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Differential response of $\delta^{13}C$ and water use efficiency to arbuscular mycorrhizal infection in two aridland woody plant species

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Abstract During a revegetation field experiment in Southeast Spain, we measured foliar carbon isotope ratios ($\delta^{13}C$) and gas exchange parameters in order to evaluate the influence of arbuscular mycorrhizal (AM) infection on the water use efficiency (WUE) of two semiarid woodland species. WUE during drought was significantly enhanced by inoculation with *Glomus intraradices* in *Olea europaea* ssp *sylvestris*, but not in *Rhamnus lycioides*. While *Olea* is a long-lived, slow-growing evergreen tree with a conservative water use strategy, *Rhamnus* is a drought-deciduous shrub with a shorter lifespan; these differences may explain their dissimilar patterns of physiological response to inoculation with the same AM fungus. Differences in $\delta^{13}C$ and WUE between *Olea* and *Rhamnus* were larger when comparing AM inoculated than non-inoculated seedlings. This result suggests that some of the interspecific variability in $\delta^{13}C$ observed for aridland plant communities may be due to different physiological responses to mycorrhization.

Keywords Water stress · Carbon isotope discrimination · Life form · *Olea europaea* · *Rhamnus lyicioides*

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Introduction

Arbuscular mycorrhizal (AM) symbiosis generally results in increased plant transpiration and stomatal conductance (Augé 2001). AM effects on stomatal conductance have been observed under a wide range of soil moisture regimes, but are often greatest under drought conditions (Augé et al. 1992; Davies et al. 1993; Duan et al. 1996). While some authors have suggested that the positive effects of AM fungi on plant water relations can be explained simply in terms of the improved P nutrition and larger size of mycorrhizal plants (Fitter 1988), non-nutritional mechanisms have been demonstrated to play a role too. These include changes in hormonal signaling (Duan et al. 1996), changes in root:shoot ratio, root hydraulic conductivity and specific root length (Khotari et al. 1990), induction of osmoregulatory adjustments (Augé et al. 1986), or substantial water uptake and transport by extraradical hyphae (Ruíz Lozano and Azcón 1995; Augé 2001).

Consistent with AM effects on stomatal conductance (g_s) and transpiration, mycorrhizal plants often show higher photosynthetic rates (A_{max}) than their experimental non-mycorrhizal counterparts (Koide 1993; Augé 2001). Photosynthesis is almost always partially limited by g_s in C3 plants. Increases in A_{max} are therefore usually associated with increases in g_s. Besides g_s, A_{max} is determined by biochemical factors (such as amount and activity of Rubisco) that can be modulated by nutrient availability. AM infection can enhance A_{max} through improved nutrition, but also through increased carbon sink strength of mycorrhizal roots (Koide 1993).

The influence of AM infection on plant water use efficiency (WUE: ratio of photosynthesis to transpiration) has received comparatively little attention, and the studies addressing this aspect have produced contradictory results. Plant WUE has been reported to increase, decrease or remain unchanged with AM colonization, depending on the plant/fungal species combination considered (Augé 2001 and references therein).

Carbon isotope ratio ($\delta^{13}C$) determinations in plant leaf tissue provide time-integrated measures of plant physiological activities. $\delta^{13}C$ is an indicator of overall trade-offs between carbon gain and water loss and is positively related to the intrinsic WUE, defined as the ratio between A_{max} and g_s (Farquhar et al. 1989). Many features other than leaf-level physiology may show strong correlations with the $\delta^{13}C$ value, including life form, longevity, hydraulic architecture, xylem anatomy, root to shoot ratios, and mineral nutrition (Ehleringer 1993b).

The few greenhouse studies that have looked at the influence of AM infection on $\delta^{13}C$ values show that responses may vary widely between different plant species (Di and Allen 1991; Koide and Li 1991; Handley et al. 1993; Davies et al. 2002). Theory predicts that as long as A_{max} and g_s are simultaneously affected by AM infection (and increase or decrease proportionally), there will be little effect on WUE and $\delta^{13}C$. However, changes in the balance between A and g_s in AM plants will alter WUE and consequently the $\delta^{13}C$ of foliar tissue (Rundel and Sharifi 1993; Scheidegger et al. 2000).

In the present study, we used carbon isotope techniques and gas exchange measurements to evaluate the influence of AM infection on the WUE and $\delta^{13}C$ of two Mediterranean woody plant species of different life form under field conditions.

Materials and methods

Study site

The experimental area was located on the El Picarcho range in the Province of Murcia (South-East Spain) (coordinates: 1°10'W and 38°23'N). The climate is semiarid Mediterranean, with an average annual rainfall of 312 mm and a mean annual temperature of 15.3°C; the potential evapo-transpiration reaches 813 mm year^{-1}. Most of the precipitation occurs between fall and early spring, while summers are very hot and dry. The predominant soils are Petrocalcic Xerosol, Petric Calcisol and Haplic Calcisol types (FAO 1988) developed from limestones, with a silt loam texture.

Materials

Two representative, semiarid shrubland/woodland species, namely *Olea europaea* L. ssp. *sylvestris* and *Rhamnus lycioides* L., were used during the revegetation experiment.

Mycorrhizal inoculation of seedlings

The mycorrhizal fungus used in the experiment was *Glomus intraradices* Schenck & Smith, obtained from the collection of the experimental field station of Zaidín, Granada, Spain (BEG registration in progress). AM inoculum consisted of a mixture of rhizospheric soil from trap cultures, containing spores, hyphae and mycorrhizal root fragments. Once germinated, seedlings were transplanted into the growing substrate, consisting of peat and cocopeat (1:1, v:v, Paisajes del Sur, Granada) mixed with *G. intraradices* inoculum (5%). The same amount of autoclaved inoculum, supplemented with a filtrate (<20 μm) of the mixture to provide the microbial populations accompanying the mycorrhizal fungi, was added to the control plants. Seedlings were grown for 8 months under greenhouse conditions, without any fertilization treatment, at Paisajes del Sur, Granada.

Experimental design and layout

The revegetation experiment was a randomized block design with two factors (plant species and AM inoculation) and eight replication blocks. In September 1999, an area of 1,200 m^2 was mechanically prepared with a subsoiler. Eight rows (1 m wide, 25 m long, 3 m apart) were established. In early November, the seedlings were planted in individual holes 1 m apart from each other. At least 40 seedlings per replication block were planted (10 seedlings x 2 plant species x 2 mycorrhization treatments in each block).

Field measurements, sampling and laboratory procedures

Instantaneous measurements of the A_{max}, and g_s under field conditions were determined using a portable gas analyzer system (ADC, LCA4 configured with PLC4C chamber, ADC, Hoddesdon, Hertfordshire, UK) according to the methodology developed by Long et al. (1996). Measurements were made on intact branches using fully expanded new leaves that had developed after field planting (4–8 months old at times of measurement). The chamber (5.5×5.5×5.5 cm) was oriented directly towards the sun during measurements. All measurements were conducted during the morning (10:30–12:00 a.m.). Measurements were obtained from one plant per block and treatment (32 plants in total). After the A_{max} and g_s measurements had been taken, the branches used for gas exchange measurement were taken to the laboratory and their total leaf surface area was calculated according to Johnson (1984). A_{max} and g_s were expressed on a total leaf surface area basis. Gas exchange measurements were conducted twice: in March (beginning of growing season with high soil moisture content; T =22.5°C; Relative humidity =64%; atmospheric pressure =974 mbar) and June (at the end of the growing period with very low soil moisture content; T =30°C; Relative humidity =38%; atmospheric pressure =990 mbar). Conditions during measurements in the fan-stirred plant chamber differed widely between March (photosynthetically active radiation, PAR =1,572±13 μmol m^{-2} s^{-1}; leaf temperature, T_{leaf}=19.8±0.3°C; vapor pressure deficit, VPD =7.1±0.7 mbar) and June (PAR =1,866±8.6 μmol m^{-2} s^{-1}; T_{leaf}=27±0.1°C; VPD =12.7±0.1 mbar). CO_2 concentration in the chamber was 365±0.8 cm^3 m^{-3} on both dates. Conditions in the chamber during measurements did not differ significantly (P>0.1 for all parameters) between mycorrhizal treatments or species.

Eight months after planting, one plant per block and treatment was harvested (32 plants in total). The sampling was carried out in June 2000, immediately after the second gas exchange measurements. Dry (70°C, 48 h) weight of seedling aerial biomass was measured. Foliar concentrations of nitrogen and phosphorus were measured after digestion in nitric-perchloric acid (5:3). The P content was determined by colorimetry (Murphy and Riley 1962) and the N was determined by the Kjeldhal method. Only new leaves produced after field planting were used for nutrient analyses.

The percentage of root length colonized by AM fungi was calculated by the gridline intersect method (Giovannetti and Mosse 1980), after staining roots with trypan blue (Phillips and Hayman 1970).

Subsamples of leaves from the same branches used for gas exchange measurements in June were oven dried, ground and analyzed for carbon isotope ratio ($\delta^{13}C$). Only new leaves produced after field planting were used for $\delta^{13}C$ measurement. Analyses were conducted on a continuous flow mass spectrometer (Europa Scientific Hydra 20/20, Chelshire, England) at the Stable Isotope Facility of the University of California-Davis. The value for carbon isotope composition of a plant leaf (in ‰) is the molar abundance ratio of the plant leaf relative to that of a standard, the PDB carbonate (Ehleringer 1991).

Species and mycorrhizal treatment effects on growth, nutrient status, mycorrhizal colonization, gas exchange and carbon isotope ratios were determined by using a two-way ANOVA.

Results

Olea europaea

Inoculation with *Glomus intraradices* significantly enhanced AM colonization, growth and nutrition in *Olea europaea* seedlings (Table 1). Eight months after field planting, the percentage of mycorrhizal infection of the roots was still much higher in the *G. intraradices* inoculated seedlings, despite evidence of some limited native AM colonization in their non-inoculated counterparts. Shoot biomass increment of the inoculated seedlings was 8 times as much as that of the controls. N and P foliar concentrations were higher in inoculated plants by 20 and 100%, respectively.

Significant differences in gas exchange parameters between inoculated and non-inoculated *O. europaea* seedlings were found both in March and June. A_{max} was 48% higher in the *G. intraradices* inoculated plants than in the controls when measured during a wet period in March. g_s was 90% higher and intrinsic WUE was 39% lower in the inoculated seedlings than in their non-inoculated counterparts at this time of the year.

When measured during drought (June), A_{max} was 50% higher in *G. intraradices* inoculated seedlings than in their counterparts. Inoculation also increased g_s by 10%, and intrinsic WUE by 35%. The $\delta^{13}C$ of new foliar tissue (produced after field planting) was 1.2‰ higher in the *G. intraradices* than in the control treatment. For *Olea*, 31% of the variation in $\delta^{13}C$ could be attributed to mycorrhizal treatment effects.

Rhamnus lycioides

AM colonization, growth and nutrition significantly improved with *G. intraradices* inoculation in *Rhamnus* seedlings (Table 1). Control seedlings were still virtually non-mycorrhizal at time of harvest, while the inoculated ones showed more than 40% infection. Shoot biomass increment of the inoculated seedlings was 10 times as much as that of the controls. N and P concentrations in foliar tissue were 37% and 70% higher in the inoculated plants than in the controls.

Both A_{max} and g_s were significantly higher (87% and 81%, respectively) in the *G. intraradices* than in the control treatment when measured in March. Intrinsic WUE was not affected by the mycorrhizal status of the seedlings at this time of the year.

During drought (June), the only gas exchange parameter that was enhanced by *G. intraradices* inoculation in *R. lycioides* was g_s (16% increase, Table 1). A_{max} and intrinsic WUE were not significantly different between treatments (although intrinsic WUE tended to be lower in

Table 1 Shoot dry weight (*SDW*) at times of planting and harvest (g), biomass increment (mg g^{-1}), photosynthetic activity (A_{max}, μmol m^{-2} s^{-1}), stomatal conductance (g_s, μmol m^{-2} s^{-1}), instantaneous water use efficiency (WUE, A_{max}/g_s) and carbon isotope ratio ($\delta^{13}C$, ‰) in AM inoculated or non-inoculated *Olea europea* and *Rhamnus lycioides* seedlings. Mean and standard error values are shown, n=8

	SDW at planting	SDW at harvest	Biomass increment	AM infection	Foliar P	Foliar N	March 2000			June 2000			$\delta^{13}C$
							A_{max}	g_s	WUE (A_{max}/g_s)	A_{max}	g_s	WUE (A_{max}/g_s)	
Olea Control	1.30±0.06	1.58±0.09	21	14±2	1.1±0.1	10.4±0.6	12.1±0.6	69±8	0.192±0.023	8.7±0.8	39±1	0.227±0.023	−27.7±0.4
Olea+*Glomus intraradices*	1.58±0.08[a]	4.12±0.33[a]	160	65±5[a]	2.2±0.1[a]	13.2±1.2[a]	17.9±0.9[a]	131±5[a]	0.138±0.009[a]	13.2±0.8[a]	43±1[a]	0.306±0.015[a]	−26.5±0.2[a]
Rhamnus Control	0.46±0.05	0.86±0.07	82	1±1	0.9±0.1	11.1±0.8	10.7±0.5	108±8	0.103±0.008	8.6±0.5	55±3	0.163±0.016	−28.3±0.6
Rhamnus+*G. intraradices*	0.24±0.04[a]	2.25±0.25[a]	818	43±7[a]	1.5±0.1[a]	15.1±0.6[a]	20.1±0.6[a]	195±8[a]	0.103±0.003	8.3±0.7	64±3[a]	0.133±0.017	−27.8±0.4
Significance													
Species	0.000	0.000	–	0.000	0.000	NS	NS	0.000	0.000	0.002	0.000	0.000	0.016
AM	0.030	0.000	–	0.000	0.000	0.000	0.000	0.000	0.047	0.013	0.012	NS	0.059
Species×AM	0.000	0.013	–	NS	0.010	NS	0.017	NS	0.047	0.004	NS	0.005	NS

[a] Significantly different from their non-inoculated controls at P<0.05

AM plants). The $\delta^{13}C$ of new foliar tissue was not significantly different between inoculated and non-inoculated plants either. For *Rhamnus,* only 4% of the variation in $\delta^{13}C$ could be attributed to mycorrhization treatment effects.

Olea versus *Rhamnus*

Overall, *O. europea* showed significantly higher $\delta^{13}C$ than *R. lycioides* (Table 1). However, differences between the two species were larger when comparing the *G. intraradices* inoculated plants only (1.3‰ difference between means). Non-inoculated *Olea* and *Rhamnus* were not significantly different from each other (0.6‰ difference between means).

A similar pattern was found as regards gas exchange parameters during drought (June): differences between the two species were larger when only the *Glomus* inoculated plants were included in the analyses. *Olea* showed higher A_{max} than *Rhamnus* during drought, but this difference was almost exclusively attributable to the mycorrhizal treatments (difference 4.8 μmol m^{-2} s^{-1}). Non-inoculated *Olea* and *Rhamnus* showed very similar values (0.1 μmol m^{-2} s^{-1} difference). g_s was higher in *Rhamnus* than in *Olea* across treatments, but again interspecific differences were larger between mycorrhizal (21 mmol m^{-2} s^{-1} difference) than between non-mycorrhizal (16 mmol m^{-2} s^{-1} difference) seedlings. Intrinsic WUE was higher in *Olea* than in *Rhamnus* during drought, yet larger differences were observed when comparing inoculated (0.173 difference) than non-inoculated (0.064 difference) seedlings.

Shoot biomass increment over the entire growing season was much higher in *Rhamnus* than in *Olea*, especially when comparing the mycorrhizal treatments.

Discussion

The responses of *Olea* and *Rhamnus* to *Glomus intraradices* infection were very dissimilar in WUE. The intrinsic WUE of the *Glomus*-inoculated *Olea* seedlings increased by 122% during the March-June soil dry down period, while the control seedlings showed a much smaller increase (16%). Conversely, *Glomus*- inoculated *Rhamnus* seedlings increased their intrinsic WUE by just 25% between March and June, half as much as their non-inoculated counterparts (48% increase).

In *Olea*, the intrinsic WUE of the inoculated plants was higher than the controls during drought only. Allen and Allen (1986) suggested that water may be one of the main factors controlling the effectiveness of mycorrhizas in semiarid environments, and hypothesized that AM symbiosis may become particularly important during an "ecological crunch" such as drought. Differential enhancement of A_{max} over g_s during drought may have been the result of increased concentration or activity of photosynthetic enzymes thanks to improved nutritional status in mycorrhizal *Olea* seedlings (Koide 1993). A_{max} may have also been differentially stimulated by the increased sink strength arising from the additional carbon requirements of the mycorrhizal fungus colonizing the roots (Wright et al. 1998). Higher $\delta^{13}C$ in mycorrhizal *Olea* seedlings was associated with increased growth, which further suggests higher intrinsic photosynthetic capacity in the inoculated plants during the soil dry down period. High $\delta^{13}C$ does not necessarily mean lesser growth in aridland plant species, contradicting the general expectation that increased WUE is associated with decreased carbon gain and biomass accumulation (Donovan and Ehleringer 1994).

In contrast to *Olea*, *Rhamnus* showed much greater responsiveness to *G. intraradices* infection during a wet period than during drought. Mycorrhizal seedlings exhibited almost double A_{max} than their non- inoculated controls during the wetter part of the growing season, but this difference completely tapered off as soil moisture availability decreased. Neither intrinsic WUE nor foliar $\delta^{13}C$ were significantly influenced by the mycorrhizal status of *Rhamnus*, indicating that *G. intraradices* infection affected both A_{max} and g_s in a correlated manner throughout the soil dry down period (Rundel and Sharifi 1993; Scheidegger 2000).

The dissimilar patterns of physiological response of *Rhamnus* and *Olea* to inoculation with the same AM fungus may be related to the different life history characteristics of these two plant species (Ehleringer 1993b). Carbon isotope ratios have been used to rank or group plant species according to their physiological and life history characteristics (Smedley et al. 1991; Brooks et al. 1997). The significant difference in $\delta^{13}C$ found between *Olea* and *Rhamnus* paralleled disparities in longevity and life form. *Olea* is an evergreen small tree characterized by its slow growth rate and remarkably long life span, while *Rhamnus* is a drought-deciduous shrub with a shorter life expectancy. Long-lived species tend to have higher $\delta^{13}C$ than shorter-lived species (Schuster et al. 1993). Although interspecific differences in carbon isotope discrimination are usually larger between adults, *Olea* showed significantly higher $\delta^{13}C$ values than *Rhamnus* even at the seedling stage (Sandquist et al. 1993). Life form likely influenced carbon isotope discrimination too, since evergreen trees generally show higher $\delta^{13}C$ than deciduous shrubs (Brooks et al. 1997).

Variations in carbon isotope discrimination between species of different life form are directly related to different patterns of utilization of water resources. A difference of 1‰ in $\delta^{13}C$ is considered sufficient to unambiguously rank plant species with respect to long term WUE (Ehleringer, 1993b). Many drought tolerant species from arid environments show a conservative strategy in their use of soil moisture, reducing water consumption to maintain photosynthetic activity during dry periods. This adaptation to drought is based on a finely adjusted stomatal control of transpiration. Mycorrhizal *Olea*, with its high $\delta^{13}C$, slow growth rate and ability to sharply increase WUE in the face of severe

drought, is clearly a representative of this conservative strategy (Ehleringer 1993a, 1993b; Donovan and Ehleringer 1994). However, high WUE may have little adaptive value in arid environments when competition for water is intense, especially for plants with shallow roots. Many species therefore adopt a different strategy, trading off water conservation for higher instantaneous carbon gain during favorable periods (Ehleringer 1993a, 1993b). These spender species "wastefully" consume water resources while available, and typically show lower intrinsic WUE and $\delta^{13}C$ values. Poorer stomatal control of transpiration is often compensated by their ability to drop some or all leaves during extended drought to reduce water losses. The high A_{max}, g_s and growth increment of mycorrhizal *Rhamnus* during the wetter part of the growing season, as well as the sharp decline of A_{max} under unfavorable dry conditions, are consistent with this spender strategy (Ehleringer 1993b). Niche differentiation based on dissimilar plant physiological responses to a common fungal associate has previously been reported on grasses from semiarid environments (Allen et al. 1984).

Differences in WUE and $\delta^{13}C$ between *Olea* and *Rhamnus* were clearly greater when comparing inoculated than non-inoculated plants. Therefore, these differences could not be entirely explained in terms of variation in genetically predetermined plant traits (such as life form or xylem anatomy), which would be expected to remain largely unaffected by AM infection. We hypothesize that a substantial proportion of the variability in $\delta^{13}C$ observed among plant species in natural communities (Smedley et al. 1990; Brooks et al. 1997) may be related to their different patterns of physiological response to mycorrhization.

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References

Allen EB, Allen MF (1986) Water relations of xeric grasses in the field: interactions of mycorrhizas and competition. New Phytol 104:559–571

Allen MF, Allen EB, Stahl PD (1984) Differential niche response of *Bouteloua gracilis* and *Pascopyrum smithii* to VA mycorrhizae. Bull Torrey Bot Club 111:361–365

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Augé RM, Schekel KA, Wample RL (1986) Osmotic adjustment in leaves of VA mycorrhizal and nonmycorrhizal rose plants in response to drought stress. Plant Physiol 82:765–770

Augé RM, Stodola AJ, Brown MS, Bethlenfalvay GJ (1992) Stomatal response of mycorrhizal cowpea and soybean to short-term osmotic stress. New Phytol 120:117–125

Brooks JR, Flanagan LB, Buchman N, Ehleringer JR (1997) Carbon isotope composition of boreal plants: functional grouping of life forms. Oecologia 110:301–311

Davies FT, Potter JR, Linderman RG (1993) Drought resistance of mycorrhizal pepper plants independent of leaf P-concentration. Response in gas exchange and water relations. Physiol Plant 87:45–53

Davies FT, Olalde-Portugal V, Aguilera-Gomez L, Alvarado MJ, Ferrera-Cerrato RC, Boutton TW (2002) Alleviation of drought stress of Chile ancho pepper (*Capsicum annuum* L. cv. San Luis) with arbuscular mycorrhiza indigenous to Mexico. Sci Hortic 92:347–359

Di JJ, Allen EB (1991) Physiological responses of six wheatgrass cultivars to mycorrhizae. J Range Manage 44:336–341

Donovan L, Ehleringer JR (1994) Carbon isotope discrimination, water use efficiency, growth and mortality in a natural shrub population. Oecologia 100:347–354

Duan X, Neuman DS, Reiber JM, Green CD, Saxton AM, Augé RM (1996) Mycorrhizal influence on hydraulic and hormonal factors implicated in the control of stomatal conductance during drought. J Exp Bot 47:1541–1550

Ehleringer JR (1991) $^{13}C/^{12}C$ fractionation and its utility in terrestrial plant studies. In: Coleman DC, Fry B (eds) Carbon isotope techniques. Academic Press, San Diego, pp 187–200

Ehleringer JR (1993a) Variation in leaf carbon isotope discrimination in *Encelia farinosa*: implications for growth, competition, and drought survival. Oecologia 95:340–346

Ehleringer JR (1993b) Carbon and water relations in desert plants: an isotopic perspective. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon-water relations. Academic Press, San Diego

FAO (1988) Soil map of the world: revised legend, final draft. Food Agriculture Organization of the United Nations, Rome

Farquhar GD, Ehleringer JR, Hubick KT (1989) Carbon isotope discrimination and photosynthesis. Annu Rev Plant Physiol Mol Biol 40:503–537

Fitter AH (1988) Water relations of red clover *Trifolium pratense* L. as affected by VA mycorrhizal infection and phosphorus supply before and during drought. J Exp Bot 39:595–603

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–499

Handley LL, Daft MJ, Wilson J, Scrimgeour CM, Ingleby K, Sattar MA (1993) Effects of the ecto- and VA-mycorrhizal fungi *Hydnangium carneum* and *Glomus clarum* on the delta nitrogen-15 and delta carbon-13 values of *Eucalyptus globulus* and *Ricinus comunis*. Plant Cell Environ 16:375–382

Johnson JD (1984) A rapid technique for estimating total surface area of pine needles. For Sci 30:913–921

Khotari SK, Marschner H, George E (1990) Effect of VA mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize. New Phytol 116:303–311

Koide RT (1993) Physiology of the mycorrhizal plant. Adv Plant Pathol 9:33–54

Koide RT, Li M (1991) Mycorrhizal fungi and the nutrient ecology of three oldfield annual plant species. Oecologia 85:403–412

Long SP, Farage PK, García RL (1996) Measurement of leaf and canopy photosynthetic CO_2 exchange in the field. J Exp Bot 47:1629–1642

Murphy J, Riley JP (1962) A modified single solution method for determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Ruíz-Lozano JM, Azcón R (1995) Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiol Plant 95:472–478

Rundel PW, Sharifi MR (1993) Carbon isotope discrimination and resource availability in the desert shrub *Larrea tridentata*. In: Ehleringer JR, Hall AE, Farquhar GD (eds) Stable isotopes and plant carbon-water relations. Academic Press, San Diego

Sandquist DR, Schuster WSF, Donovan L, Phillips SL, Ehleringer JR (1993) Differences in carbon isotope discrimination between seedlings and adults of southwestern desert perennial plants. Southwest Nat 38:212–217

Scheidegger Y, Saurer M, Bahn M, Siegwolf R (2000) Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: a conceptual model. Oecologia 125:350–357

Schuster WSF, Sandquist DR, Phillips SL, Ehleringer JR (1992) Comparisons of carbon isotope discrimination in populations of aridland plant species differing in lifespan. Oecologia 91:332–337

Smedley MP, Dawson TE, Comstock JP, Donovan LA, Sherrill DE, Cook CS, Ehleringer JR (1991) Seasonal carbon isotope discrimination in a grassland community. Oecologia 85:314–320

Wright DP, Scholes JD, Read DJ (1998) Effects of VA mycorrhizal colonization on photosynthesis and biomass production of *Trifolium repens* L. Plant Cell Environ 21:209–216