Do pine plantations provide mycorrhizal inocula for seedlings establishment in grasslands from Patagonia, Argentina?

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Do pine plantations provide mycorrhizal inocula for seedlings establishment in grasslands from Patagonia, Argentina?

**María Eugenia Salgado Salomón · Carolina Barroetaveña ·
Mario Rajchenberg**

Abstract   We investigated if *Pinus ponderosa* plantations in Patagonia are able to produce viable mycorrhizal inocula towards adjacent grasslands, which only harbor endomycorrhizal vegetation. We hypothesized that these inocula have the potential to contribute to the establishment of naturally disseminated seedlings. Also, we determined the main fungal taxa involved in this process. Seven plantations in the onset of their reproductive phase and located in the Patagonian native forest/steppe ecotone (Argentina) were selected. Soil samplings were obtained at nine points along a 450 m long, W-E transect established in each plantation. Soil bioassays were performed in a greenhouse, with *P. ponderosa* seedlings acting as hosts for mycorrhizal inocula present in soil samples, during 12 months. Mycorrhization percentage, morphotype richness and morphotype composition was determined through morphological evaluation. Viable ecto- and ectendomycorrhizal inocula were found disseminated outside plantations. The amount of mycorrhizal inoculum followed a decreasing function with distance to plantation edges. Mycorrhizal fungal genus *Rhizopogon* and "E-strain" mycorrhizal types appeared as pioneering taxa regarding seedlings colonization, being the most persistent and frequent symbionts found. Plantations, thus, facilitate the surrounding terrain for newcoming seedlings through the dispersion of mycorrhizal fungal inocula.

M. E. Salgado Salomón · C. Barroetaveña · M. Rajchenberg
Patología Forestal, Departamento de Ingeniería Forestal, Facultad de Ingeniería, Universidad Nacional de la Patagonia S.J. Bosco, 9200 Esquel, Chubut, Argentina
e-mail: mesalgadosalomon@gmail.com

C. Barroetaveña
e-mail: cbarroetavena@ciefap.org.ar

M. E. Salgado Salomón · C. Barroetaveña · M. Rajchenberg
Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Buenos Aires, Argentina

C. Barroetaveña · M. Rajchenberg (✉)
Centro de Investigación y Extensión Forestal Andino Patagónico, C.C. 14, 9200 Esquel, Chubut, Argentina
e-mail: mrajchenberg@ciefap.org.ar

Introduction

The eastern slope of the Andes in Patagonia (Argentina) presents vast grass and shrublands located between the native forests on the west and the steppe on the east, creating a narrow but long strip suitable for afforestation with fast growing non-native conifers. Plantations were initiated around 50 years ago and approximately 70,000 ha are currently forested mainly with *Pinus ponderosa* Dougl. ex Laws. (80% of planted surface area), *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus contorta* Dougl.; yet 2,225,000 ha of suitable lands are still not forested (Andematten et al. 2002). This activity aims to produce high quality timber in order to foster social and economical regional development, and also to ameliorate or even reverse erosion in areas that have been heavily overgrazed. Nevertheless, it is a fact that this activity impacts native vegetation through its alteration, displacement or invasion, both in native forests and/or stepparian areas (Richardson et al. 2008).

It is well known that fast growing pine species with light and small seeds and short rotations have invading characteristics in disturbed habitats (Grotkopp et al. 2002). Studies carried out in NW Patagonia have demonstrated that exotic conifers have an invading behavior, both into the steppe (mainly by *P. contorta*) and the native forests [mainly by *P. menziesii* into the endemic *Austrocedrus chilensis* (D. Don) Pic. Serm. & Bizzarri (Cupressaceae) forests (Sarasola et al. 2006; Richardson et al. 2008)]. Several documented situations have shown that *Pinus ponderosa*, which grows naturally on the eastern side of the Cascades of north western North America (Oliver and Ryker 1991), is prone to produce invasions (Steinauer and Bragg 1987; Richardson et al. 2008). On the contrary, Sarasola et al. (2006) found that plantations from Patagonia have low invading capacity, although that could be a transitional stage considering most of them have not yet reached the effective initial recruitment age (Richardson et al. 2000).

Little research has considered the belowground conditioning factors of trees invasion processes. The net balance of the interactions between plants and soil organisms determines growth and competitive capacity of each plant species in the community where they interact (Wardle et al. 2004) and explains, at least in part, the mechanism of invasion. The symbiotic associations established between some species of fungi and the roots of most of terrestrial plants (i.e., mycorrhizas) are an example. In Patagonia no studies have focused yet on the micro-organisms present in adjacent grasslands surrounding pine plantations.

Mycorrhizas are classified according to their specific structure and associations as ecto- (EM), ectendo- (ECM) or endomycorrhizas (AM), together with other smaller groups (Smith and Read 2008). All *Pinus* species have ectomycorrhizal fungal symbionts necessary for their normal growth (Kropp and Langlois 1990), and they do not obtain an effective establishment without the symbiosis (Marx and Cordell 1989; Richardson et al. 2000; Simberloff et al. 2002). Ectomycorrhizal fungal species richness associated with *P. ponderosa* in Patagonia has been reported as low, with some species very abundant and widely distributed (Barroetaveña et al. 2005, 2007). Though AM fungi colonizing seedlings of *Pinus* species have been reported (Cázares and Smith 1996; Smith et al. 1998), their function is still unclear (Horton et al. 1998; Smith and Read 2008) and seems limited to the seedling stage (Kovacic et al. 1984). Contrary to *Pinus* spp. condition, grass and shrub species of Patagonia exclusively form endomycorrhizal symbiosis (Fontenla et al. 1998)

and do not offer EM inoculum to plantations. Thus, as pines have been planted in Patagonian grasslands/shrublands they depended exclusively on the ectomycorrhizas provided by the nursery for their proper establishment and growth. Nowadays, as plantations grow and produce seeds that could colonize adjacent lands, it is possible that EM colonization of seedlings can occur from inocula coming from those plantations.

Ectomycorrhizal colonization may occur from vegetative or spore inocula. Vegetative colonization occurs through the contact with mycelium growing from roots of mycorrhized, mature plants, or by dormant structures as sclerotia. Spores might be wind-dispersed from fruiting bodies into the neighbouring landscape or through vectors, i.e. animals that feed from the fruiting bodies and distribute spores in patches with their faeces. Inoculum potential and fungal community structure may vary sensibly in richness and abundance according to each particular situation and dispersion method (Kranabetter and Wylie 1998; Thiet and Boerner 2007). Recently, Núñez et al. (2009) have reported that the lack of belowground mutualism hinders invasion by exotic Pinaceae in climaxing native *Nothofagus-Austrocedrus* forests in Patagonia. Afforested areas in the steppe, though, constitute a completely different situation where the generally square and well delimited exotic plantations are surrounded exclusively by native grasses and shrubs.

The aim of this research was to evaluate the presence and abundance of viable mycorrhizal inocula produced by plantations of *P. ponderosa* in Patagonia towards surrounding stepparian grounds and also to evaluate their nursery capacity towards eventual seeds produced and dispersed by plantations. We hypothesized that ponderosa pine plantations in Patagonia are an effective source of mycorrhizal inocula, which establish and persist in adjacent ground but have a short dispersion that abruptly decreases with distance to plantations edge.

Methods

Study sites

The study was conducted in several private ranches in NW Patagonia, Argentina, located in stepparian habitats belonging to the Occidental Patagonian District, Patagonian Province, Andino-Patagonian Domain (Cabrera and Willink 1980). The vegetation formations in this area are grasslands and sub-arbustive shrublands dominated by species of *Stipa*, *Poa*, *Festuca*, *Mulinum*, *Acaena*, *Trifolium*, *Berberis* and *Colletia*. Seven pine plantations were selected in the neighbourhoods of Esquel city (Chubut), near Los Alerces National Park (Chubut) and Cuesta del Ternero road (Río Negro; Table 1). Soils are classified as Mollisols or Andisols orders (Salazar Lea Plaza et al. 1990), with either low (i.e. Mollisols) to high (i.e. Andisols) P retention, slightly acids, high cationic exchange capacity, deep, with 3–11% organic matter content in the upper layer, and 0.85–1.0 g cm^{-3} bulk density (Ortiz 1976; Colmet Dâage et al. 1988; Broquen et al. 1995; López 1996). Predominant wind direction is W-E, intensity values exceeding 100 km/h during spring (Servicio Meteorologico Nacional 2001).

Plantation selection and characterization

Plantations were chosen according to the following features: (a) pure masses of *P. ponderosa* older than 16 years, at the beginning of their reproductive phase; (b) flat terrains with no more than a 5% slope, to minimize variations in soil humidity; (c) fenced sites preferably,

Table 1 Plantations location and description

Plantation	Lat/Long	Density (trees/ ha)	Age (years)	Forestry management	Mean annual precipitation (mm)[a]
1. Ello	42°51′54.24″S/71°24′52.67″O	904	20	Without management	503
2. La Zeta	42°53′26.08″S/71°18′52.68″O	1,666	18	Prunned	503
3. El Principio	42°59′46.95″S/71°29′41.72″O	2,500	22	Without management	639
4. Andrés	41° 57′58.52″S/71°21′02.96″O	2,500	17–22	Without management	348
5. Cuesta del Ternero	41°58′14.98″S/71°20′28.39″O	2,500	18	Without management	348
6. Rocco	41°58′19.56″S/71°21′18.33″O	1,111	18–20	Prunned	348
7. Regimiento Esquel	42°52′55.32″S/71°16′53.68″O	1,111	18–22	Without management	503

[a] Servicio Meteorologico Nacional (2001)

with known anthropogenic history (i.e., cattle/ sheep grazing); (d) plantations surrounded by steppe/grasslands suitable to be afforested, without neighboring pine plantations and with enough open landscape to establish a 400 m, W-E transect coinciding with prevailing wind direction in Patagonia, that could favored seeds and fungal dispersion; (d) absence of EM (but endomycorrhizal) native vegetation surrounding plantations. Information of GPS location, mean annual precipitation, stand age, density and silvicultural management for each plantation is presented in Table 1.

Soil sampling

A 450 m long, W to E direction transect was established in each plantation, from the eastern edge onwards. Nine sampling points (i.e. Treatments) were determined along them, the first 50 m towards the interior of the stand (Treatment-50) and the others at 10 m (Treatment 10), 20 m (Treatment 20), 30 m (Treatment 30), 50 m (Treatment 50), 100 m (Treatment 100), 200 m (Treatment 200), 300 m (Treatment 300) and 400 m (Treatment 400) from the stand edge eastward (Fig. 1). Sampling point 1 was considered the tester situation regarding the EM and ectendomycorrhizal abundance and richness in each
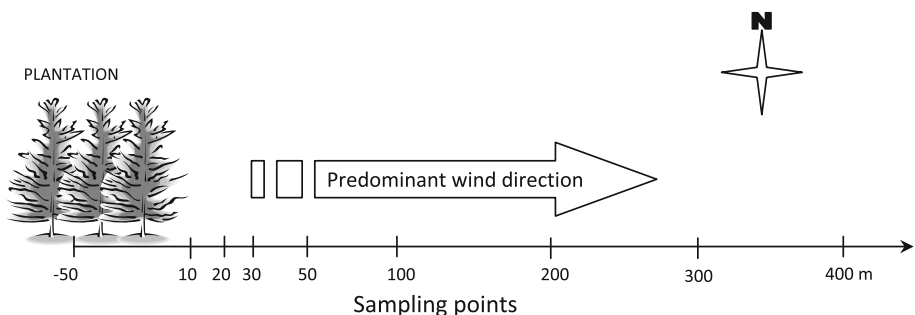

Fig. 1 Sampling design

plantation. A composite, 2 kg soil sample conformed by three sub samples obtained along a perpendicular, 2 m line was taken at each sampling point, and put together in brand-new plastic bags. Any living, decayed or dead vegetal cover was removed from each sampling point and samples were labeled and stored outdoors in the shadow, under fresh atmosphere until its processing, during not more than 45 days.

Soil bioassay setup

A soil bioessay with seedlings acting as baites was set up to evaluate the EM and ECM inoculum potentials and compositions of soil at each sampling distance.

Clean, brand-new flowerpots 250 cm^3 were filled with soil obtained from each sampling point. *Pinus ponderosa* seeds of commercial quality were properly cleaned and conditioned according to Willan (1991), and stratified during 24 h in water and afterwards at 4°C for 60 days in a refrigerator. Each flowerpot was sown with two seeds to select the most vigorous seedling after germination. Flowerpots were lodged during 12 months in a greenhouse devoted exclusively to this experiment, watered regularly according to weather conditions, with water obtained directly from a well through pipes, in order to minimize EM or ECM contamination.

A blocks design was applied, with plantations considered as blocks and distance along transects as treatments (seven blocks with nine treatments). Twenty repetitions (flowerpots) were included per sample unit (each distance).

Soil bioessay evaluation

The mycorrhizal status of each tramp plant was determined after 12 months, through the evaluation of mycorrhizal percentage and morphotype richness including both EM and ECM. As outcome variables the overall mycorrhization percentage, morphotypes richness and mycorrhization percentage by morphotype were considered.

Mycorrhization percentage (%M) was estimated following Brundrett et al. (1996), by quantifying the percentage of mycorrhizal tips (MT) from the total root tips on each root system, expressed as:

$$\%M = (\text{number of mycorrhizal tips/total root tips}) \times 100.$$

The amount of mycorrhizas was also expressed as total mycorrhizal tips by total root length in m, expressed as:

$$MT/RM = \text{number of mycorrhizal tips/total root length (m)}.$$

Morphotype richness was expressed as the number of morphotypes found in each sample unit. Morphotype composition for each Treatment was also quantified as the average percentage of each morphotype from all the sample units in that treatment.

Morphotype classification

Morphotypes were characterized, determined and classified according to Goodman et al. (1996) and the reference works by Barroetaveña (2004), Barroetaveña and Rajchenberg (2003) and Barroetaveña et al. (2005), in which all morphotypes so far found in *P. ponderosa* plantations and forest nurseries in Patagonia were described. Mycorrhizal morphotyping was chosen as an appropriate, quick and non-expensive method to check the hypothesis of this research.

Statistical analysis

The %M and MT/RM were analyzed with a lineal regression analyses by the minimum squares method (Steel and Torrie 1988). Normality and Homocedasticity of data was always verified using Shapiro-Wilk and Levene tests, respectively.

Morphotypes richness data were analyzed with Friedman test for non-parametric data and Wilcoxon multiple comparisons test with the application of Bonferroni's correction (Steel and Torrie 1988).

Similarity in morphotype composition between all Treatments was analyzed through a model of hierarchic clusters, with Ward test. The matrix included the number of plants of each Treatment that presented each morphotype.

All the analyses were run with the statistical packages SPSS for Windows version 11.5 and WINSPAD version 5.5.

Results

Percentage of mycorrhization

Percentage of mycorrhization displayed a lineal distribution showing a progressive decrease with distance to plantation (Fig. 2, Table 2).

The predicted rate of mycorrhization change with distance is 0.0758 (σ: 3.4098). The high R^2 value shows a very good fit to the equation. The estimated %M is 44.246 ($\pm$6.8196) at 10 m and 14.684 ($\pm$6.8196) at 400 m.

A significant drop in mycorrhization percentage occurred at 30 m outside plantations (Table 2, Fig. 2).

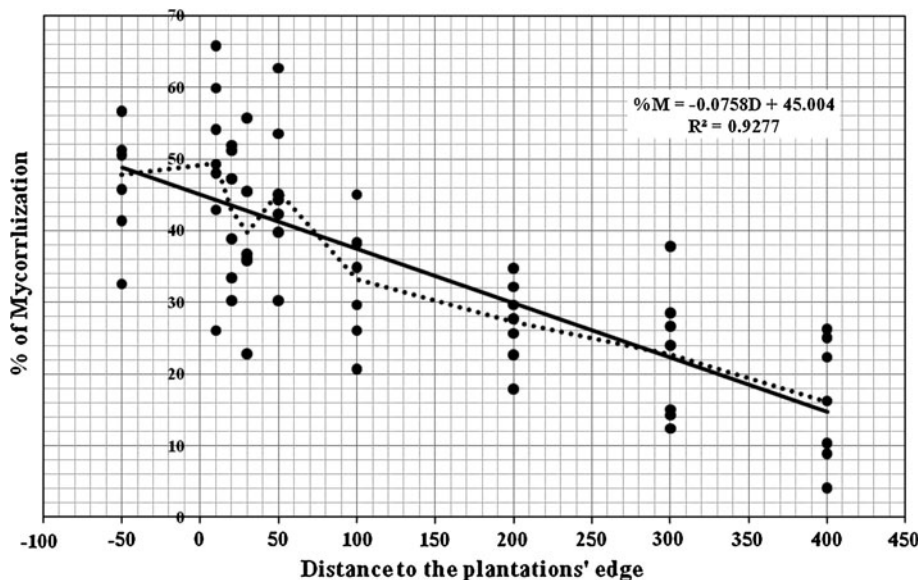$$\%M = -0.0758D + 45.004$$
$$R^2 = 0.9277$$

Fig. 2 Lineal regression of mycorrhization percentage along distance (*solid line*) and %M average by distance (*dotted line*). *%M*, percentage of mycorrhization; *D*, distance to the edge plantation

Table 2 Values of mycorrhization percentage (%M) and mycorrhizal tips per root meter (MT/RM) obtained through the bioassay

| Plantation	Ello		La Zeta		El Principio		Andrés		Cuesta del Ternero		Rocco		Regimiento Esquel	
Treatment / Variable	%M	MT/RM	%M	MT/RM	%M	MT/RM	%M	MT/RM	%M	MT/RM	%M	MT/RM	%M	MT/RM
−50	56.61 (18.89)	68.20 (35.05)	32.57 (14.93)	41.30 (25.54)	56.72 (7.86)	73.73 (24.00)	45.77 (8.21)	50.12 (15.95)	51.27 (7.84)	57.48 (13.09)	41.43 (12.43)	43.64 (17.75)	50.54 (11.6)	59.12 (15.82)
10	65.75 (12.44)	85.35 (29.17)	42.89 (13.73)	44.86 (19.14)	59.89 (12.33)	65.00 (18.91)	47.99 (10.35)	43.65 (9.52)	54.09 (10.89)	50.53 (12.84)	26.08 (14.83)	23.20 (14.49)	49.29 (8.93)	58.12 (21.89)
20	51.94 (10.09)	78.67 (24.22)	47.31 (17.78)	56.02 (25.44)	47.26 (9.67)	57.23 (16.38)	51.16 (11.41)	51.62 (14.67)	38.84 (5.36)	39.11 (14.65)	30.25 (10.71)	32.57 (16.70)	33.39 (16.4)	32.36 (18.63)
30	55.68 (14.55)	70.25 (27.71)	35.79 (18.93)	30.65 (20.11)	45.45 (11.87)	63.60 (24.78)	36.75 (11.58)	35.26 (13.81)	45.52 (12.08)	37.11 (14.01)	22.85 (11.75)	22.17 (13.71)	36.09 (16.0)	36.34 (22.35)
50	53.54 (19.9)	75.04 (36.17)	45.14 (11.90)	59.23 (23.49)	42.32 (9.78)	58.50 (20.14)	44.30 (9.81)	40.55 (11.72)	62.66 (21.11)	67.70 (27.02)	30.24 (17.26)	28.25 (16.24)	39.79 (17.0)	46.21 (21.70)
100	45.05 (15.93)	82.73 (49.50)	26.06 (13.94)	28.27 (16.49)	38.20 (12.52)	59.06 (12.59)	34.85 (9.22)	41.93 (14.04)	38.39 (14.02)	37.52 (16.39)	20.70 (23.22)	14.49 (14.31)	29.64 (19.4)	33.22 (28.36)
200	34.78 (14.95)	41.68 (35.23)	17.91 (16.38)	17.38 (19.78)	25.66 (7.09)	28.03 (9.01)	22.67 (11.22)	23.45 (14.87)	32.15 (9.68)	31.12 (11.14)	27.74 (14.14)	25.00 (15.76)	29.64 (19.4)	33.22 (28.36)
300	15.04 (15.86)	16.16 (18.90)	12.42 (19.79)	13.92 (27.30)	26.68 (12.05)	30.33 (18.92)	24.05 (6.56)	22.86 (11.61)	37.83 (10.14)	42.57 (20.67)	28.53 (20.02)	29.01 (25.81)	14.26 (16.1)	14.20 (28.36)
400	8.85 (12.27)	10.63 (16.27)	4.12 (6.94)	4.91 (9.55)	16.23 (14.15)	14.48 (14.08)	25.11 (7.91)	24.08 (12.63)	22.34 (7.25)	19.90 (6.71)	26.25 (7.19)	23.49 (7.95)	10.36 (6.77)	7.76 (5.65)

Standard deviation between brackets
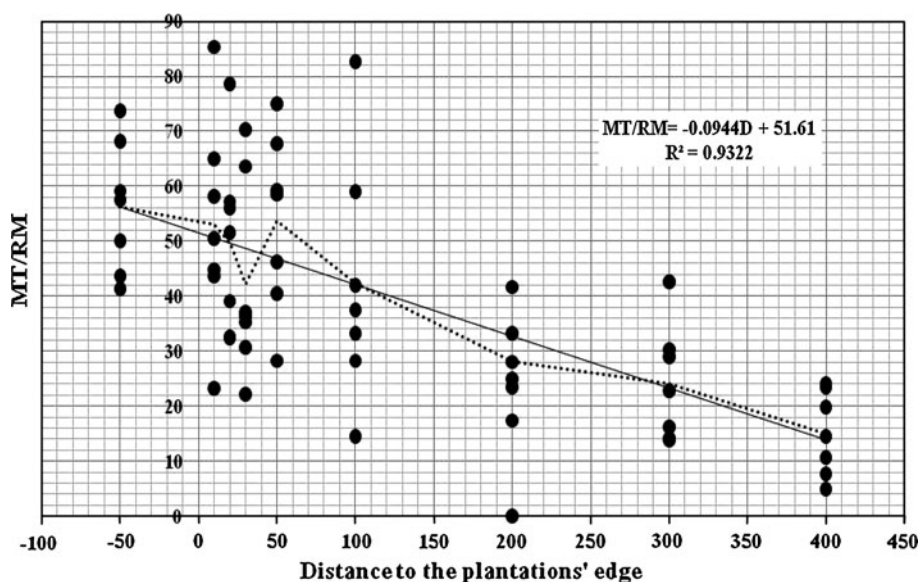
Fig. 3 Lineal regression of mycorrhizal tips per root meter along distance (*solid line*) and MT/RM average by distance (*dotted line*). *MT/RM*, mycorrhizal tip per meter of root; *D*, distance to the edge plantation

Mycorrhizal tips per root meter (MT/RM) displayed, in average, the same decreasing tendency with distance to plantation, with very similar features. It showed a lineal distribution (Fig. 3, Table 2).

The predicted rate of mycorrhization change with distance is 0.0944 (σ: 4.0972). The estimated MT/RM is 50.67 ($\pm$8.1944) at 10 m and 13.85 ($\pm$8.1944) at 400 m.

Morphotype richness

Only six out of 17 previously described mycorrhizal tips from *P. ponderosa* plantations and forest nurseries in Patagonia were found (Barroetaveña 2004, 2005; Barroetaveña and Rajchenberg 2003; Table 3). In addition, an unrecorded morphotype that corresponded to an ECM arbitrarily named as "E-strain IV" was also registered.
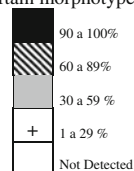
Morphotype richness varied between 2–6 morphotypes along transects. The tester sampling point (50 m within plantations) displayed the maximum mycorrhizal richness in four out of the seven transects. Outside plantation edges richness varied irregularly along transects, equaling the tester in 1-several sampling points.

The similarity analysis of morphotype composition by distance to plantation, defined three subgroups (Fig. 4). The dendrogram shows that Cluster 1, grouping treatments within or immediate to plantations (Treatment-50 and some Treatments 10) presented the greater morphotype richness, including all the seven morphotypes found in this study. Morphotypes *Rhizopogon* and "E-Strain" were the most abundant (Table 3) and *Amphinema byssoides* and 'Tuber type' were present exclusively within this cluster. Cluster 2 grouped treatments close to plantations (Treatments 20, 30 and 50), and only *Rhizopogon*, "E-Strain I", "E-Strain III" and "Brown mycelium" morphotypes were present. Cluster 3 grouped treatments between 200 and 400 m from plantations, and only *Rhizopogon*, E-Strains I, III

Table 3 Morphotypes presence along distance to plantations' edge in each treatment

Note: Cells contain graphic shading indicating percentage of plants with a certain morphotype; only the "+" symbols (1 a 29 %) are rendered as text below.

Plantation	Treatment (m)	N° of seedlings	Rhizopogon	Amphinema	E-strain III	E-strain II	Tuber type	E-strain IV	Brown mycelium
Ello	-50	10			+				
	10	10			+				
	20	10							
	30	10				+			
	50	10							
	100	10							
	200	10			+				
	300	10	+		+			+	
	400	10				+			
La Zeta	-50	10	+	+	+				
	10	10				+			
	20	10							
	30	10							
	50	10				+			
	100	10			+				
	200	6							
	300	10							
	400	10	+		+	+			
El Principio	-50	10							
	10	10							
	20	10							
	30	10			+				
	50	10							
	100	9			+	+			
	200	9				+			
	300	10		+					
	400	9	+						
Andrés	-50	10							
	10	10				+			
	20	10							
	30	10							
	50	10				+			
	100	10							
	200	9							
	300	10							
	400	10							

Plantation	Treatment (m)	N° of seedlings	Rhizopogon	Amphinema	E-strain III	E-strain II	Tuber type	E-strain IV	Brown mycelium
Cuesta del Ternero	-50	10							
	10	10							
	20	10							
	30	10							
	50	10							
	100	10							
	200	10							
	300	10							
	400	10							
Rocco	-50	10							
	10	10							
	20	9							
	30	10							+
	50	7							
	100	10							
	200	10				+			+
	300	10							+
	400	10							
Regimiento Esquel	-50	8		+	+			+	
	10	6							
	20	6							
	30	6							
	50	10		+					
	100	10							
	200	6			+				
	300	10			+				
	400	10			+				

Percentage of plants with a certain morphotype.

- 90 a 100%
- 60 a 89%
- 30 a 59 %
- + 1 a 29 %
- Not Detected

and IV morphotypes were present. Treatments 10 and 100 were variable in their morphotypes' composition, thus the former appeared in all three Clusters and the latter in Clusters 2 and 3.
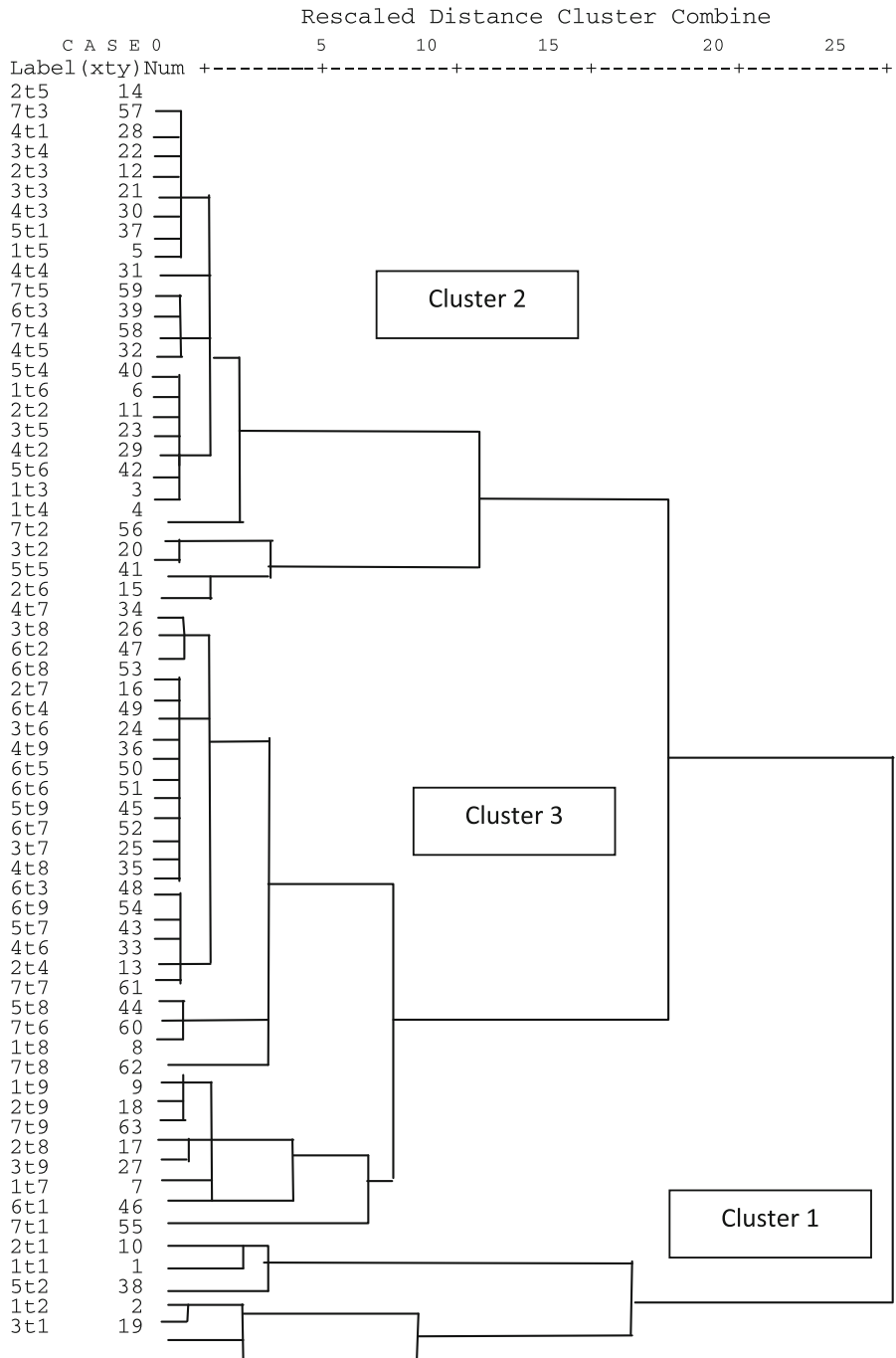
Fig. 4 Dendrogram showing the similarity analysis of morphotypes composition by distance to plantation's edge. *Label* indicates transect number (xt) followed by treatment number (y). *1t* Ello, *2t* La Zeta, *3t* El Principio, *4t* Andrés, *5t* Cuesta del Ternero, *6t* Rocco, *7t* Regimiento Esquel. *y1* −50 m, *y2* 10 m, *y3* 20 m, *y4* 30 m, *y5* 50 m, *y6* 100 m, *y7* 200 m, *y8* 300 m, *y9* 400 m

Analyzing mycorrhizal richness between transects, although without statistical support, those installed in the driest areas (i.e., Cuesta del Ternero, Rocco and Andrés plantations; Table 1) presented the lowest richness values (2, 2 and 3 morphotypes, respectively).

Discussion

This research showed, contrary to our hypothesis that EM and ECM inocula would drop drastically beyond the plantations' edge, that ponderosa pine plantations at the onset of their reproductive life built an inoculum potential that was diverse in abundance and diminished progressively. Although no quantification of possible EM contaminants was carried out in this study, so we can not affirm whether 400 m constitute or not the true limit of inocula dispersion, the diminishing trend of %M and MT/RM was evident at each increasing sampling distance. These results agree with Richardson et al. (1994) and Reinhart and Callaway (2006) who suggested that fungi introduced to a region may not limit the spread of trees for long owing to the high dispersal potential of fungi. It also agrees with what Núñez et al. (2009) reported from an invaded *Nothofagus-Austrocedrus* forest from Patagonia: even though they worked with exotic conifers planted in a matrix of native forest, a very different environment compared with a dense plantation surrounded by grasslands, they showed a drop in EM inoculum potential and diversity but with some colonization still as far as >1,000 m from original exotic plantings.

A significant drop in mycorrhization percentage occurred at 30 m outside plantations, and recovered at 50 m (Table 2, Fig. 2). This could be due to an edge effect through the reduction of mycelial inocula associated with roots at increasing distance, and/or the low presence of wind dispersed propagules due to a wind-shadow effect produced by plantations. This topic needs to be evaluated with broader considerations including the role of possible mammal vectors such as those in genera *Abrothrix*, *Chlemys* and *Ctenomys* that are present in the studied area (Pardiñas et al. 2003; Pearson 1995). Sarasola et al. (2006) established that the maximum effective recruitment distance for 18–22 years old *P. ponderosa* plantations is 50 m in the Patagonian steppe. Our data showed that the mycorrhizal inocula available for seed establishment widely surpasses the effective range of seed dispersion. Nevertheless, *P. ponderosa* plantations studied by Richardson et al. (2000) and Sarasola et al. (2006) were in the Effective Initial Recruitment Age, suggesting that at a later time, with older plantations, this distance would possibly increase.

We recovered only some (seven out of 17) of the mycorrhizal morphotypes previously recorded from ponderosa pine plantations in Patagonia (Barroetaveña and Rajchenberg 2003; Barroetaveña 2004). This is not surprising as only seven plantations were selected for this study comparing with the full survey undertook in the other studies. Also, it is possible that with the systematic elimination of the organic cover in the tester soil samples part of the available inocula has been removed, preventing the expression of some morphotypes. This might be also the reason why not all Treatments −50 displayed the highest morphotype richness.

Different mycorrhizal fungal morphotypes behaved differently regarding their dispersion. At 400 m from plantations' edge, only *Rhizopogon* sp. and ectendomycorrhizas of the "E-strain" I and III types were found in all transects. *Rhizopogon* Fr. & Nordholm (Rhizopogonaceae, Boletales) is an ectomycorrhizal fungal genus widely distributed and with a high infecting and colonization capacity, as had been observed

by Barroetaveña ([2004](#)) and Barroetaveña et al. ([2005](#)) for Patagonia, agreeing with results found for *Pinus jeffreyi* Balf. by Izzo et al. ([2006](#)) in Sierra Nevada and for *P. muricata* D. Don and *P. ponderosa* by Kjøller and Bruns ([2003](#)), both in California (USA). In this work it is demonstrated that it also displays an efficient dispersion outside an established plantation, in close correspondence to the pioneering character that has been assigned to several species of this genus (Molina et al. [1999](#)). "E-strain I" would also have the same features that Izzo et al. ([2006](#)) detected for the ectendomycorrhizas formed by a species of the genus *Wilcoxina* Chin S. Yang & Korf (Otideaceae, Pezizales). These ectendomycorrhizas are world-wide distributed and found in diverse environments (Yu et al. [2001](#)) and display a strong capacity for an early colonization of seedlings, also in severe conditions of lack of humidity and irradiation (Mikola [1988](#); Izzo et al. [2006](#)). All this was also observed by Barroetaveña ([2004](#)) for ponderosa pine in Patagonia.

Amphinema byssoides and "*Tuber*" type displayed a short distance dispersion mode. This could be related to a low abundance, to a very low fruiting bodies production, or to an inefficient dispersion strategy. It is intriguing why *Amphinema byssoides* (Fr.) J. Eriks. (Atheliaceae, Atheliales), widely spread and fructifying abundantly in *P. ponderosa* plantations from Patagonia (Barroetaveña [2004](#)), showed such strong declining dispersion pattern (only 50 m outside plantations edge). One reason could be the fact that it develops very thin, corticioid basidiomes unlikely to be eaten by potential small mammals acting as dispersion vectors for its spores. Regarding "*Tuber*-type" it should be considered that there are no records of this species fruiting in pine plantations from Patagonia, whereas its finding in forest nurseries has been scanty (Barroetaveña et al. [2005](#)).This could indicate the taxon is still very scarce in Patagonia and still needs to build up a proper amount of inoculum in order to be more widely found in plantations. It could also be explained by a restricted fructification pattern of this taxon in Patagonia, perhaps due to environmental factors. All this in spite that *Tuber* species have similar characteristics as *Rhizopogon* species, i.e. the capacity to form hypogeous, edible fruiting bodies capable to be dispersed by small mammals as pointed out above.

Our results differ from Nuñez et al. ([2009](#)), who reported the presence of *Suillus luteus* as the most abundant species far from plantations, and *Wilcoxina* (E-Strain) and *Rhizopogon* as the most abundant close to plantations. Different environmental conditions in their study sites regarding wind intensity and understory hight and density could have probably reduce inocula dispersion by wind, being one reason for such differences. They also discarded mammals mentioned above as possible vectors; those animals, having herbivore dietary habits that are not fully studied yet, could incorporate fungi or colonized roots on their diet, and their habits and movements might be very different in grassland compared with a climaxing forest environment.

Little information exists regarding EM-ECM inocula longevity in natural conditions. Our handling process of soil samples did not keep them intact as tubular cores. This could damage in part, though not necessarily all, the inocula present as hyhpal nets or cords. Presumably, spores or sclerotia would play a major role in maintaining a viable, long-lived inoculum bank in the soil. This was confirmed in few studies (Miller et al. [1994](#), Kjøller and Bruns [2003](#)) that showed the capability of certain EM taxa, as *Rhizopogon*, to persist and maintain its viability in soil at least a decade long (Bruns et al. [2009](#)). Our study did not address this question and it is difficult to answer whether the mycorrhizal inoculum bank found during this research was built and maintained along several years or was the product of the year in which the experiment was carried out.

Conclusion

We could confirm the presence of viable mycorrhizal inocula necessary for the establishment of *P. ponderosa* as an exotic plant outside plantations edges in the Patagonian steppe. It is possible to assert that: (1) plantations of *P. ponderosa* produce EM and ECM inocula that is disseminated outside the plantations, at least in the W to E studied direction, and that it is viable in order to establish symbiosis with *P. ponderosa* seedlings; (2) the amount of mycorrhizal inoculum follows a decreasing function with distance to plantation edges and, (3) *Rhizopogon* sp. and the "E-strain" mycorrhizas appeared as pioneering taxa regarding the colonization of seedlings, being the more persistent and frequent symbionts found.

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