Impact of mycorrhizae and irrigation in the survival of seedlings of *Pinus radiata* D. Don subject to drought

Impacto de la inoculación con micorrizas y el riego en la sobrevivencia de plántulas de *Pinus radiata* D. Don sometidas a sequía

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ABSTRACT

In drought condition**,** plants increase survival chance by adjusting their functional traits and by biological associations. Mycorrhizae association and artificial watering have been shown to increase plant survival under drought, especially at early developmental stages when plants are more susceptible. In Chile, *Pinus radiata* is the most important forest species. It is grown mainly in Central Chile, where precipitations are predicted to drop in 40% and change in frequency in the future due to climate change. *Rhizopogon luteolus* is an ectomycorrhizae usually associated with *Pinus* species and has been found to increase drought tolerance. We addressed the effect of *R. luteolus* inoculation on survival and functional traits of *P. radiata* seedlings exposed to two watering treatments. These treatments simulated control (50 ml) and 40% reduced precipitations (20 ml). We also evaluated the combined effect of watering quantity (20 and 50 ml) and frequency (every 5, 10 and 30 days) on the same variables. *R. luteolus* inoculation increased seedling survival, but reduced plant size. Watering quantity affected plant survival only at intermediate watering frequencies, but not at the high and low frequencies. The lowest frequency, normal for the summer of Central Chile, resulted in ~80% seedling mortality. Most of the functional traits measured were not affected neither by watering frequency nor quantity, but they were affected by mycorrhization. Mycorrhizae inoculation, together with some sort of artificial watering could be a possible strategy to cope with prolonged drought events.

KEYWORDS: *Pinus radiata*, *Rhizopogon luteolus*, climate change, ectomycorrhizae, drought tolerance.

RESUMEN

En condiciones de sequía, las plantas aumentan la probabilidad de sobrevivir ajustando sus rasgos funcionales y por medio de asociaciones biológicas. Las asociaciones micorrícicas y el riego artificial aumentan la sobrevivencia en condiciones de sequía, especialmente en etapas tempranas del desarrollo, cuando las plantas son más sensibles. En Chile, *Pinus radiata* es la especie forestal más importante. Se planta principalmente en Chile central, donde se predice que las precipitaciones disminuirán en un 40% y cambiará su frecuencia en el futuro debido al cambio climático. *Rhizopogon luteolus* es una ectomicorriza usualmente asociada a especies de *Pinus* y se ha encontrado que aumenta la tolerancia a la sequía. Se analizó el efecto de la inoculación de *R. luteolus* en la sobrevivencia y rasgos funcionales de plántulas de *P. radiata* expuestas a dos tratamientos de riego. Estos tratamientos simulan precipitaciones control (50 ml) y reducidas a un 40% (20 ml). También se evaluó el efecto combinado de la cantidad (20 y 50 ml) y frecuencia (cada 5, 10 y 30 días) de riego en las mismas variables. La inoculación con *R. luteolus* aumentó la sobrevivencia pero redujo el tamaño de las plántulas. La cantidad de riego afectó la sobrevivencia de las plantas sólo en la frecuencia intermedia de riego, pero no en las frecuencias alta y baja. La frecuencia más baja, normal para el verano de Chile central, resultó en ~80% de mortalidad de plántulas. La mayoría de los rasgos funcionales no fueron afectados por la frecuencia o cantidad de riego, pero si por la micorrización. La inoculación

con micorrizas, en conjunto con algún tipo de riego artificial, podría ser una posible estrategia para lidiar con eventos de sequía prolongada.

PALABRAS CLAVE: *Pinus radiata*, *Rhizopogon luteolus*, cambio climático, ectomicorrizas, tolerancia a la sequía.

INTRODUCTION

Water is one of the most important factors in plant productivity across ecosystems. Water availability affects plant survival and impact several morphological and physiological traits (Atala & Gianoli 2009, Grace 1997, Molina-Montenegro *et al.* 2011, Pedrol *et al.* 2000). Water limitation usually results in reduced water loss and increased water use efficiency. Plants exposed to drought show reduced leaf area, higher root/shoot ratio, and smaller tracheid radius (Atala & Gianoli 2009, Gianoli & González-Teuber 2005, Hunt & Nicholls 1986, Molina-Montenegro *et al.* 2011). Additionally, low water potential rapidly induces stomatal closure, reducing stomatal conductance (Schulze 1986), and hence limiting photosynthesis. Overall, growth is reduced in water-limited plants, mainly due to reduced carbon gain.

It has been shown that plant species have developed morpho-physiological mechanisms to cope with stressful environments (Molina-Montenegro *et al.* 2011). Plants have also developed association with microorganisms as another successful strategy to cope with environmental stress. Ectomycorrhizae are a type of mycorrhizal fungi in which the mycelium is located in the outer layer of the roots, and are commonly found associated with forest species (Gurevitch *et al.* 2006). This type of fungi has been shown to provide drought tolerance to some vascular plants (Harris-Valle *et al.* 2009, Ortega *et al.* 2004, Steinfield *et al.* 2003), particularly in early ontogenetical stages (Lamhamedi *et al.* 1990, 1992, Ortega *et al.* 2004). *Rhizopogon luteolus* is an ectomycorrhizic fungus that grows associated with pine species, particularly in the sandy soils of Bío-Bío district in Chile (Pereira *et al.* 2007, 2010a). *R. luteolus* has been shown to improve plant performance and establishment under water stress (Castellano 1996, Steinfield *et al.* 2003, Theodorou 1971, Theodorou & Bowen 1970).

Precipitations are projected to decline in the future in some areas due to climate change (IPCC 2007). The precipitations in Central Chile have declined in the last 50 years and some models predict that they will be further reduced in the future reaching a decrease of 40-50% (IPCC 2007). Climatic change could result in salinization and desertification of the soils in Central Chile, reducing the possibilities for agriculture and forestry.

Watering is a common and expensive practice for many crops, and helps maintain an adequate moisture level and assures continuous growth (Johnson 1986). The amount and frequency of watering depends on age, time of the year, and climatic conditions (Starkey 2002). Still it is not clear whether the frequency or the amount of watering is the most relevant factor for plant survival, but probably the relative importance of frequency and quantity depends on the species, and developmental stage.

Individuals of *Pinus radiata* D. Don are used for the forestry industry worldwide, mainly due its growth rate and wood quality. In Chile, *Pinus radiata* is the most important commercial timber species. In 2003, Chile had 1.4 million ha planted with this species (INFOR 2009), and 61,560 ha were established in 2005 (INFOR 2009). By 2008 *Pinus radiata* plantations corresponded to 63.32% of the total area of planted and managed forest species (INFOR 2009). In a future scenario of global change, with reduced precipitation, some regions in Chile will not be fit to support *Pinus radiata* growth and survival. This could negatively impact the forestry industry, an important source of income for the country.

The present study addresses the effect of water frequency and quantity in seedlings of *Pinus radiata* with and without *Rhizopogon luteolus* inoculation. We evaluated plant survival, growth, and some morphological, anatomical and allocation traits. Specifically, we addressed the following hypothesis: *i*) if inoculation with *R. luteolus* can increase survival in *P. radiata* seedling when exposed to drought and *ii*) if watering quantity or watering frequency is more relevant for plant survival and growth.

MATERIALS AND METHODS

SEEDLING ESTABLISHMENT

Pinus radiata plants were grown in polyethylene trays with 260 ml sockets, filled with soil made from composted pine bark. We used separated trays for uninoculated plants (C) and for plants inoculated with *Rhizopogon luteolus* (I). Separated trays prevented contamination of control plants. Trays were put in a greenhouse and moved weekly to avoid blocking effect. We planted 3-4 pine seeds per socket, and after establishment, only one seedling were left in each socket. Plants were watered to field capacity (50 ml) with tap water every day until the initiation of the treatments (see below). One uninoculated tray was used for the mycorhizae experiment as a control, and another one was used for the watering frequencies experiment.

SPORE SUSPENSION AND INOCULATION

We prepared a suspension containing *Rhizopogon luteolus*

spores in a glass bottle. The bottle was cover in foil in order to maintain a dark environment. The suspension was put in a shaker at 80 rpm and maintained in constant agitation at 24°C for 24 h. This ensured the hydration of the spores that were obtained from dry carpophore samples. After 24 h the solution was kept at 4°C. To check for effective spore concentration, the spore suspension was filtered and 1 mL of hydrated spores was diluted in 10 ml of distilled water. A sample of this diluted solution was put in a cover and observed under the microscope. Spores were counted and a final concentration of 5.4×10^8 spores/ml was obtained. We applied 1 ml of the spore suspension to *Pinus radiata* plants obtaining inoculated individuals (I). The inoculation was repeated three times to ensure mycorrhizal association.

Watering treatments

We measured the percentage of field capacity with a soil moisture tester (Kel Instruments Co., Inc., Japan) and established that 50 ml were sufficient to reach field capacity. Plants with and without mycorrhizae were randomly assigned to 2 watering treatments ($n = 28$ plant per treatment). Control plants were watered every 10 days with 50 ml of tap water. Plants exposed to drought were watered every 10 d with 40% the water necessary to reach field capacity (i. e. 20 ml). Separately, 30 plants were randomly assigned to three watering frequencies (every 5, 10 or 30 days, $n = 10$ plant per treatment). The full watering experiment lasted 60 days (two 30 days cycles).

Before the beginning of the experiment, one plant per treatment was sacrificed to check microscopically for mycorrhizae presence. At the beginning of the watering treatments all plants were 1 year old and of similar size (ANOVA, $p<0.05$, data not shown) and condition.

The experiment was conducted in a greenhouse at Universidad de Concepción, Campus Los Ángeles, Chile in spring-summer 2010. The greenhouse did not have any temperature or moisture levels controlling mechanism.

Measured traits

We measured plant height and crown width every 10 days from the beginning of the experiment up to 60 days. We also measured percentage of field capacity to 10 randomly selected sockets twice to check for the effects of the watering treatments in the soil. Plant survival was registered daily. Relative growth rate (RGR) was calculated as RGR = [final height - initial height]/initial height.

After 60 days from the beginning of the watering treatments, the experiment was concluded and all plants were collected for biomass measurements. Plants were separated in roots, leaves, and stem. We counted leaves per plant, and cut a transversal section of the stem close to the crown. The transversal section was photographed using a microscope (Primo Star, Carl Zeiss, Germany) connected to a digital camera (Cannon EOS), and digital images were

obtained. Using image software (Axiovision 4.6, Carl Zeiss MicroImaging GmbH, Jena, Germany) we measured tracheid lumen area. All plant portions were then put in an oven at 75 °C for 72 h. Dry weight of roots and shoot (stem + leaves) was obtained using an analytical scale. We registered plant height and stem width every 2 weeks for 60 days.

Statistical analysis

The effects of water quantity and water frequency on growth and biomass allocation traits were compared using two-way ANOVA's. For all two-way ANOVA's, the assumptions of normality and homogeneity of variances were tested using the Shapiro-Wilks and Bartlett tests, respectively (Zar 1999). To compare the survival curves of *P. radiata* seedlings with and without mycorrhizae inoculation and with different quantity and frequency of water were estimated by means of the Kaplan-Meier method, and statistical differences were assessed with the Cox-Mantel test (Fox 1993).

RESULTS

Mycorrhiza inoculation

No mortality was recorded before the beginning of the watering treatments. After the initiation of the watering treatments, many individuals died, especially in the drought treatment, and without mycorrhizae (Fig. 1). At the end of the experiment, survival for inoculated plants watered with 50 ml was around 60% and close to 50% for control plants (Fig. 1). While half of the inoculated plants watered with 20 ml survived after 60 days, only a quarter of the uninoculated plants under the same watering survived (Fig. 1). Overall, mycorrhizae infection significantly increased plant survival in both watering treatments (Cox-Mantel test, $p = 0.012$, Fig. 1).

Mycorrhization affected all measured plant traits (Table I, two-way ANOVA, $p < 0.05$) except RGR (Table I, two-way ANOVA, $p > 0.05$). Watering affected only RGR (Table I, two-way ANOVA, $p < 0.05$). The only significant interactions found between *R. luteolus* inoculation and watering were for RGR and root/shoot ratio (Table I, two-way ANOVA, $p < 0.05$). Mycorrhization decreased plant size and weight in both watering treatments and increased the root/shoot ratio, but only in the 50 ml treatment (Table II, Tukey test, $p < 0.05$). Inoculation also decreased stem width and RGR but only in plants watered with 50 ml (Table II, Tukey test, $p < 0.05$). Plants watered with 50 ml had wider stems and higher RGR compared to plants watered with 20 ml, but only in the control (uninoculated) treatment (Table II, Tukey test, $p < 0.05$).

Watering frequencies

After 30 days of the initiation of the watering treatments the first plants begun to die (Fig. 2). Plants watered every 5 days

had almost no mortality in both watering quantities (Cox-Mantel test, p = 0.012, Fig. 2). Plants watered every 10 days with 50 ml survived significantly more than plants watered every 10 days with 20 ml and less than plants watered every 5 days (Cox-Mantel test, p = 0.012, Fig. 2). Plants watered every 30 days with 20 or 50 ml had the higher mortality, similar to plants watered every 10 days with 20 ml (Cox-Mantel test, p = 0.012, Fig. 1).

Watering frequency significantly affected leaves number, and watering quantity affected stem width (Table III, two-way ANOVA, p < 0.05). The only significant interaction found between watering frequency and quantity was for stem width (Table III, two-way ANOVA, p < 0.05). Marginally significant effects were found for the effect of watering frequency on plant height and RGR, and for the effect of the interaction between frequency and quantity on root/shoot ratio (Table III). Plants watered every 5 days with 50 ml had thicker stems compared to plants watered with 20 ml every 5 and 30 days (Table IV, Tukey test, p<0.05). Plants watered every 5 days and with 20 and 50 ml had more leaves compared to plants watered every 30 days with 20 ml (Table IV, Tukey test, p < 0.05).
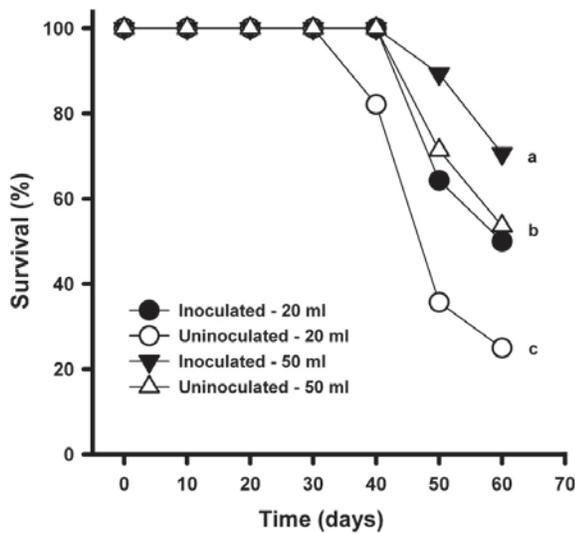

Figure 1. Survival of *Pinus radiata* seedling inoculated and not-inoculated with *Rhizopogon luteolus* under two watering treatments. Different letters show statistically significant differences (Cox-Mantel test, p = 0.012). *n* = 28 plants per treatment.

Figura 1. Sobrevivencia de plántulas de *Pinus radiata* inoculadas y no inoculadas con *Rhizopogon luteolus* bajo dos regímenes de riego. Letras diferentes muestran diferencias significativas (Cox-Mantel test, p = 0.012). *n* = 28 plantas por tratamiento.
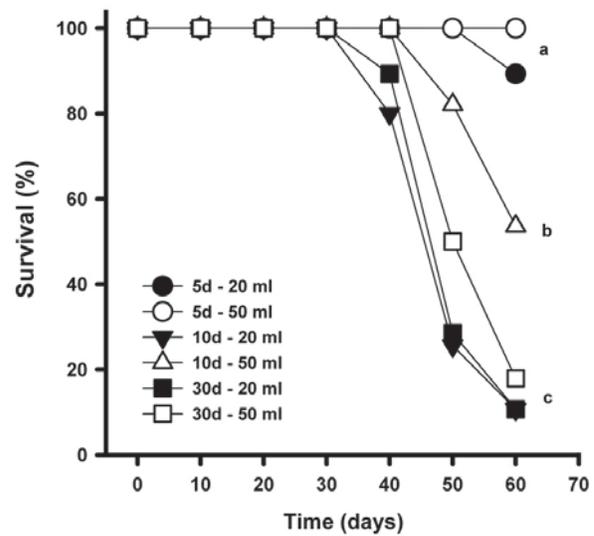
Figure 2. Survival of *Pinus radiata* seedling exposed to a combination of three watering frequencies and two watering quantities. Different letters show statistically significant differences (Cox-Mantel test, p = 0.009). *n* = 10 plants per treatment.

Figura 2. Sobrevivencia de plántulas de *Pinus radiata* expuestas a una combinación de tres frecuencias de riego y dos cantidades de riego. Letras diferentes muestran diferencias significativas (Cox-Mantel test, p = 0.009). *n* = 10 plantas por tratamiento.

Table I. *p*-values from the two-way ANOVA testing the effect of *Rhizopogon luteolus* inoculation and watering quantity on *Pinus radiata* functional traits. *n*=28 plants per treatment.

Tabla I. Valores de p del ANOVA de dos vías testeando el efecto de la inoculación con *Rhizopogon luteolus* y la cantidad de riego en rasgos funcionales de *Pinus radiata*. *n*=28 plantas por tratamiento.

	Final Stem width (mm)	Final height (cm)	RGR (cm day^{-1})	Number of leaves	Leaves weight (g)	Root weight (g)	Stem weight (g)	Root/ Shoot
Inoculation	<0.0001	<0.0001	0.1063	<0.0001	<0.0001	<0.0001	<0.0001	0.0150
Watering	0.5900	0.6472	0.0220	0.6585	0.6629	0.6491	0.9511	0.2701
Inoculation x watering	<0.0001	0.2102	0.9187	0.3848	0.3200	0.7817	0.0902	0.0057

TABLE II. Average ± SE of functional traits of *Pinus radiata* seedlings inoculated and not-inoculated (control) with *Rhizopogon luteolus* under two water regimens. Different letters show statistically significant differences (Tukey test, p<0.05). *n*=28 plants per treatment.

TABLA II. Valor promedio ± ES de rasgos funcionales de plántulas de *Pinus radiata* inoculadas y no inoculadas (control) con *Rhizopogon luteolus* bajo dos regímenes hídricos. Letras diferentes muestran diferencias significativas (Tukey test, p<0.05). *n*=28 plantas por tratamiento.

	FINAL STEM WIDTH (mm)	FINAL HEIGHT (cm)	RGR (cm day^{-1})	NUMBER OF LEAVES	LEAVES WEIGHT (g)	ROOT WEIGHT (g)	STEM WEIGHT (g)	ROOT/SHOOT
CONTROL-20ml	3.61±0.08[b]	22.32±0.59[b]	0.038±0.005[a]	404±23[b]	0.83±0.05[b]	0.68±0.04[b]	0.64±0.04[b]	0.48±0.02[ab]
INOCULATED-20ml	3.43±0.08[b]	18.09±0.63[a]	0.028±0.004[a]	328±15[a]	0.49±0.02[a]	0.42±0.02[a]	0.42±0.03[a]	0.47±0.01[a]
CONTROL-50ml	4.02±0.11[c]	23.32±0.63[b]	0.057±0.010[b]	433±22[b]	0.89±0.05[b]	0.68±0.03[b]	0.70±0.04[b]	0.44±0.02[a]
INOCULATED-50ml	3.10±0.06[a]	17.61±0.46[a]	0.041±0.006[a]	323±15[a]	0.47±0.03[a]	0.45±0.03[a]	0.37±0.02[a]	0.55±0.03[b]

TABLE III. *p*-values from the two-way ANOVA testing the effect watering frequency and watering quantity on *Pinus radiata* functional traits.

TABLA III. Valores de *p* del ANOVA de dos vías testeando el efecto de la frecuencia y la cantidad de riego en rasgos funcionales de *Pinus radiata*.

	FINAL STEM WIDTH (mm)	FINAL HEIGHT (cm)	RGR (cm day^{-1})	NUMBER OF LEAVES	LEAVES WEIGHT (g)	ROOT WEIGHT (g)	STEM WEIGHT (g)	ROOT/SHOOT
FREQUENCY	0.2403	0.0777	0.0721	0.0347	0.9661	0.2521	0.6087	0.1707
QUANTITY	0.0017	0.2631	0.0873	0.4032	0.4606	0.9074	0.2997	0.2180
FREQUENCY X QUANTITY	0.0293	0.9593	0.8679	0.8645	0.6281	0.6774	0.6029	0.0682

TABLE IV. Average ± SE of functional traits of *Pinus radiata* seedlings exposed to a combination of three watering frequencies (every 5, 10 and 30 days), and two watering quantities (20 and 50 ml). Different letters show statistically significant differences (Tukey test, p < 0.05). Letters are missing when there was no significant effect from the two-way ANOVA for a trait. *n*=10 plants per treatment.

TABLA IV. Promedio ± ES de rasgos funcionales de plántulas de *Pinus radiata* expuestas a una combinación de tres frecuencias de riego (cada 5, 10 y 30 días)y dos cantidades de riego (20 and 50 ml). Letras diferentes muestran diferencias significativas (Tukey test, p<0.05). No se muestran letras cuando no se encontró un efecto significativo en el ANOVA de dos vías para el rasgo. *n*=10 plantas por tratamiento.

	FINAL STEM WIDTH (mm)	FINAL HEIGHT (cm)	RGR (cm day^{-1})	NUMBER OF LEAVES	LEAVES WEIGHT (g)	ROOT WEIGHT (g)	STEM WEIGHT (g)	ROOT/SHOOT
20ml -5 d	3.51±0.17[a]	23.3±1.29	0.052±0.01	463±47[b]	0.86±0.11	0.76±0.06	0.64±0.06	0.53±0.04
50ml -5 d	4.41±0.15[b]	24.0±0.54	0.076±0.03	464±44[b]	0.85±0.09	0.71±0.04	0.76±0.05	0.46±0.03
20ml -10 d	3.78±0.14[ab]	22.9±0.60	0.024±0.01	405±32[ab]	0.79±0.09	0.67±0.09	0.65±0.09	0.49±0.04
50ml -10 d	3.84±0.17[ab]	23.8±1.45	0.044±0.01	446±25[b]	0.94±0.05	0.68±0.05	0.72±0.07	0.41±0.02
20ml -30 d	3.53±0.11[a]	20.8±0.93	0.038±0.01	345±29[a]	0.83±0.08	0.60±0.06	0.64±0.05	0.40±0.02
50ml -30 d	3.85±0.17[ab]	22.0±0.75	0.053±0.01	385±49[ab]	0.86±0.11	0.66±0.08	0.62±0.07	0.46±0.04

DISCUSSION

The survival of *Pinus radiata* seedling exposed to drought was increased by previous inoculation with *R. luteolus*. This increased drought tolerance due to mycorrhizae inoculation has been previously reported for different plant species (Ortega *et al.* 2004, Steinfeld *et al.* 2003). It has been shown that mycorrhizae can induce root hair bifurcation, growth and survival (Brundrett *et al.* 1996, Duan *et al.* 1996). In our work, inoculated plants had higher root/shoot ratio compared to control plants. A higher root/shoot results in greater effective absorption surface and exploration of new areas in the soil (Hunt & Nichols 1986). Mycorrhization also increased average tracheid radius. This implies greater water conductance, and hence, higher carbon gain (Hubbard *et al.* 2001, Meinzer & Grantz 1990, Sperry & Pockman 1993). Mycorrhization could ameliorate the negative effects of drought via increasing root surface and sustaining higher levels of water supply to the leaves, increasing photosynthesis. *Rhizopogon* inoculation has been found to increase the photosynthetic rate and stomatal conductance in *Pseudotsuga menziesii* seedlings (Dosskey *et al.* 1990). It has been suggested that higher photosynthetic rate enhance the biomass, growth and survival in conifer seedlings (Pinto *et al.* 2012). This way, mycorrhization can improve the performance of trees in two ways: maintaining higher gas exchange in shoot enhancing the carbon up-take, and avoiding the negative effects of water shortage in root zone through of higher absorptive capacity for water and mineral nutrients (Kipfer 2011).

Despite the positive effect on survival, mycorrhization reduced plant size (height and dry weight) and did not significantly affect RGR. This could be explained by carbon movement from the plant to the fungi. Mycorrhizae obtain their carbon mostly from their host plant (Read 1987, Harris-Valle *et al.* 2009), resulting in a greater carbon demand in mycorrhizated roots compared to non-infected roots (Paul *et al.* 1985, Harris-Valle *et al.* 2009). Carbon export to mycorrhizae has been found in *Rhizopogon vinicolor* infecting other Pinaceae species (Dosskey *et al.* 1990). Mycorrhizae inoculation can also cause increased transpiration (Pallardy *et al.* 1995), and hence, reduce the leaf water potential, increasing stomatal limitations for carbon gain.

At the highest and lowest watering frequencies (every 5 and 30 days respectively), watering quantity did not have any significant effect on plant survival. At the intermediate frequency (every 10 days), however, higher watering quantity resulted in increased survival. In a global change scenario with reduced precipitation (IPCC 2007), watering could be a possible strategy to increase *Pinus radiata* survival for the forestry industry; especially in early developmental stages. According to our results, a high watering frequency with relatively low watering quantity should avoid/reduce seedling mortality. The sparser watering frequencies (every 10 and 30 days) imposed severe drought stress on plant, impacting survival and growth. Previous studies on *Pinus* species have used less severe watering frequencies (Villar *et al.* 2000, Puértolas 2003), but in order to assess a more realistic scenario, the treatments used in our study represent the rain frequency usually recorded in summer for central Chile (Di Castri & Hajek 1976). In a future scenario of global change, with reduced precipitation, as suggested for several models (IPCC 2007), some regions in Chile will not be fit to support *Pinus radiata* growth and survival. This could negatively impact the forestry industry, an important source of income for the country. Watering in Chilean forestry is currently an uncommon practice, used only for some intensive plantations (Reyna & Colina 2007, Pereira *et al.* 2010b). If precipitations decline as predicted, watering could become a necessary managing strategy in the future.

In the forestry industry, plants are initially grown in nurseries. This helps in maintaining an adequate moisture level to avoid growth limitations (Landis *et al.* 1989, Lopushinsky 1990, Prieto *et al.* 2004). Watering is commonly used in order to maintain the plant water potential above -0.5 MPa (Landis *et al.* 1989, Prieto *et al.* 2004). Watering at the nursery improves plant quality and the likelihood of survival at later stages (Birchler *et al.* 1998). Mycorrhization and watering at early ontogenetical stages could be a feasible strategy to cope with plant mortality due to water stress after seedling establishment.

Several eco-physiological traits that enhance performance under drought are frequently looked for in plants to be used in reforestation of Mediterranean ecosystems (Vallejo *et al.* 2005). However, in stressed plants, different functional traits and management strategies are mixed, and evidence for which combinations could be advantageous in new climatic scenarios is scant and often speculative. For instance, our study suggested that high drought tolerance coupled with a conservative water use (condition observed with mycorrhizae inoculation) seems to render good results under current climatic conditions, where chronic drought alternates with seasonal rainfall. But mycorrhization as a successful strategy to enhance performance under future scenarios, characterized by extreme aridity and more unpredictable rainfall, can not be definitively concluded, and some sort of irrigation will be necessary to sustain forestry production.

CONCLUSIONS

Rhizopogon luteolus inoculation ameliorates drought stress on *Pinus radiata* seedling, but limits growth when water availability is higher. An increase in 60% in watering quantity is only relevant for seedling survival at intermediate watering frequencies (every 10 days). When frequencies were low (every 30 days) or high (every 5 days), the amount

of water supplied was irrelevant for seedling survival. At the intermediate watering frequency (every 10 days), survival is similar in inoculated plants watered with 20 ml and uninoculated plants watered with 50 ml. Thus, mycorrhizae inoculation could replace a higher watering quantity in order to maintain seedling survival, reducing the demand for irrigation water.

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