

## BRIEF COMMUNICATION

## Photosynthetic and transpiration rates of *Olea europaea* subsp. *sylvestris* and *Rhamnus lycioides* as affected by water deficit and mycorrhiza

F. CARAVACA<sup>1</sup>, E. DÍAZ<sup>2</sup>, J.M. BAREA<sup>3</sup>, C. AZCÓN-AGUILAR<sup>3</sup> and A. ROLDÁN<sup>2</sup>

CSIC-CEBAS, Department of Soil and Water Conservation,  
P.O. Box 4195, Campus de Espinardo, E-30100 Murcia, Spain<sup>2</sup>

CSIC-Institute of Natural Resources and Agrobiology, P.O. Box 1052, E-41080 Sevilla, Spain<sup>2</sup>  
CSIC-EEZ, Department of Microbiology, Profesor Albareda 1, E-18008 Granada, Spain<sup>3</sup>

### Abstract

This study examined the effect of mycorrhizal colonization with *Glomus intraradices* on physiological parameters and foliar nutrient concentrations in *Olea europaea* L. subsp. *sylvestris* and *Rhamnus lycioides* L. seedlings subjected to well-watered or drought-stressed conditions. Under drought stress, mycorrhizal *O. europaea* seedlings showed significantly higher photosynthetic and transpiration rates, stomatal conductance and foliar P concentration than its similarly-sized non-mycorrhizal counterpart. The intrinsic water use efficiency (photosynthetic rate to stomatal conductance ratio) was not change in *O. europaea* and decreased in *R. lycioides* seedlings due to mycorrhizal colonization under both well-watered and drought-stress conditions.

*Additional key words:* intrinsic water use efficiency, *Glomus intraradices*, Mediterranean ecosystems, stomatal conductance, water stress.

Successful programmes of revegetation in soils where the water supply limits plant growth may require improvement of plant drought resistance, through mycorrhizal inoculation (Requena *et al.* 2001). Mycorrhizal symbiosis can increase the drought tolerance of plants, by enhancing nutrient and especially P uptake, changing hormonal signalling, and increasing the hydraulic conductivity of the roots and the water use efficiency of the plant defined as the ratio between photosynthesis and stomatal conductance (Augé 2001, Morte *et al.* 2001). Stomatal conductance of water vapour, transpiration and the photosynthetic rate are stimulated by arbuscular mycorrhizal (AM) fungal inoculation under drought conditions, effects which are dependent on the fungus involved in the symbiosis (Ruiz-Lozano *et al.* 1995). Likewise, the influence of AM infection on plant transpiration efficiency values shows that responses may vary widely between different plant

species (Augé 2001). The objective of this study was to evaluate interactive effects between mycorrhizal inoculation with a arbuscular mycorrhizal fungus (*Glomus intraradices* Schenck & Smith) and water stress on stomatal conductance, transpiration and photosynthetic rates, intrinsic water use efficiency and nutrient contents in leaves of *Olea europaea* L. subsp. *sylvestris* and *Rhamnus lycioides* L. seedlings.

The experiment was conducted as two completely randomised factorial designs (one per plant species) with two factors. The first factor had two levels: non-inoculation and inoculation with *G. intraradices*, and the second factor had two levels: well watered and drought stressed conditions. Five replicates per treatment were set up, making a total of 20 seedlings per plant species. Soil water deficit was imposed for six weeks (from 20 December until 31 January). For each plant species, well-watered plants were maintained at a substrate water

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*Abbreviations:* AM - arbuscular mycorrhizal; E - transpiration rate;  $g_s$  - stomatal conductance;  $P_N$  - net photosynthetic rate.

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<sup>1</sup>Corresponding author; fax: (+34)968396213; e-mail: fcb@cebas.csic.es

potential equivalent to field capacity (-0.03 MPa) and stressed plants were maintained at a substrate water potential close to wilting point (averaging -0.60 MPa).

After the induced water stress period, basal stem diameters and heights of plants were measured with callipers and rules. Plants were harvested, and the roots were washed free from soil under a stream of cold tap water. Fresh and dry (60 °C, 24 h) masses of shoots and roots were recorded. Plant tissues were ground before chemical analysis. The foliar concentrations of nitrogen, phosphorus and potassium were determined after digestion in nitric-perchloric acid (5:3) for 6 h. N was determined by the Kjeldahl method, the P concentration was determined by colorimetry (Murphy and Riley 1962) and the K uptake was estimated by flame photometry. The percentage of root length colonised by arbuscular mycorrhizal fungi was calculated by the gridline intersect method (Giovannetti and Mosse 1980), after staining with trypan blue (Phillips and Hayman 1970). Instantaneous measurements of the net photosynthetic and transpiration rates and stomatal conductance were made using a portable gas analyser system (LCA4 configured with a PLC4C chamber, ADC, Hoddesdon, UK), according to the methodology developed by Long *et al.* (1996).

After the water stress period, mycorrhizal inoculation and water regime had no significant effect on the height, the basal diameter or shoot dry mass of the *O. europaea* and *R. lycioides* seedlings (Table 1). Under drought stress, *G. intraradices*-colonised *O. europaea* seedlings showed significant increases in photosynthetic rate ( $P_N$ ), transpiration rate (E) and stomatal conductance ( $g_s$ ) over their similarly-sized non-mycorrhizal counterparts (Fig. 1). However, there were no significant differences in  $P_N$  of inoculated and non-inoculated *R. lycioides* seedlings subjected to drought stress (Fig. 1). In inoculated *O. europaea* seedlings,  $P_N$  may have been stimulated by the increased sink strength arising from the additional carbon requirements of the mycorrhizal fungus.

Table 1. Growth parameters, mycorrhizal colonisation and foliar nutrients of *O. europaea* and *R. lycioides* seedlings as affected by the inoculation treatment and the water stress. Means  $\pm$  SE,  $n = 5$ . For each plant species, values sharing the same letter are not significantly different ( $P < 0.05$ ) by the LSD test.

Species	Treatment	Treatment	Height	Diameter	Shoot d.m.	Mycorrhizal	Leaf N	Leaf P	Leaf K
			[cm]	[mm]	[g shoot $^{-1}$ ]	root length [%]	[g kg $^{-1}$ (d.m.)]	[g kg $^{-1}$ (d.m.)]	[g kg $^{-1}$ (d.m.)]
<i>O. europaea</i>	well-watered	control	20.6 $\pm$ 7.4a	2.5 $\pm$ 0.4a	0.82 $\pm$ 0.53a	6.4 $\pm$ 5.2a	6.8 $\pm$ 1.7a	1.1 $\pm$ 0.3a	14.5 $\pm$ 0.9a
		AM	24.8 $\pm$ 5.2a	2.6 $\pm$ 0.5a	0.81 $\pm$ 0.21a	63.1 $\pm$ 18.2b	9.1 $\pm$ 1.6a	2.3 $\pm$ 0.6b	14.8 $\pm$ 1.4a
	drought-stressed	control	22.3 $\pm$ 5.6a	2.4 $\pm$ 0.4a	0.99 $\pm$ 0.45a	0.46 $\pm$ 1.0a	7.1 $\pm$ 0.9a	1.0 $\pm$ 0.2a	13.8 $\pm$ 0.5a
		AM	25.3 $\pm$ 6.2a	2.9 $\pm$ 0.3a	0.97 $\pm$ 0.21a	73.9 $\pm$ 8.6b	9.4 $\pm$ 1.6a	2.0 $\pm$ 0.5b	15.2 $\pm$ 1.7a
<i>R. lycioides</i>	well-watered	control	8.2 $\pm$ 1.3a	1.5 $\pm$ 0.1a	0.15 $\pm$ 0.04a	0.0 $\pm$ 0.0a	13.4 $\pm$ 2.6a	1.8 $\pm$ 0.8a	4.1 $\pm$ 0.9a
		AM	7.3 $\pm$ 2.5a	1.3 $\pm$ 0.4a	0.12 $\pm$ 0.06a	14.0 $\pm$ 4.3b	24.1 $\pm$ 3.6b	1.9 $\pm$ 0.6a	10.5 $\pm$ 1.6b
	drought-stressed	control	8.1 $\pm$ 1.4a	1.4 $\pm$ 0.2a	0.18 $\pm$ 0.05a	0.0 $\pm$ 0.0a	15.2 $\pm$ 2.9a	1.9 $\pm$ 0.7a	5.8 $\pm$ 0.9a
		AM	10.7 $\pm$ 1.3a	1.8 $\pm$ 0.4a	0.19 $\pm$ 0.06a	14.9 $\pm$ 5.1b	22.6 $\pm$ 2.7b	2.0 $\pm$ 0.9a	12.1 $\pm$ 3.0b

colonising the roots (Wright *et al.* 1998). Thus, variations in physiological responses to water deficit between

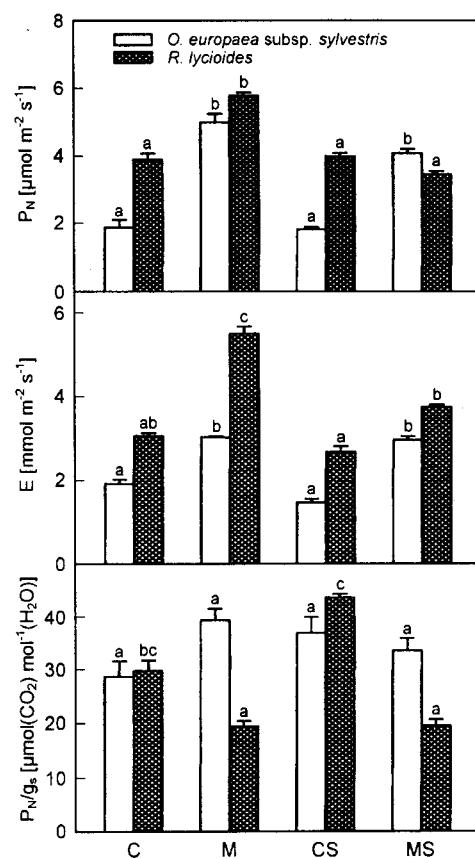


Fig. 1. Photosynthetic rate ( $P_N$ ), transpiration rate (E) and  $P_N/g_s$  ratio in *O. europaea* and *R. lycioides* seedlings as affected by the inoculation treatment (C, M - control and mycorrhizal, respectively) and the water stress (CS, MS). For each plant species, columns with the same letter are not significantly different at  $P < 0.05$ , according to LSD test.

*O. europaea* and *R. lycioides* seedlings may have been due to the percentage AM infection of the seedlings. After the water stress period, the *G. intraradices*-inoculated *O. europaea* seedlings had significantly higher percentages of root colonisation (about 74 %) than the inoculated *R. lycioides* seedlings (about 15 %), which in turn may enhance the beneficial effects of AM fungi, such as increased uptake of water by *O. europaea* roots (Table 1).

Water deficit in inoculated and non-inoculated *O. europaea* seedlings affected  $P_N$  and  $g_s$  in equal proportion, and thus the intrinsic water use efficiency ( $P_N/g_s$ ) was not altered by AM colonisation. However, the higher increases in  $g_s$  with respect to  $P_N$  in inoculated *R. lycioides* seedlings decreased  $P_N/g_s$  with respect to non-inoculated seedlings. The intrinsic water use efficiency is a physiological indicator of the drought tolerance of plants (Díaz and Roldán 2000). Under well-watered conditions, mycorrhizal *R. lycioides* seedlings showed higher instantaneous carbon gain at the expense of consuming available water. In contrast, the photosynthetic activity decreased under drought conditions. To avoid water losses by transpiration, these types of "spender" species are capable of dropping some or all leaves during extended drought.

The P status of the *O. europaea* seedlings was improved by mycorrhizal inoculation under both well-watered and drought-stress conditions (Table 1). Several authors have found that plants colonised by AM fungi are much more efficient in taking up soil P than uncolonised plants, particularly under drought conditions

(Subramanian and Charest 1997). Improved P nutrition due to AM fungi during water deficit has been postulated as a potential mechanism for enhancing host-plant drought tolerance. Subramanian and Charest (1997) reported that improved nutritional status may help AM plants to exploit available soil moisture under moderate drought conditions. In contrast, others have reported that improvement of water status by mycorrhizal fungi during drought was independent of host plant P status (Davies *et al.* 1993). AM colonisation had a positive influence on the N and K concentrations in *R. lycioides* leaves. The external mycelium of AM fungi plays a critical role in uptake and transport of N when water availability is limited (Subramanian and Charest 1999). The hyphal N contribution to the improvement of plant nutritional status could help the host plant to withstand drought conditions. However, a negative correlation was found between the intrinsic water use efficiency and N concentration in leaves of *R. lycioides* seedlings ( $r = -0.625$ ,  $P < 0.01$ ).

On the basis of the physiological parameters, nutritional status and percentage of mycorrhizal colonisation tested, it can be stated that *O. europaea* seedlings inoculated with *G. intraradices* would be better suited to semiarid environments than theirs non-mycorrhizal counterparts. Colonisation by AM fungi diminished the drought tolerance of *R. lycioides*, resulting in lower intrinsic water use efficiency than for non-inoculated seedlings. These results may be of great importance in the selection of mycorrhizal *O. europaea* seedlings to be used in revegetation programmes for these areas.

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