon'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):

$$SH = eH'$$

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 Levenne 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 Kruskall 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 ($df = 5$; $F = 4.79$;