

Effectiveness of mycorrhizal inoculation in the nursery on growth and water relations of *Pinus radiata* in different water regimes

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Summary We compared the performance of mycorrhiza-inoculated and non-inoculated radiata pine (*Pinus radiata* D. Don) seedlings in two field studies. In the first study, above-ground growth of 2-year-old container-grown trees was compared after planting in two areas that differed in water availability. Mycorrhizal inoculation significantly improved aboveground growth in the 2 years after planting in both areas, especially at the drier site. In the second study, the influence of *Rhizopogon roseolus* (Corda ex Sturm) Th. Fries and *Scleroderma citrinum* Pers. on xylem water potential and hydraulic conductance at the soil–root interface of trees established at the same sites was assessed. Nursery inoculations with *R. roseolus* and *S. citrinum* improved tree growth during the first 2 years after field outplanting, particularly at the drier site.

Keywords: assimilation rate, field performance, fluorescence, hydraulic conductance, mycorrhizal trees, water potential.

Introduction

Radiata pine (*Pinus radiata* D. Don) is an economically important source of timber in northern Spain and has been widely used in reforestation programs for many decades. Currently, this species is the largest component of annual seedling production in regional tree nurseries that are producing stock for areas with low rainfall (400–600 mm). At present, seedlings are grown in containers to optimize the production process and to increase stock quality. However, the seedlings are not intentionally inoculated with mycorrhizal fungi, nor have any studies been conducted on their mycorrhizal status.

The lack of mycorrhizal fungi on root systems is a leading cause of poor plant establishment and growth in a variety of forest, restoration, agricultural, suburban and urban landscapes. At afforestation sites devoid of mycorrhizal propagules (e.g., cutover lands, treeless areas, mining spoils), non-mycorrhizal seedlings never develop fully and often die. Moreover, it has been demonstrated that specific endomycorrhizal or ectomycorrhizal fungi are able to improve the

survival and early growth of various tree species in the field. Mycorrhizal association is estimated to occur in 95% of native vegetation from undisturbed sites, whereas it occurs in less than 1% of vegetation from disturbed sites. However, mycorrhizal symbiosis can be reestablished, and the benefits of mycorrhizal association can be achieved by inoculating seedlings before they are transplanted to disturbed sites.

Considerable data on ectomycorrhizal fungi and their effects on reforestation under various ecological conditions have been reported (Castellano and Molina 1989, Kropp and Langlois 1990, Marx et al. 1991, Castellano 1996, Roldan et al. 1996, Garbaye and Churin 1997). Different fungal associations do not provide the same benefit to the host. A pronounced variability in response depending on the nature of the fungal–plant association has been observed (Tyminska et al. 1986, Chalot et al. 1988, Guehl et al. 1990, Stenström and Ek 1990). Thus, for successful afforestation, it is essential that nurseries produce tree seedlings associated with mycorrhizal fungi that are ecologically adapted to the tree species and the planting sites.

Water stress plays a major role in limiting the success of conifer seedling regeneration (Livingston and Black 1987). Previous studies have demonstrated that seedlings of *Pinus pinaster* Ait. inoculated with different dikaryotic cultures of *Pisolithus* spp. differed in their growth and physiological response to drought (Lamhamedi et al. 1990, 1992). Plants that form ectomycorrhizae with fungi that produce abundant rhizomorphs (such as *Rhizopogon* and *Scleroderma* species) may be more drought-tolerant, benefiting from the role of rhizomorphs in water transport (Duddridge et al. 1980).

However, large variation in response to inoculation arises from factors such as the degree of host–fungus compatibility, mycorrhizal dependency of the host, fungal effectiveness in relation to biotic and abiotic site conditions, and the abundance and effectiveness of indigenous fungi. Therefore, it is essential to carry out field experiments in a range of situations representative of major plantations to evaluate the possible economic advantage of ectomycorrhizal inoculation. Mycorrhizal inoculation cannot become a routine practice unless

large quantities of efficient and inexpensive inoculum are available. Although several methods of inoculation can be used in practice, only some species of the genera *Laccaria*, *Hebeloma*, *Paxillus*, *Pisolithus*, *Scleroderma*, *Suillus* or *Rhizopogon* have been routinely used (Castellano 1996).

The objective of this work was to evaluate the effectiveness of nursery mycorrhizal inoculation of container-grown radiata pine seedlings with *Rhizopogon roseolus* (Corda ex Sturm) Th. Fries and *Scleroderma citrinum* Pers. in improving the establishment of seedlings in plots differing in biogeoclimatic conditions. Specifically, we determined the effects of these inoculations on water relations and gas exchange parameters of *Pinus radiata* trees growing on sites differing in water availability.

Materials and methods

Nursery production of mycorrhizal *Pinus radiata*

In August 1999, radiata pine seeds (provenance NZ GF-13) were sown over a mixture of brown and black peat (70:30 v/v) in plastic trays. Each tray comprised 35 cavities 5.3 × 5.5 cm wide, 12.5 cm deep (Model 35/200, Arnabat, Barcelona, Spain). Seedlings were grown in a greenhouse for 2 months. Standard cultivation procedures included weekly fertilization with soluble fertilizer low in nitrogen and phosphorus (Peters, Scotts, Tarragona, Spain).

After 2 months in the greenhouse, seedlings were transferred to an open greenhouse and randomized into three groups of 36 trays each: non-inoculated seedlings as controls, and seedlings inoculated with spore suspensions of *R. roseolus* or with spore suspensions of *S. citrinum*.

Sporocarps of these fungi had been collected from two mature radiata pine stands of different ages located in northern Spain. After collection, sporocarps were cleaned, dried at 40 °C for 48 h and kept at room temperature until used. Spore slurry used for inoculation was prepared according to Castellano and Trappe (1985). Each sporocarp was blended in distilled water at high speed for 2–3 min. Initial spore density was determined with a haemocytometer, and the spore suspension was adjusted to a spore density of 10⁵–10⁶ spores ml⁻¹. Seedlings were inoculated by watering each cavity with 25 ml of the spore suspension once per month from October to January.

The seedlings were grown for 8 months, after which 20 seedlings per treatment were harvested at random. Shoot height, stem basal diameter and dry mass (80 °C, 48 h) of shoots and roots were determined. To assess mycorrhizal colonization, root systems were thoroughly and carefully washed free of soil and separated into three parts: upper, middle and lower root systems. Samples of each part were selected at random to determine the number of root tips that had formed well-developed mycorrhizas per total number of short roots formed.

Field experiments

In April 2000, trees were planted in commercial plantations at Zekutze (43°11' N, 3°9' W, altitude = 375 m) and Somokurcio (43°10' N, 2°48' W, altitude = 252 m). There is no significant air pollution in these areas. Both sites were surrounded by non-inoculated *P. radiata*, and were at a distance greater than 150 m from the nearest mature stands of *P. radiata*. Rainfall during summer was 295.9 and 169.1 mm at Zekutze and Somokurcio, respectively.

Plantations were established in a fully randomized block design with three treatments (non-inoculated trees, *R. roseolus* inoculated trees and *S. citrinum* inoculated trees), eight replicates per treatment and 25 trees per block.

Tree sampling

Two years after plantation establishment, all trees showed free growth. Total tree height and diameter at about 10 cm above the soil surface were measured. Needles were taken from separate branches at two canopy positions: the uppermost whorl of the tree (apical) and the basal northern quadrant. A needle sample was taken from the current, mature, fully exposed fascicles situated near the ends of branches of each canopy position. Each needle sample (10 mg) was stored in a bag in liquid nitrogen and returned to the laboratory. The plant material was oven-dried (80 °C for 48 h) and subsequently ground for isotope measurements.

¹³C Partitioning

To study possible variation in ¹³C associated with branch canopy position, duplicate carbon isotope measurements were made on 3 g of plant material composed of 2-cm segments taken from the central position of needles collected for the purpose. The dry needle material was finely ground and the ¹³C isotopic composition (δ¹³C) was analyzed with a Carbo Erba Elemental Analyzer (EA 1108 CHNS/O) connected to an isotope mass spectrometer (DELTAplus of Finningan MAT, Servicio de Investigación, Universidad de La Coruña, Spain).

Survival of inoculated fungi

To determine the survival and development of the inoculant of ectomycorrhizal fungi on roots of planted radiata pine trees, soil around trees was carefully hand-excavated at a distance of 0.5 m and a depth of 20 cm from the root collar, as described by Pera et al. (1999). Samples of lateral roots were taken and short roots were examined with the aid of a microscope for the presence of ectomycorrhizas of the inoculated fungi. Different types of ectomycorrhiza were macroscopically and microscopically characterized on the basis of the methodology and descriptions of Agerer (1987–1996, 1991). Results were expressed as number of mycorrhizal root tips per treatment in the field.

Soil water content and water relations parameters

Soil water content was determined gravimetrically. In September 2001, five soil cores were taken per treatment (50 mm di-

ameter and 20 cm depth) for gravimetric calculation of water content.

Hydraulic conductance (L_p) of the soil to needle pathway was estimated based on measurements in the same branches used for foliage sampling and $\delta^{13}\text{C}$ analysis. Hydraulic conductance was estimated as $K = E/(\psi_s - \psi_{md})$, as described by Hubbard et al. (1999).

Predawn and midday plant water potentials (ψ_{pd} , ψ_{md}) and transpiration rate (E) were measured in September 2001, 1 week after abundant rains. Five trees from each block and treatment were harvested, fascicles were cut from intact branches with a razor blade, and xylem water potential was measured immediately with a pressure chamber (Model 3005, Soil Moisture Equipment, Santa Barbara, CA).

Predawn water potential was used as an estimate of soil water potential (ψ_s), taking into account the hydrostatic gradient. Rates of transpiration were measured with an open infrared gas analyzer (IRGA, ADC type LCA-4, Analytical Development, Hoddesdon, Herts, U.K.) under ambient conditions.

Gas exchange parameters and chlorophyll fluorescence

Photosynthesis (A) and stomatal conductance (g_s) of five trees from each block and treatment were measured on several clear days during September 2001. On these days, photosynthetically active radiation (PAR) exceeded $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ between 0900 and 1700 h, and the vapor pressure deficit of the air remained above 1.27 kPa. Gas exchange parameters were measured with an ADC portable IRGA fitted to an open system as described by Lacuesta et al. (1993).

Chlorophyll a fluorescence was measured with an ADC portable modulated fluorimeter (Model OXI-5). Initial (F_o) and maximal fluorescence (F_m) were measured in dark-adapted leaves (60 min) with a saturating flash. The maximal apparent efficiency of Photosystem II was estimated as $F_v/F_m = (F_m - F_o)/F_m$.

Statistical analyses

Statistical evaluation of the data was performed with the SPSS (Chicago, IL) software package. For each measured parameter, plot and treatment variation were subjected to analysis of variance (ANOVA). All effects were considered random. The relationship between variables was analyzed by Pearson's correlation.

Results

Mycorrhization and biometric parameters

At the end of the nursery stage, mycorrhizal colonization of the root systems was similar for both inoculated fungi (*R. roseolus* and *S. citrinum*), with about 30% of the short roots infected (data not shown). In all treatments, inoculated and non-inoculated seedlings showed some short roots colonized by ectomycorrhizal fungi that were native to the nursery, but this percentage of mycorrhization was below 15%.

Two years after field outplanting, fine roots of inoculated trees at both field sites were colonized by the inoculant ecto-

mycorrhizal fungi. The number of mycorrhizal short roots formed by *R. roseolus* was higher than the number formed by *S. citrinum* (Figure 1). The number of mycorrhizal fine roots was higher at Somokurcio, which had lower water availability, than at Zekutze. At both sites, inoculated and non-inoculated trees were also colonized by other mycorrhizal fungi that formed morphotypes that differed in color and morphology from those formed by the native nursery fungi. We assumed that roots were being colonized by resident ectomycorrhizal fungi, including *Pisolithus tinctorius* (Pers.) Coker & Couch, sporocarps of which were found at the Somokurcio site during the measurement period. Mycorrhizal status at the time of field planting had a marked effect on the final size of the tree at both sites (data not shown).

Comparisons of soil volumetric water content (%) at the planting sites indicated that soil water availability was less at Somokurcio than at Zekutze (12 versus 20%). Trees growing at the Zekutze site showed significantly higher hydraulic conductance (L_p) than trees growing at Somokurcio (Figure 2). Two-way ANOVA also revealed a significant mycorrhizal effect on all the measured hydraulic parameters. A one-way ANOVA across stock types within the Somokurcio and Zekutze sites indicated a significant mycorrhizal effect on the hydraulic properties of apical branch needles at both sites, with the largest differences occurring in trees at the drier site (Somokurcio).

The ectomycorrhizal fungi *R. roseolus* and *S. citrinum* increased total L_p , especially in trees at Somokurcio, where the mean increase for the apical branch was 61 and 65%, respectively, and 27 and 24%, respectively, for basal branches.

One-way ANOVA across stock types with respect to branch position (apical and basal) indicated a significant site effect on hydraulic properties, mainly for the apical branch. Compared with trees at Zekutze, total L_p was lower in trees at Somokurcio, especially in control trees. Basal branch needles of Somokurcio trees exhibited on average 36, 53 and 78%

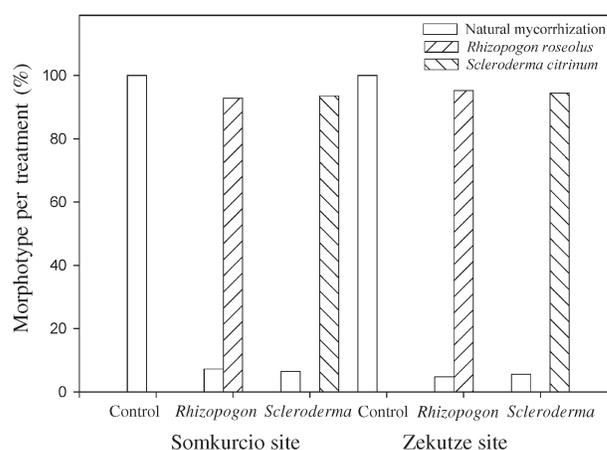


Figure 1. Morphotypes in soil from Somokurcio and Zekutze sites 2 years after nursery inoculation with *Rhizopogon roseolus* or *Scleroderma citrinum*. Measurements were made on 250 ml of soil taken randomly around five *Pinus radiata* trees from each block.

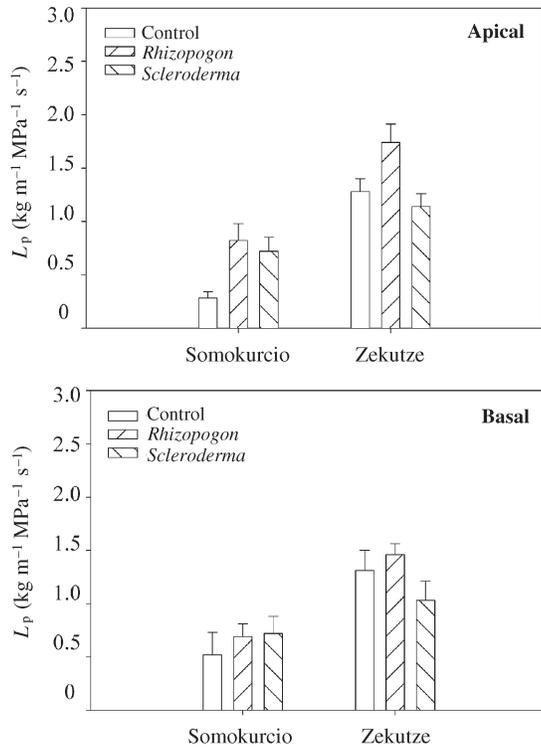


Figure 2. Hydraulic conductance (L_p) of *Pinus radiata* apical shoots and basal branches from trees differing in mycorrhizal status (*Rhizopogon roseolus* or *Scleroderma citrinum*) and location (Somokurcio and Zekutze sites).

(*R. roseolus*, *S. citrinum* and control, respectively) lower total L_p than the corresponding apical branch needles.

Gas exchange and chlorophyll parameters

Two years after planting, A of trees inoculated with *R. roseolus* or *S. citrinum* was higher than that of non-inoculated trees. Stomatal conductance and thus E were lower in trees at Somokurcio than in trees at Zekutze (Figure 3). At both sites, A was higher in apical needles than in basal needles, especially in mycorrhizal trees. Moreover, at the drier Somokurcio site, A , g_s and E decreased in both apical and basal branch needles with decreasing ψ_{pd} . Both A and g_s were more sensitive to decreasing ψ_{pd} ($P \leq 0.001$) in apical needles than in basal needles, particularly in non-inoculated trees.

At the Somokurcio and Zekutze sites, A was correlated with E ($r = 0.951$ and 0.610 , respectively) and with g_s ($r = 0.89$ and 0.78 , respectively) (Figure 3). However, seedlings at the two sites differed significantly in the response of A to E and to g_s . Seedlings at Somokurcio had a higher correlation because of a higher range of A and g_s values that showed treatment differences, whereas in seedlings at Zekutze, there was a narrow range in these values, with no differences between treatments.

When water deficit increased at Zekutze, the slope of the A/g_s curve increased, indicating that the decrease in A was largely attributable to stomatal closure rather than to an intrinsic effect of photosynthetic capacity (Figure 3). However, in

seedlings at Somokurcio, when g_s declined, reducing transpiration and thus preventing the development of excessive water deficits, internal CO_2 concentration increased, indicating that the decline in A was caused by stomatal limitations and not by non-stomatal factors.

In vivo chlorophyll fluorescence, expressed as F_v/F_m ratio, corroborated these results (Figure 4). In trees at Zekutze, significant differences between treatments did not occur, indicating that photosystem II was undamaged. Therefore, the decrease in A must have been caused by stomatal limitations (data not shown). Mean F_v/F_m (0.715) was lower in Somokurcio trees than in Zekutze trees (0.800), indicating that the lower water availability at Somokurcio affected the photosynthetic apparatus, although serious damage was not observed. Likewise, there were significant differences between treatments in the Somokurcio trees, revealing the advantage of the mycorrhizal status for the tree. Radiata pine seedlings mycorrhizal with *R. roseolus* or *S. citrinum* showed significantly higher F_v/F_m ratios than non-inoculated trees. Moreover, the decrease in A was clearly related to the decrease in F_v/F_m (Figure 4). Among treatments, both apical and basal needles of control trees showed the lowest F_v/F_m values, as a result of an increase in F_o and a decrease in F_m (data not shown).

¹³C Partitioning

Site and branch canopy position had significant effects on $\delta^{13}\text{C}$, whereas the influence of branch length was negligible. Maximum ¹³C depletion occurred in foliage with the least exposure to direct solar radiation (north to west). The general pattern observed on the sampled trees is shown in Figure 5. At Somokurcio, $\delta^{13}\text{C}$ values were around -26‰ in apical needles, and below -27‰ in basal needles. Variation in $\delta^{13}\text{C}$ in trees growing at Zekutze (higher water availability) was significantly higher than in trees growing at Somokurcio ($P < 0.001$). The mycorrhizal status \times site interaction was significant for $\delta^{13}\text{C}$ ($P < 0.001$), indicating that $\delta^{13}\text{C}$ values of mycorrhizal trees change with water availability.

Based on these comparisons of $\delta^{13}\text{C}$ variation in foliage collected from the ends of branches of various lengths, canopy positions and heights, we infer that the shorter a branch and the less its exposure to solar radiation, the more favorable its water relations should be. Physiological measurements made in the morning and afternoon on a clear day confirmed that water relations were more favorable for foliage on shaded branches than on sun-exposed branches (although the differences were not significant), and that water relations were more favorable for mycorrhizal trees than for non-mycorrhizal trees, especially at Somokurcio.

A plot of tree height versus $\delta^{13}\text{C}$ indicated that the most water-stressed trees were the control trees at Somokurcio (Figure 5), which exhibited lower A and were shorter than the mycorrhizal trees at Somokurcio. Comparing sites, $\delta^{13}\text{C}$ values were less negative at Somokurcio than at Zekutze, indicating that trees growing at Somokurcio were more water stressed. However, nursery-inoculated trees were taller than

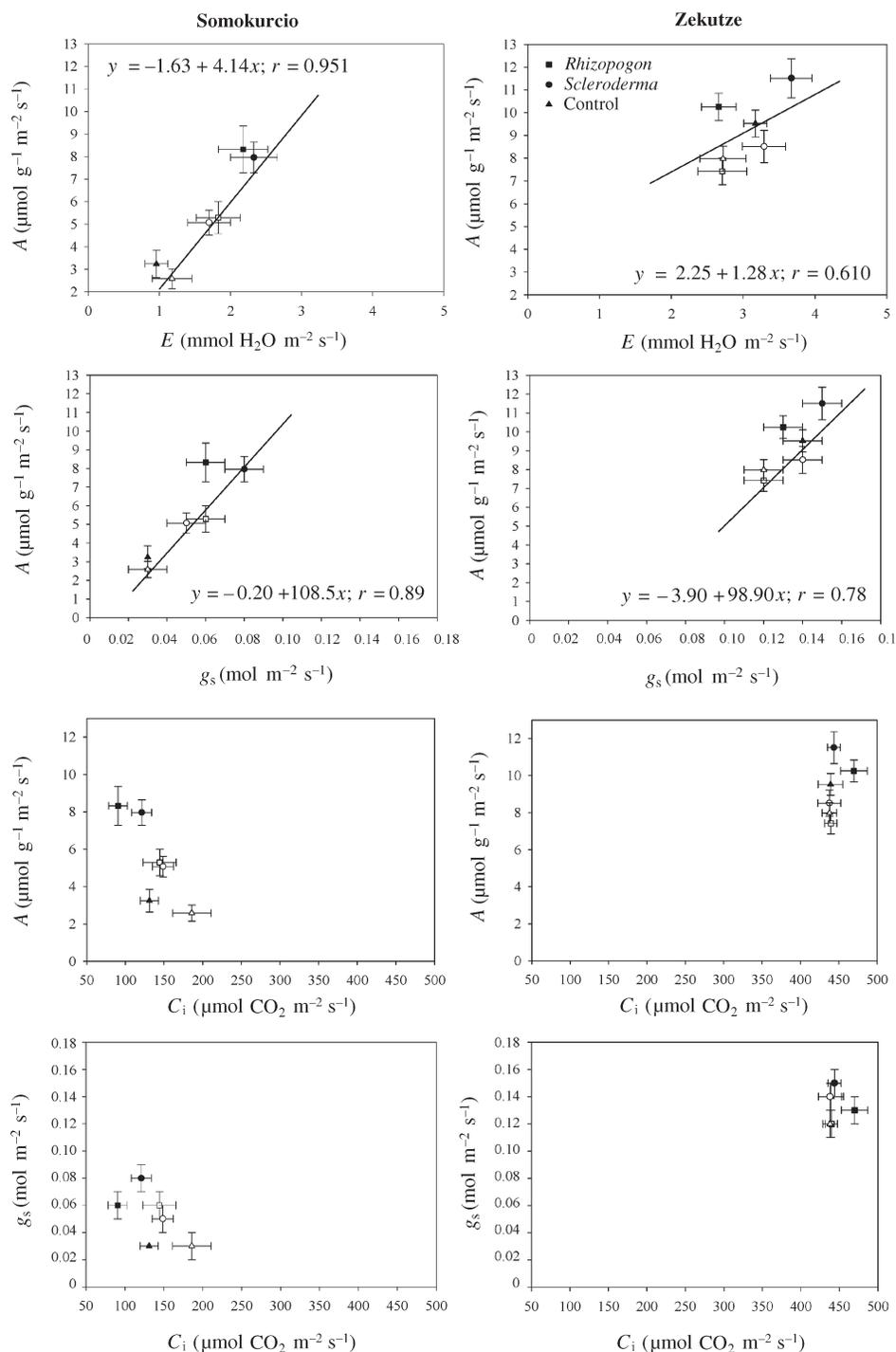


Figure 3. Relationships between net photosynthesis (A) and transpiration (E), stomatal conductance (g_s) and internal CO_2 concentration (C_i) of *Pinus radiata* apical shoots and basal branches from trees differing in mycorrhizal status (*Rhizopogon roseolus* or *Scleroderma citrinum*) and location (Somokurcio and Zekutze sites). Solid symbols represent apical needles and open symbols represent basal needles.

control trees, and the trees at Somokurcio were taller than the trees at Zekutze, especially the *R. roseolus* mycorrhizal trees.

Discussion

Mycorrhization and biometric parameters

Our field results indicate that good forest tree nursery practice and compatible inoculation programs can ensure that radiata

pine seedling roots are infected with mycorrhizal fungus, resulting in improved growth after field planting. The degree of mycorrhization necessary, if artificial fungal inoculation is to be effective after the nursery stock has been transplanted to the field, has been studied for only a few ectomycorrhizal species. For *Pisolithus tinctorius* (Marx 1980, Ruehle et al. 1981), infection of approximately 50% of roots is necessary to ensure effective inoculation (Last et al. 1990, Trofymov 1990). For other species such as *Amanita muscaria* (L.:Fr.) Hook.,

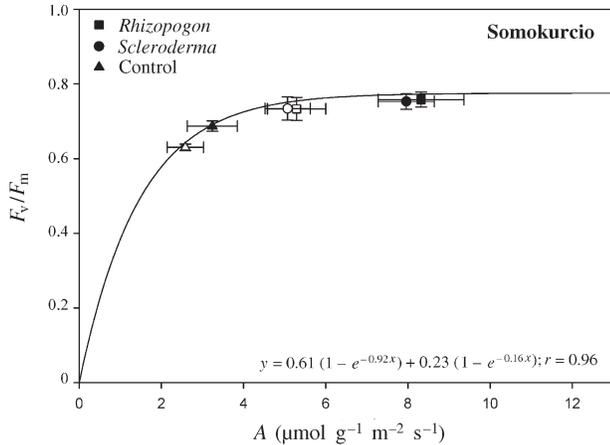


Figure 4. Relationship between maximal apparent efficiency of photosystem II (F_v/F_m) and net photosynthesis (A) in *Pinus radiata* needles during September 2001. Measurements were made on needles fully exposed to radiation on apical branches (solid symbols) and on basal-north branches (open symbols), from trees differing in mycorrhizal status (*Rhizopogon roseolus* or *Scleroderma citrinum*) from the Somokurcio site.

Lactarius rufus (Scopoli) Fr. or *Tricholoma albobrunneum* (Pers.:Fr.) Kummer, good results have been obtained with lower percentages of initial mycorrhization (Stenström and Ek 1990).

In our study, a low degree of mycorrhizal colonization was obtained in the nursery following inoculation with *R. roseolus* and *S. citrinum*, presumably because of the high water content of the substrate and competition by native nursery fungi (mainly *Thelephora terrestris* Ehrh.:Fr. and *Laccaria* spp.) that are well-adapted to standard nursery conditions in northern Spain. These native mycorrhizas had no beneficial effects on the tree, and when the trees were established in the field,

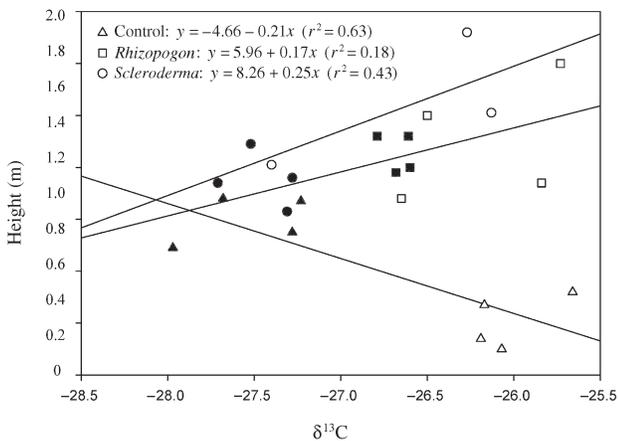


Figure 5. Effects of mycorrhizal status and site on the relationship between ^{13}C isotopic composition ($\delta^{13}\text{C}$) and height of *Pinus radiata* trees. Solid and open symbols represent trees grown at Zekutze and Somokurcio, respectively.

these fungi disappeared or were replaced by naturally occurring fungi from the plantation sites.

Despite the low degree of mycorrhizal colonization at the time of field planting, mycorrhizal infection had a pronounced effect on tree growth, and a positive effect on tree water relations, enhancing adaptability to low soil water availability. Needle water potential was less negative in Zekutze trees than in Somokurcio trees. Soil water content and gas exchange parameters were lower at Somokurcio than at Zekutze, indicating that water availability at Somokurcio partially limited tree development.

Morphotypic analysis showed that different fungi responded differently to soil water potential, and that the field site with lower water availability had an increased percentage of morphotypes corresponding to the original inoculated species. There is evidence that soil water status influences the specificity of colonization of radiata pine roots. Theodorou (1978) showed that colonization by *Rhizopogon luteolus* Fr. & Nordh. was greatest at soil water contents of 13% (−120 kPa osmotic potential) and was significantly depressed at 6.5% (−510 kPa) and 27% (−40 kPa).

Gas exchange parameters and chlorophyll parameters

Plant hydraulic conductance has been used to quantify tree behavior in the soil–plant–atmosphere continuum (Nelsen 1987, Lamhamedi et al. 1992) and to assess the functional significance of mycorrhizal fungi in water transport (Sands and Theodorou 1978, Sands et al. 1982, Nelsen 1987). Safir et al. (1971) and Morte et al. (2000) suggested that mycorrhization could increase water uptake by increasing effective root hydraulic conductivity. Likewise, we found that hydraulic conductance was increased by ectomycorrhizas formed by *R. roseolus* and *S. citrinum* in juvenile radiata pine, especially at the site with lower water availability.

We observed the presence of abundant and well-developed rhizomorphs on the root systems of trees inoculated with *R. roseolus* and *S. citrinum*. The architecture of the extramatrical phase of mycorrhizas influences resistance to water flow through the soil–root interface, and rhizomorphs have been shown to absorb water and facilitate its transport over ecologically significant distances (Duddridge et al. 1980, Foster 1981, Brownlee et al. 1983, Read and Boyd 1986, Cairney 1992).

Recently, Hubbard et al. (1999) compared old and young *Pinus ponderosa* var. *ponderosa* Dougl. trees to test the hypothesis that lower hydraulic conductance limits photosynthesis. They suggested that water transport in trees is regulated by the hydraulic conductance of the soil–root–shoot–leaf pathway, whereas whole-tree hydraulic conductance is a function of path lengths and permeability of these tissues. Because stomatal conductance and photosynthesis depend on the transport of water from soil to leaf and atmosphere, changes in whole-tree hydraulic conductance may affect leaf gas exchange.

Water relations, measured as gas exchange, were significantly affected by inoculation with *R. roseolus* and *S. citrinum*, mainly when soil water availability was low. Contradictory

results have been reported with respect to the ability of mycorrhizal associations to neutralize the effects of water limitation on tree growth. Early studies showed lower shoot water potentials in drought-stressed ectomycorrhizal seedlings compared with non-mycorrhizal seedlings of *Pinus radiata* (Sands and Theodorou 1978), *Pseudotsuga menziesii* (Mirb.) Franco (Parke et al. 1983) and *Picea sitchensis* (Bong.) Carr. (Lehto 1992). Moreover, Sands and Theodorou (1978) detected no significant difference in transpiration rates between drought-treated non-inoculated radiata pine seedlings and drought-treated *Rhizopogon*-inoculated seedlings. In contrast, other authors have found positive effects of mycorrhization under drought conditions. Pallardy et al. (1983) studied tissue water relations of mycorrhizal and non-mycorrhizal *Pinus echinata* Mill. seedlings during drying cycles and found that trees inoculated with *Pisolithus tinctorius* showed significantly greater root and shoot dry mass than non-inoculated trees.

Our results are consistent with studies showing that stomatal conductance and transpiration rates are higher in drought-treated mycorrhizal trees than in drought-treated non-mycorrhizal trees, as a result of enhanced water uptake (Augé et al. 1987, Subramanian et al. 1995, Duan et al. 1996), which allows the maintenance of higher photosynthetic rates and higher water contents in the mycorrhizal trees (cf. Morte et al. 2001).

Chlorophyll fluorescence parameters indicated that trees mycorrhizal with *R. roseolus* or *S. citrinum* suffered less severe stress than control trees when soil water availability was low. The decrease in F_v/F_m is a good indicator of photo-inhibitory damage caused by light when plants are subjected to environmental stresses (Ögren and Öquist 1985). At low water availability, leaves exhibiting low assimilation rates may be exposed to high irradiance stress, especially at midday. Under such conditions, the photosynthetic apparatus suffers photo-damage as a result of over-excitation of photosystem II (Powles 1984, Kyle 1987). The decrease in F_v/F_m may be caused by an increase in F_o , as a result of the destruction of the photosystem II reaction centers, or by a decrease in F_v , indicating an increase in non-photochemical quenching (Björkman and Powles 1984, Björkman 1986, Baker and Horton 1988). In our study, *R. roseolus* and *S. citrinum* mycorrhizal trees showed a decrease in F_v/F_m with respect to control trees, suggesting a mitigation of the negative effects of water deficit.

Under conditions of low water availability, inoculation of radiata pine seedlings with *R. roseolus* or *S. citrinum* resulted in increased water uptake that was sufficient to permit the stomata to remain open for most of the day. The ability to maintain open stomata and photosynthesis during the early stages of a drought could increase the supply of carbon for growth, particularly new root growth, which requires current photosynthates (van den Driessche 1987). Moreover, an increased rate of photosynthesis also supplies additional carbon for osmotic adjustment, thereby promoting turgor maintenance (Tan et al. 1992) and permitting higher water uptake from dryer soils. Therefore, inoculation of radiata pine with *R. roseolus* or *S. citrinum* provides the tree with a greater capacity to tolerate limited soil water availability.

¹³C Measurements

Several authors have discussed the effects of variation in leaf and branch length on the stable isotope composition of plant tissue (Waring and Silvester 1994, Walcroft et al. 1996, Warren and Adams 2000). However, there have been few field studies, particularly with conifers, to determine the effects and interactions of different environmental stresses conditioned by ectomycorrhizal status on $\delta^{13}\text{C}$.

Our young juvenile trees presented the same kinetics observed by Waring and Silvester (1994) with adult radiata pine. They observed that, in needles from trees with 0.5- to 10-m-long branches representing extremes in sun exposure, ^{13}C enrichment varied consistently and linearly with branch length. Branch length, rather than branch height, appeared to be the major contributor to variation in $\delta^{13}\text{C}$. At the Somokurcio site, we observed significant decreases in hydraulic conductance of apical branches exposed to direct radiation for more than 4 h (midday) on a day with prolonged high irradiance and high humidity deficits. These decreases were associated mainly with a reduction in stomatal conductance rather than with a change in water potential. At the same site, the control trees reduced their internal CO_2 concentration (C_i), resulting in less ^{13}C depletion in leaves in response to changes in water-conducting properties.

We conclude that the $\delta^{13}\text{C}$ in juvenile foliage of radiata pine is an accurate reflection of environmental conditions during needle expansion and carbon accumulation. Similarly, Warren and Adams (2000) showed that $\delta^{13}\text{C}$ of adult foliage of *Pinus pinaster* reflects environmental conditions during needle expansion. We note that $\delta^{13}\text{C}$ can be affected by the induced ectomycorrhizal status.

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