BIOLOGIA PLANTARUM 44 (2): 263-267, 2001

Growth and water relations in mycorrhizal and nonmycorrhizal *Pinus halepensis* plants in response to drought

A. MORTE*, G. DÍAZ*, P. RODRÍGUEZ**,***, J.J. ALARCÓN***, M.J. SÁNCHEZ-BLANCO***[a]

*Dpto. Biologia Vegetal, Facultad de Biología, Universidad de Murcia, E- 30071, Murcia, Spain**
*Instituto Nacional de Ciencias Agrícolas, Gaveta Postal 1, 32700, San José de las Lajas, La Habana, Cuba***
Dpto. Riego y Salinidad, Centro de Edafología y Biología Aplicada del Segura (CSIC),
*P.O. Box 4195, E-30080, Murcia, Spain****

Abstract

Mycorrhizal and nonmycorrhizal *Pinus halepensis* plants were subjected to water stress by withholding irrigation for four months and then rehydrated for 30 d. Water stress affected plants growth and mycorrhizal association was unable to avoid the effects of drought on plant growth. However, when irrigation was re-established the increase in height, number of shoots, total dry mass, and chlorophyll content in the mycorrhizal plants were greater than in non-mycorrhizal plants. The decrease in soil water content decreased the leaf water potential, leaf pressure potential and stomatal conductance. These decreases were higher for nonmycorrhizal than for mycorrhizal plants, indicating that the mycorrhizal fungi permit a higher water uptake from the dry soils. The total content of inorganic solutes was not changed by presence of mycorrhizae.

Additional key words: chlorophyll, dry mass, ectomycorrhizal fungi, fresh mass, pine, osmotic potential, pressure potential, stomatal conductance, water potential.

Introduction

Nutritional aspects of conifer mycorrhizal associations have been studied more often than water relations (Augé *et al.* 1986, Fitter 1988). While different modification of host plant water relations induced by the fungus have been reported in the arbuscular mycorrhizal symbiosis, but little information exists of ectomycorrhizal associations. Stomatal conductance, transpiration rate and leaf water potential is often higher in mycorrhizal plants under drought because of a higher water uptake (Augé 1987, Duan *et al.* 1996, Subramanian *et al.* 1995). However, other authors have suggested that ectomycorrhiza may cause an increase in water stress, since stimulation of transpiration may cause lower leaf water potentials (Pallardy *et al.* 1995).

Pinus halepensis Mill., is well adapted to semiarid conditions and widely used in reforestation programmes in the Mediterranean area, in symbiosis with ectomycorrhizal fungi. The response to water deficit, osmotic adjustment (Meier *et al.* 1992, Newton *et al.* 1989), enhanced tissue elasticity (Meier *et al.* 1992, Emadian and Newton 1989), and the regulation of gene expression (Funkhouser *et al.* 1993) were observed. However, the influence of ectomycorrhizal symbiosis on these mechanisms is still unknown.

The objective of the present work was to study the effects of drought/rewatering cycle and ectomycorrhizis on the water relations and growth of *Pinus halepensis* plants.

Received 30 May 2000, *accepted* 18 September 2000.

Abbreviations: g_s - stomatal conductance; DM - dry mass; FM - fresh mass; Ψ_l - leaf water potential; Ψ_p - leaf pressure potential;. Ψ_s - leaf osmotic potential.

Acknowledgements: The authors are grateful to Mrs. M.D. Velasco and Mrs. M. García. This research was supported by INCO-DC MYRISME project (ERB 3514 PL 96 1889) from the European Comunity.

[a] Corresponding author; fax: (+34) 968 396213, e-mail: quechu@natura.cebas.csic.es

Materials and methods

Carpophores of *Suillus mediterraneensis* were collected in October 1995 under *P. halepensis* in La Puerta, Moratalla, Murcia, Spain. Isolations were made from carpophore tissue on modified Melin-Norkrans (MMN) solid medium (Marx 1969), and then transferred to fresh media every three months. 500 cm^3 bottles were filled with mixture of peat:vermiculite (1:4 v/v), sterilised twice by autoclaving (60 min, 120 °C), saturated with MMN liquid medium, and again sterilised by autoclaving (20 min, 120 °C). The bottles were inoculated with a culture of *S. mediterraneensis* in MMN liquid medium and then incubated at 23 °C in the dark for 8 weeks.

P. halepensis Mill. plants were from in Poliforest® containers on a substrate consisting of a 2:1:1 (v/v) mixture of *Sphagnum* peat / black peat / perlite + vermiculite, sterilised by autoclaving (115 °C, 60 min). Seeds were sterilised in H_2O_2 for 30 min before sowing. The total amount of macronutrients received by each seedling was 35 mg N, 27 mg P, 61 mg K. Controlled inoculation was carried out three months after sowing, when the seedlings had developed the secondary roots suitable for mycorrhization, inoculum being placed on the root surface at a rate 1:10 (v/v). The formation of mycorrhizae was noted 1 - 2 months after inoculation, while three months after inoculation all the plants were assessed for mycorrhiza formation. Seedlings which showed more than 50 % of mycorrhizal roots were selected for the water stress experiment. One-year-old seedlings were transferred to individual pots and maintained in the greenhouse. When the plants were 18-months old, the half of them was subjected to water stress by withholding irrigation water for four months (from 20 October until 20 February). After this period, these plants were irrigated to run off and were subsequently maintained at sufficient water supply for 30 d. During the experiment, temperatures ranged from 10 to 20 °C, and the relative humidity was between 50 and 80 %. Control, uninoculated seedlings were produced under the same conditions. A total of 60 plants were used, 15 plants per treatment.

At the end of the stress and also at the end of the recovery, five plants per treatment were harvested and weighed. The percentage of colonisation was determined before they were dried for 48 h at 100 °C. Chlorophyll was extracted in acetone. The absorbance of the chlorophyll extract was assessed with a spectrophotometer (*Gricel-RDJ-55*, Barcelona, Spain), and the chlorophyll concentration was calculated with the equations of Inskeep and Bloom (Inskeep and Bloom 1985).

At the beginning of water stress (October), 2 months after imposition of stress (December), at the end of the stress (February) and at the end of recovery (March), leaf water potential (Ψ_l), leaf osmotic potential (Ψ_s) and leaf pressure potential (Ψ_p) were measured at midday on 6 apical twigs taken from each treatment. Water potential was determined using a pressure chamber (*Soil Moisture Equipment Corp.*, Santa Barbara, USA), according to Scholander *et al* (1965). Ψ_s was estimated using a vapour pressure osmometer (*Wescor 5500*, Logan, USA) in excised leaves, immediately frozen and stored at -30 °C. Before the measurements were made, samples were thawed and leaf sap expressed for osmotic potential determination according to Gucci *et al.* (1991). Leaf pressure potential (Ψ_p) was calculated as the difference between Ψ_l and Ψ_s.

At the same times, leaf gas exchange was measured at saturated photon flux density (PFD >1000 $\mu mol\ m^{-2}\ s^{-1}$) on atached leaves, using a *Licor LI-1600* (Lincoln, USA).

At the end of the water stress period samples of five plants per treatment were weighed, dried and stored. These dry shoot samples were used to determine nutrient content. Mineral concentrations (N, P, K, Ca, Mg, Fe, Cu, Mn, Zn, B and Na) in both shoots and roots tissues were determined. Subsamples were digested in nitric : perchloric acid mixture (5:3) for 6 h and phosphorus contents was determined colorimetrically with the malachite green reagent (Fernández *et al.* 1985) and other elements by atomic absorption spectrophotometry (*Perkin-Elmer 1100B*, USA).

Results

Water stress did no affect the percentage of root mycorrhizal colonization but did affect *P. halepensis* growth (Table 1). Mycorrhizal and nonmycorrhizal control plants showed a slightly higher plant height, shoot numbers and dry mass compared to mycorrhizal and nonmycorrhizal water stressed plants. Values of fresh mass and FM/DM ratio in the mycorrhizal plants were higher than in nonmycorrhizal plants, independently of soil water conditions (Table 1). In general, the chlorophyll content was higher in mycorrhizal plants, the mycorrhizal control plants presented the highest values (Table 1). Survival was also higher in mycorrhizal than in nonmycorrhizal plants at the end of the water stress period, when soil moisture percentage in the stressed treatments was about 23 % of that in control treatments.

During recovery, the plant water content increased in the previously stressed plants, and this increase was greater in mycorrhizal than in nonmycorrizhal plants, the height, number of shoots, the total dry mass and chlorophyll content increased substantially (Table 1). Thus, at the end of the experiment, these parameters were close to level in control plants (Table 1).

No significant differences were observed in the shoot mineral content between mycorrhizal and nonmycorrhizal plants, and control and stressed plants (Table 2).

Table 1. Shoot height, number of branches, total fresh mass, dry mass, and leaf chlorophyll content for *Pinus halepensis* subjected to four different tretaments (C+M, mycorrhizal control; C-M, nonmycorrhizal control; S+M, mycorrizal stressed; S-M, nonmycorrhizal stressed) at end of water stress, end of recovery period. Means whithin a column and time that do not have a common letter are significantly different by $LSD_{0.05}$ test.

	Treatment	Shoot height [cm]	Number of branches	Fresh mass [g plant^{-1}]	Dry mass [g plant^{-1}]	FM/DM	Chlorophyll [μg g^{-1}(DM)]
Stress	C+M	17.1 a	13.5 a	14.8 a	5.0 a	2.96 a	647 a
	C-M	17.2 a	13.0 a	10.7 b	4.5 a	2.38 b	590 b
	S+M	15.0 b	11.5 b	5.5 c	2.4 b	2.30 b	464 c
	S-M	14.5 b	11.3 b	4.5 d	2.2 b	2.05 c	317 d
Recovery	C+M	17.5 a	13.8 a	15.1 a	5.1 a	2.97 a	721 a
	C-M	17.6 a	13.3 a	11.0 b	4.6 a	2.39 b	660 b
	S+M	16.3 b	12.4 b	7.8 c	2.6 b	3.00 a	631 c
	S-M	15.1 c	11.8 c	5.5 d	2.3 b	2.41 b	374 d

Table 2. Concentrations of N, P, K, Ca, Na, Mg, Fe, Cu, Mn, Zn and B [%] measured in both shoots and root tissues for *Pinus Halepensis* subjected to four different treatments (C+M, mycorrhizal control; C-M, nonmycorrhizal control; S+M, mycorrizal stressed; S-M, nonmycorrhizal stressed) at the end of the water stress period. Means whithin a element that do not have a common letter are significantly different by $LSD_{0.05}$ test.

Treatment	N	P	K	Ca	Na	Mg
C+M	0.81 a	0.21 a	0.99 a	0.57 a	0.14 a	0.27 a
C-M	0.70 a	0.22 a	0.95 a	0.47 a	0.15 a	0.25 a
S+M	0.85 a	0.22 a	0.96 a	0.43 a	0.19 a	0.21 a
S-M	0.75 a	0.28 a	0.94 a	0.54 a	0.17 a	0.27 a
	Fe	Cu	Mn	Zn	B	
C+M	0.00655 a	0.00027 a	0.02865 a	0.00498 a	0.00418 a	
C-M	0.00749 a	0.00013 a	0.02405 a	0.00588 a	0.00406 a	
S+M	0.00836 a	0.00032 a	0.02371 a	0.00539 a	0.00403 a	
S-M	0.00993 a	0.00014 a	0.02392 a	0.00561 a	0.00406 a	

The midday leaf water potential was high in the control plants throughout the experimental period, and no significant differences between mycorrhizal and nonmycorrhizal plants were noted (Fig. 1*A*). In the water stress treatments a progressive decrease of Ψ_l was detected from October to February. At the end of the stress period, the differences between mycorrhizal and nonmycorrhizal plants was about 0.4 MPa. The leaf osmotic potential remained unchanged during the stress period in all treatments (Fig. 1*B*). Leaf pressure potential (Ψ_p) behaved similarly to Ψ_l (Fig. 1*C*). When irrigation was resumed, a slow recovery of Ψ_l and Ψ_p in stressed plant was noted, Ψ_l reaching values of about -1.04 MPa and -0.85 MPa in nonmycorrhizal and mycorrhizal plants, respectively. At the same time, the Ψ_p values of mycorrhizal plants were generally higher than those of nonmycorrhizal plants.

Stomatal conductance (g_s) of mycorrhizal and nonmycorrhizal plants did not differ at high soil moisture (Fig. 2). Once the soil had dried, a substantial drop in g_s was observed, particularly in stressed nonmycorrhizal plants. This parameter recovered after rewatering in mycorrhizal plants to reach similar values to those of control plants and higher than those of nonmycorrhizal plants (Fig. 2).

Discussion

The mycorrhizal fungi permit a higher water uptake, because they increase the total root surface (Augé *et al.* 1987, Duan *et al.* 1996, Subramanian *et al.* 1995). In our experiment, the water content was higher in the mycorrhizal plants. However, it was unable to avoid the effects of drought on plant growth (Table 1), because probably other factors in addition to the plant water status were involved. However, when the plants were rewatered, the increase in biomass in the inoculated plants was greater than in uninoculated plants, which demonstrates the substantial benefits of the ectomycorrhizal fungus.

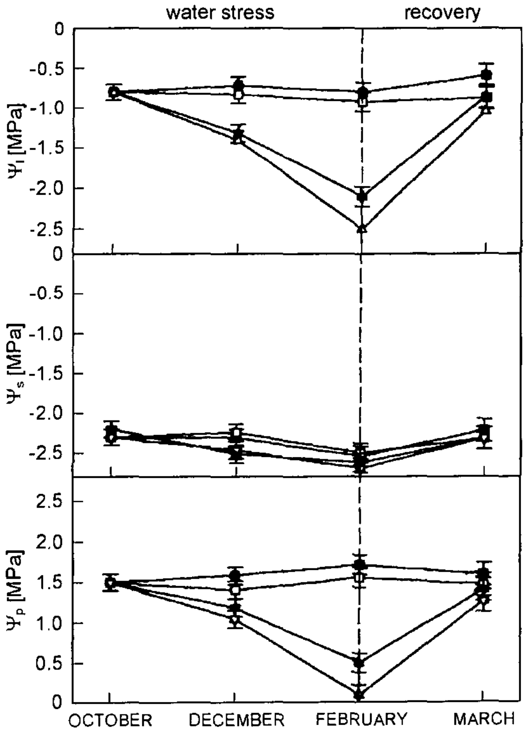

Fig. 1. Seasonal course of leaf water potential (Ψ_l), leaf osmotic potential (Ψ_s), and leaf pressure potential (Ψ_p) at midday for *Pinus halepensis* control plants (mycorrhizal - *closed circles*, nonmycorrizal - *open circles*), and water stressed plants (mycorrhizal - *closed triangles*, nonmycorrhizal - *open triangles*), at four different times of the growing season (October, December, February and March). Means of 6 measurements, *vertical bars* represent SE (not shown when smaller than the symbols). The *discontinuous line* indicates the irrigation time for the water stressed plants.

In well watered soil the effect of mycorrhizae on Ψ_l was not statistically significant, but under water stress mycorrhizal plants exhibited midday Ψ_l and Ψ_p values that were more than 0.4 MPa higher than in nonmycorrhizal plants.

Stomatal conductance was higher in mycorrhizal plants in similar soil water content similarly as pointed out by Augé *et al.* (1992) and Ebel *et al.* (1997). It has been demonstrated that mycorrhizal symbiosis can alter stomata sensitivity to ABA or change the amount of ABA transferred to shoots. Another possibility for higher g_s and shoot Ψ_l is the more efficient extraction of soil moisture by mycorrhizal root systems in dry soil (Duan *et al.* 1996). According to Sands and Mulligan (1990), the importance of mycorrhizae in stimulating water absorption is greater as the soil becomes drier.

After rewatering, leaf Ψ_p recovered in stressed plants, being higher in mycorrhizal than in non-mycorrhizal plants, as has been also described by Bryla and Duniway (1997). This may explain the enhancement in growth observed during this phase in the mycorrizhal plants. Similar results have been found for *Pinus virginia* (Walker *et al.* 1982) and for *Pinus taeda* (Walker *et al.* 1989).

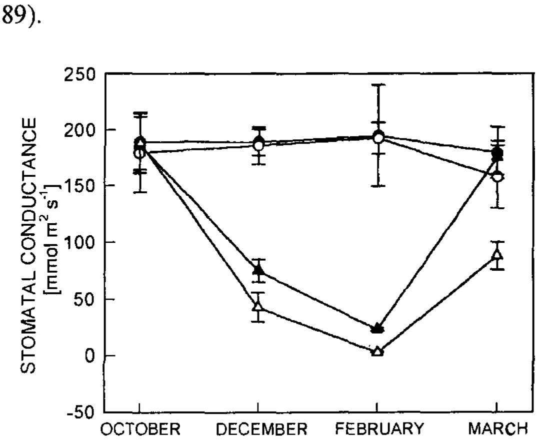

Fig. 2. Seasonal course of stomatal conductance at midday for *Pinus halepensis* control plants (mycorrhizal - *closed circles*, nonmycorrizal - *open circles*) and water stressed plants (mycorrhizal - *closed triangles*, nonmycorrhizal - *open triangles*), at four different times of the growing season (October, December, February and March). Means of 6 measurements, *vertical bars* represent SE (not shown when smaller than the symbols).

The decrease in Ψ_l during water stress was not accompanied by a clear decrease in Ψ_s which may be due to water loss from the apoplast (Joly and Zaerr 1987). These reductions in the water content of the apoplast and the increase in wall cell rigidity could be the main consequences of the drought stress in our assay. Such effects of water stress on tissue elasticity have been described previously in *Pinus* sp. (Meier 1992, Emadian 1989).

In *Pinus* species, osmotic adjustment has also been shown to act as a mechanism for turgor maintenance under water stress conditions (Nguyen and Lamant 1989), although great genotypic variation has been observed in this respect (Seiler 1990). For example, Newton *et al.* (1989) reported no osmotic adjustment in *Pinus taeda* under severe drought stress. Similarly, water stress conditioning in the nursery and applied in the autumn did not induce osmotic adjustment in *P. halepensis* seedlings (Villar-Salvador 1999). In our experiment, the absence of changes in the ψ_s and no accumulation of inorganic solutes indicate absence of osmotic adjustment.

The content of total inorganic solutes was not changed by the presence of mycorrhizae (Table 2). Thus, the indirect effect of improved P and N nutrition in mycorrhizal plants, as described by Fitter (1988) and Harley and Smith (1983), was not observed in our assay.

Our results indicate that ectomycorrhizal fungi improve *P. halepensis* plant water relations, although they did not improve plant growth under drought applied over an extended period. However, after a reirrigation the increase in root water absorption and subsequent improvement in the plant water status induced faster growth in mycorrhizal than in nonmycorrhizal plants.

References

Augé, R.M., Brown, M.S., Bethlenfalvay, G.J., Stodola, A.J.W.: Stomatal response of mycorrhizal cowpea and soybean to short-term osmotic stress. - New Phytol. **120**: 117-25, 1992.

Augé, R.M., Schekel, K.A., Wample, R.L.: Osmotic adjustment in leaves of VA mycorrhizal and non mycorrhizal rose plants in response to drought stress. - Plant Physiol. **82**: 765-770, 1986.

Augé, R.M., Schekel, K.A., Wample, R.L.: Rose leaf elasticity changes in response to mycorrhizal colonization and drought acclimatation. - Physiol. Plant. **70**: 175-182, 1987.

Bryla, D.R., Duniway, J.M.: Growth, phosphorus uptake, and water relations of sunflower and wheat infected with an arbuscular mycorrhizal fungus. - New Phytol. **136**: 581-590, 1997.

Duan, X., Neuman, D.S., Reiber, J.M., Green, C.D., Saxton, A.M. Auge, R.M.: Mycorrhizal influence on hydraulic and hormonal factors involved in the control of stomatal conductance during drought. - J. exp. Bot. **47**: 1541-1550, 1996.

Ebel, R.C., Duan, X., Still, D.W., Augé, R.M.: Xylem sap abscisic acid concentration and stomatal conductance of mycorrhizal *Vigna unguiculata* in drying soil. - New Phytol. **135**: 755-761, 1997.

Emadian, S.F., Newton, R.J.: Growth enhancement of lobolly pine (*Pinus taeda* L.) seedlings with silicon. - J. Plant Physiol. **134**: 98-103, 1989.

Fernández, J.A., Niell, F.X., Lucena, J.: A rapid and sensitive automated determination of phosphate in natural waters. - Limmol. Oceanogr. **30**: 227-230, 1985.

Fitter, A.H.: Water relations of red clover *Trifolium pratense* L. as affected by VA mycorrhizal infection and phosphorus supply before and during drought. - J. exp. Bot. **3**: 595-603, 1988.

Funkhouser, E. A., Cairney, J., Artlip, T.S., Chang, S., Dias, M.A., Newton, R.J.: Cellular and molecular responses to water deficit stress in woody crops. - In: Pessarakli, M. (ed.): Handbook of Plant and Crop Stress. Pp. 347-362. Marcel Dekker, New York 1993.

Gucci, R., Xiloyannis, C., Flore, J.A.: Gas exchange parameters water relations and carbohydrate partitioning in leaves of field-grown *Prunus domestica* following fruit removal. - Physiol. Plant. **83**: 497-505, 1991.

Harley, J.L., Smith, D.E.: Mycorrhizal Symbiosis. - Academic Press, London 1983.

Inskeep, W.P., Bloom, P.R.: Extinction coefficients of chlorophyll *a* and *b* in N,N-dimethylformamide and 80 % acetone. - Plant. Physiol. 77: 483-485, 1985.

Joly, R.J., Zaerr, J.B.: Alteration of cell-wall water content and elasticity in Douglas-fir during periods of water deficit. - Plant Physiol. **83**: 418-422, 1987.

Marx, D.H.: The influence of ectotrophic mycorrhizal fungi on the resistence of pine roots to pathogenic infections. I. Antagonism of ectomycorrhizal fungi to root pathogenic fungi and soil bacteria. - Phytopathology **59**: 153-163, 1969.

Meier, C.E., Newton, R.J., Puryear, J.D., Sen, S.: Physiological responses of lobolly pine (*Pinus taeda* L.) seedlings to drought stress: osmotic adjustement and tissue elasticity. - J. Plant Physiol. **140**: 754-760, 1992.

Newton, R.J., Puryear, J.D., Sen, S.: Water status of lobolly pine (*Pinus taeda* L.) callus. - Plant Cell Organ Tissue Cult. **16**: 3-13, 1989.

Nguyen, A., Lamant, A.: Variation in growth and osmotic regulation of roots of water-stressed maritime pine (*Pinus pinaster* Ait) provenances. - Tree Physiol. **5**: 123-133, 1989.

Pallardy, S.G. Čermák, J., Ewers, F.W., Kaufmann, M.R., Parker, W.C., Sperry, J.S.: Water transpor dynamic in trees and stands. - In:. Smith, W.K., Hinckley, W.K (ed.): Resource Physiology of Conifers: Acquisition, Allocation and Utilization. Pp. 301-389. Academic Press, London 1995.

Sands, R., Mulligan, D.R.: Water and nutrient dynamics and tree growth. - Forest. Ecol. Manage. **30**: 91-111, 1990.

Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemingsen, E.A.: Sap pressure in vascular plants. - Science **148**: 339-346, 1965.

Seiler, J.R., Cazell, B.H.: Influence of water stress on physiology and growth of red spruce seedlings. - Tree Physiol. 6: 69-77, 1990.

Subramanian, K.S., Charest, C., Dwyer, L.M., Hamilton, R.I.: Arbuscular mycorrhizas and water relations in maize under drought stress at tasseling. - New Phytol. **129**: 643-650, 1995.

Villar-Salvador, P., Ocaña, L., Peñuelas, J., Carrasco, I.: Effect of water stress conditioning on the water relations, root growth capacity, and the nitrogen and non-structural carbohydrate concentration of *Pinus halepensis* Mill. (Aleppo pine) seedlings. - Amer. Forests **56**: 459-465, 1999.

Walker, R.F., West, D.C., McLaughlin, S.B., Amundsen, C.C.: Growh, xylem pressure potential, and nutrient absorption of loblolly pine on a reclaimed surface mine as affected by an induced *Pisolithus tinctorius* infection. - Forest. Sci. **35**: 569-581, 1989.

Walker, R.F., West, D.C., McLaughlin, S.B.: *Pisolitus tinctorius* ectomycorrhizae reduce moisture stress of Virginia pine on southern Appalachiancoal spoil. - In: Proc. Seventh North American Forest Biology Workshop. Pp. 374-383. University of Kentucky, Lexington 1982.