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Influence of arbuscular mycorrhizas on the growth rate of mist-propagated olive plantlets

A. Porras Piedra*, M. L. Soriano Martín, A. Porras Soriano and G. Fernández Izquierdo

Escuela Universitaria de Ingeniería Técnica Agrícola. Ronda de Calatrava, 7. 13071 Ciudad Real. Spain

Abstract

The aim of this work was to determine the influence of early inoculation of semi-woody olive cuttings (cv. Cornicabra), cultivated under mist propagation conditions, with arbuscular mycorrhizal fungi. A strongly positive response was seen to root inoculation with the three fungal species tested —*Glomus mosseae, G. intraradices* and *G. claroideum*— both in nursery phase plantlets, and in older plants grown in 5 L pots.

Additional key words: *Glomus*, mist propagation, mycorrhizal fungi, *Olea europaea*, plantlets.

Resumen

Influencia de las micorrizas arbusculares en el desarrollo de plantones de olivos propagados bajo nebulización

El objetivo de este trabajo ha sido valorar la influencia de la inoculación temprana con micorrizas arbusculares en el desarrollo de olivos cv. Cornicabra obtenidos por propagación bajo nebulización de estaquillas semileñosas. Los resultados muestran la respuesta altamente positiva que en el desarrollo de las jóvenes plantas de olivo tiene la colonización de sus raíces por tres hongos micorrícicos distintos (*Glomus mosseae, G. intraradices* y *G. claroideum*), tanto en la primera fase de vivero como en plantas de más edad cultivadas en macetas de 5 L de capacidad.

Palabras clave adicionales: *Glomus,* hongos micorrícicos, *Olea europaea,* plántulas, propagación.

Introduction

The olive (*Olea europaea* L.) is one of the most important crop plants in the Spanish region of Castilla-La Mancha (extension 278,443 ha). The cultivar 'Cornicabra' is the most common with 269.000 ha planted, making it the second most important in the country. Cornicabra olives are appreciated for their high lipid content (18.9%), their oleic acid content (77.1%) and the stability of their oil (106 h at 98.8ºC) (Barranco, 1998).

In recent years the Spanish olive sector has enjoyed a period of expansion, both in terms of production and in new land brought under cultivation, a phenomenon that has been accompanied by great activity in olive nurseries. According to the *Instituto Nacional de Semillas y Plantas de Vivero* (the National Institute of Seeds and Nursery Plants), 261 nurseries generated 5,598,294 olive plantlets between them in the period 1999/2000 (Anonymous, 2004).

Mist propagation of semi-woody olive cuttings has been widely adopted by nurseries since it helps produce healthier, better rooted plantlets (Caballero and Del Río, 1994; Porras *et al.,* 1999). These plantlets require a growth period of some 18 months before they can be marketed, during which time they are raised either in bags or polythene pots containing an artificial substrate (a 1:1, v v^{-1} mixture of sand and peat) (Porras *et al.,* 1991, 1997).

It has been shown that mycorrhizal fungi reduce the stress suffered by olive plantlets at transplant, and that they increase their resistance to disease, improve their water relations and nutrient uptake, increase their rate of photosynthesis, and generally improve their vigour – aspects of great importance in plant propagation systems (Davies *et al.*, 2000). In addition, these fungi can change the morphology of the root system, favouring the establishment of young plants (Citernesi *et al.*, 1998).

Mycorrhizas are a manifestation of the symbiotic relationship that develops between certain soil fungi and

* Corresponding author: andres.porras@uclm.es
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the roots systems of plants. About 90% of cultivated plants are naturally associated with mycorrhizas (Azcón-Aguilar *et al.*, 1979). The external mycelium that arbuscular mycorrhizal (AM) fungi develop following their colonisation of plant roots acts as an extension of the root system proper, increasing its contact with the soil and favouring the absorption of water and nutrients (Harper *et al.,* 1991; Ruiz-Lozano *et al.,* 1996a,b; Rinaldelli and Mancuso, 1998; Barea *et al.,* 1999). Plant growth is therefore facilitated (Vidal *et al.,* 1992). Apart from these physical features, a number of biochemical processes increase the affinity of mycorrhiza-associated roots for soil nutrients, increasing their absorption when in low concentration (Monzón and Azcón, 1996; Camprubí *et al.*, 2000). This can increase the effectiveness of any fertilizer applied (Azcón-Aguilar *et al.*, 1979; Barea *et al.,* 1987; Azcón-Aguilar *et al.,* 1999). The development of plants whose roots are associated with a mycorrhiza system is therefore favoured, especially in soils very poor in nutrients (Requena, 1997).

Numerous papers have reported that plants depend on mycorrhizas for their full development in low-fertility soils, and that the capacity of plants to absorb nutrients from such substrates is that which most affects their growth rate (Jaizme-Vega and Rodríguez-Romero, 1997; Porras *et al.*, 2003). The substrates used in the cultivation of plants in pots or other containers usually lack AM fungi. Although the use of fertilizer can substitute for them, such plants are at a disadvantage when transplanted into soils where no AM fungi are present. Early association with AM fungi therefore provides an initial benefit to plants, both in terms of transplant survival and their establishment in orchards (Camprubí *et al*., 2000). However, not all AM fungi have the same effects on plants, and the right choice needs to be made when performing artificial inoculations (Jaizme-Vega and Rodríguez-Romero, 1997; Citernesi *et al.*, 1998, Barea *et al*., 1999). The benefit gained will depend on the plant/fungus combination being appropriate.

The use of AM fungi with wild olive plants (*Olea europaea* L. ssp. *sylvestris)* has shown that mycorrhizal growth increases the activity of the hosts' antioxidant enzymes (Alguacil *et al*., 2003) as well as the rates of photosynthesis and transpiration, stomatal conductance and leaf phosphorus levels (Caravaca *et al.,* 2003a,b). It also seems to improve nutrient acquisition after transplantation to the field (Palenzuela *et al.*, 2002), the stability of rhizosphere aggregates (Caravaca *et al*., 2002), the efficiency of water use, and the biomass of the aerial component of the plant (Querejeta *et al*., 2003). The growth of mycorrhiza in the cultivated olive (*Olea europaea* L.) 'Arbequina' leads to increased plant development and production in the three years following inoculation (Estaún *et al.*, 2003). In the cultivar 'Misión', inoculation of the roots also leads to improved development and an increase in root and leaf phenolic contents (Ganz *et al*., 2002). The presence of a mycorrhizal system in 'Picual' induces enzyme activity that might be involved in the plant's defence against certain soil pathogens (Calvente *et al*., 2002). However, different cultivars have shown different responses to the same fungus. The inoculation of 'Moraiolo' and 'Frantoio' with *Glomus mosseae* leads to increased plant development, but 'Leccino' plantlets experience no benefit over controls lacking mycorrhizal systems. Differences are also seen in the degree of colonisation of roots (Citernesi *et al*., 1998). Similarly, the same cultivar can respond differently to different AM fungi. For example, the inoculation of 'Arbequina' plantlets with *Glomus intraradices* seems to stimulate development more so than inoculation with *G. mosseae* or other native endophytes (Estaún *et al.*, 2003).

The aim of this work was to determine how 'Cornicabra' plantlets would respond to the colonisation of their roots by the AM fungi *G. mosseae, G. intraradices* and *G. claroideum*, both at the nursery stage (in 2 L pots) and later in development (in 5 L pots representing micro-plots).

Material and Methods

Plant material and inoculation with AM fungi

Semi-woody cuttings of 'Cornicabra', obtained from a commercial olive plantation in Ciudad Real, central Spain, were cut to lengths of 15 cm, leaving three pairs of leaves at the top end (Porras *et al.,* 1997). The bottoms of the cuttings were treated with INABARPLANT IV, a commercial rooting compound containing 0.4% indole butyric acid (IBA), 0.4% naphthalene-acetic acid (NAA) and 15% Ziram. The cuttings were planted in perlite at a density of 1500 plants m^{-2}, and placed in a propagation tunnel equipped with a high precision environmental control system (Porras *et al.*, 1999; 2000). The substrate was warmed to 22ºC and the air temperature maintained at 20ºC. The leaves of the cuttings were automatically maintained moist using 0.8 mm angle flat spray nozzles (pressure 0.3 Mpa).

Rooted cuttings were inoculated with spores, mycelia and root fragments from cultures of *Glomus intraradices* Schenk & Smith, *G. mosseae* (Nicol & Gerd) Gerdemann & Trappe and *G. claroideum* Schenck & Smith, obtained from non-agricultural soils in the region of Murcia (southwestern Spain). All were kindly supplied by Dr. J.M. Barea. These fungi were originally multiplied in a mixture of sand and sepiolite using alfalfa (*Medicago sativa* L.) as a host.

Three grams of inoculum were deposited directly below the roots of the cuttings during their transplant into individual containers (capacity 120 cm^3) made of compressed peat and filled with a sterile substrate (a mixture of peat and sand) (1:1 v v^{-1}). The peat used had the following characteristics: total organic nitrogen 1%, total organic matter 75%, maximum ash content 10%, pH 5.5, EC 2.0 mS cm^{-1}. The substrate was tyndallised at 98ºC for 1 h for three consecutive days. Filtered (Whatman 1) leachates of each inoculum were applied to non-treated controls.

To avoid cross contamination, each treatment group was placed on a separate tray before introduction into the same propagation tunnel used for rooting the cuttings. The substrate was again heated to 22ºC and the air temperature maintained at 20ºC. After six weeks the roots began to emerge through the walls of the compressed peat containers and 192 of these plantlets (32 for testing with each fungal species plus 32 for each control group) were transferred to 2 L black polythene pots filled with sterile substrate. These were placed randomly under shade netting covered with a polythene net; micronozzle sprays controlled by an electronic sensor provided the necessary moisture (Porras *et al.*, 1997). Every month the plants were provided with 100 ml of Hewitt's solution (1952) modified as follows: 20 ml L^{-1} $MgSO_4.7H_2O$ (18.4 g L^{-1}), 10 ml L^{-1} EDTA-Fe (2.45 g L^{-1}), 1 ml L^{-1} $MnSO_4.7H_2O$ (2.23 g L^{-1}), 0.1 ml L^{-1} $CuSO_4.5H_2O$ (2.4 g L^{-1}), 0.1 ml L^{-1} $ZnSO_4.2H_2O$ (2.9 g L^{-1}), 0.1 ml L^{-1} H_3BO_3 (18.6 g L^{-1}), 0.1 ml L^{-1} $Na_2MoO_4.2H_2O$ (0.35 g L^{-1}), 10 ml L^{-1} KNO_3 (30.3 g L^{-1}), 20 ml L^{-1} $Ca(NO_3)_2$ (70.8 g L^{-1}) and 1 ml L^{-1} $NaPO_4H_2.2H_2O$ (20.8 g L^{-1}).

After eight weeks the plantlets were transferred to a greenhouse equipped with heating and cooling systems. All were watered to field capacity twice per week when the sun was at its strongest, and once per week when solar radiation was less intense. These plants were kept in the same pots for one year (from the beginning of December).

Colonisation of the roots

Six months after inoculating the plants, fine roots were taken from five plants (1 g $plant^{-1}$) of each group and stained to see whether the fungi had colonised them successfully. This was performed using the technique of Phillips and Hayman (1970), as modified by Koske and Gemma (1989). The roots were carefully washed with tap water, cut into segments of 1-2 cm in length, and submerged in a solution of 10% KOH for 20 min at 100ºC. They were then washed again in cold tap water and those with excess pigment submerged in H_2O_2 (10% vol.) to bleach them. All the roots were then submerged in 0.1N HCl for 15 min to neutralise the KOH. The acid was eliminated and the roots submerged in a solution of 0.05% trypan blue (5 trypan blue:95 lactic acid, v v^{-1}) for 15 min at 100ºC. The percentage of the total root length that became colonised by mycorrhizas was determined using the guideline intersect technique (Giovannetti and Mosse, 1980). The same technique was used to determine whether the plants remained colonised at the end of the experiment.

Data collection and statistical analysis

Plants in substrate-filled 2 L pots

Over the year following their entry into the greenhouse, the growth of 12 of the 32 plants in each group was determined by measuring their maximum height and the number of their shoots. The data collected were compared by ANOVA followed by Duncan's multiple range test. All calculations were made using Statgraphics Plus v2.1 software.

At the end of this year the plants were harvested and the dry weight of their aerial and root compartments determined by drying them in an oven at 70º C until constant weights were reached.

Plants in substrate-filled 5 L pots

In December, when the plants had been in the 2 L pots for one year, the 15 remaining plants in each treatment group, plus 15 randomly selected from each control group, were pruned, leaving them with a single stem. These were then introduced individually into the 5 L pots filled with the same substrate. Each pot was provided with bamboo guide to which the plants were attached with plastic tape to form trees suitable for

Table 1. Number of shoots per plantlet in 2 L pots during the first year of growth

Fungal species	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
G. intraradices	NI	3.0 a	3.5 a	3.6 a	3.9 a	4.1 a	4.3 a	5.1 a	5.9 a	7.1 a	7.4 a	7.5 a	7.7 a
	I	3.6 a	4.3 a	6.3 b	7.2 b	11.1 b	12.8 b	14.4 b	17.0 b	17.2 b	17.9 b	18.2 b	18.5 b
G. mosseae	NI	3.2 a	3.2 a	3.4 a	3.6 a	4.0 a	4.6 a	5.3 a	6.5 a	7.3 a	8.4 a	8.5 a	8.7 a
	I	3.5 a	4.0 a	4.6 b	6.6 b	10.0 b	13.5 b	18.6 b	22.2 b	24.6 b	26.4 b	27.0 b	27.2 b
G. claroideum	NI	3.1 a	3.5 a	4.2 a	4.9 a	5.5 a	5.9 a	6.4 a	8.6 a	8.8 a	9.1 a	9.3 a	9.3 a
	I	3.4 a	4.1 a	4.6 b	6.3 b	10.4 b	13.0 b	16.7 b	19.9 b	21.4 b	22.9 b	23.2 b	23.4 b

Values in columns with different letters indicate significant differences, as determined by Duncan's multiple range test ($p \leq 0.05$). Each value corresponds to the mean of 12 repetitions. NI: not inoculated. I: inoculated.

mechanised (vibration) harvesting. The plants were left in these pots for another full year, during which time all shoots were eliminated. Rings were drawn with indelible paint around the stem of each plant at a height of 10 and 60 cm; the stem diameter was then measured monthly at these points. The data collected were compared by ANOVA followed by Duncan's multiple range test using the same software as above.

At the end of this second year, the plants were harvested and the colonisation of the roots examined as above. The dry weights of the roots and aerial compartment were again determined.

Results

Rooting and transplantation of the olive plantlets

After an initial 75 days, the percentage rooting obtained was 56%. These roots (characteristically fragile) reached up to 12 cm in length. Only 4% of the rooted, inoculated plants died after transfer to the compressed peat containers, while 7% of the non-inoculated plants died.

Plants in substrate-filled 2 L pots

Tables 1, 2 and 3 show the effect of the AM fungi on the growth of the olive plantlets.

Table 1 shows how the number of shoots per plant clearly increased during the six months of greatest vegetative activity (April-September), while in the months of lesser activity the increase was less evident. The appearance of these new shoots, though positive for all the fungi tested, was greatest with *G. mosseae*, although the differences were not significant.

Table 2 shows how the mean length of the shoots clearly increased during the active period of vegetative growth and how this was less evident during the period of reduced activity. Shoot length increased with all three fungal species - the longest being attained with *G. claroideum*, although differences were not significant.

Table 3 shows that the height of the plants clearly increased during the period of active vegetative growth, and that these increases were considerably reduced during the period of low activity. The plants grew tallest with *G. claroideum*, but again, no significant differences were seen between the effects of the three fungi.

Table 4 shows the percentage of plants colonised by each species of *Glomus*, the dry weights of the

Table 2. Mean length of shoots (cm) per plantlet in 2 L pots during the first year of growth

Fungal species	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
G. intraradices	NI	3.1 a	4.8 a	8.3 a	10.2 a	16.3 a	24.0 a	32.9 a	39.7 a	47.6 a	53.0 a	54.6 a	56.8 a
	I	4.3 a	7.4 b	12.6 b	20.5 b	35.7 b	50.2 b	66.9 b	80.1 b	84.6 b	96.7 b	100.3 b	104.4 b
G. mosseae	NI	3.2 a	4.7 a	7.5 a	9.6 a	15.7 a	24.0 a	30.9 a	44.7 a	48.6 a	50.3 a	52.6 a	52.3 a
	I	3.4 a	5.5 a	10.5 b	18.9 b	32.0 b	43.7 b	57.0 b	63.6 b	70.0 b	71.3 b	71.9 b	75.0 b
G. claroideum	NI	3.3 a	5.0 a	7.6 a	11.1 a	19.1 a	31.0 a	37.6 a	48.4 a	49.6 a	50.3 a	50.6 a	50.8 a
	I	3.8 a	5.1 a	8.0 a	17.3 b	34.5 b	51.7 b	68.8 b	86.7 b	96.3 b	105.4 b	109.1 b	109.9 b

Values in columns with different letters indicate significant differences, as determined by Duncan's multiple range test ($p \leq 0.05$). Each value corresponds to the mean of 12 repetitions. NI: not inoculated. I: inoculated.

Table 3. Maximum mean height (cm) achieved by plantlets in 2 L pots during the first year of growth

Fungal species	Treatment	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
G. intraradices	NI	5.9 a	6.6 a	8.1 a	10.1 a	16.3 a	25.0 a	32.9 a	40.6 a	43.4 a	45.3 a	45.8 a	46.6 a
	I	7.0 a	8.1 b	14.1 b	22.2 b	35.7 b	50.2 b	67.0 b	80.1 b	81.8 b	83.6 b	85.1 b	86.2 b
G. mosseae	NI	5.5 a	6.5 a	7.2 a	9.9 a	14.7 a	22.1 a	29.2 a	41.7 a	43.6 a	45.8 a	46.5 a	46.9 a
	I	6.8 a	8.1 b	12.2 b	18.6 b	32.8 b	44.7 b	58.4 b	64.3 b	66.0 b	67.6 b	69.3 b	69.8 b
G. claroideum	NI	5.9 a	6.5 a	9.6 a	11.7 a	19.0 a	29.6 a	35.5 a	45.1 a	46.8 a	49.2 a	50.6 a	50.9 a
	I	6.9 a	7.4 a	12.7 b	19.0 b	35.8 b	53.6 b	72.0 b	90.1 b	92.0 b	94.4 b	96.3 b	96.7 b

Values in columns with different letters indicate significant differences, as determined by Duncan's multiple range test ($p \leq 0.05$). Each value corresponds to the mean of 12 repetitions. NI: not inoculated. I: inoculated

root and aerial compartments, and the ratio between these compartments. All the inoculated plants examined were colonised and showed mycorrhizal development; none were cross-contaminated. These results show the affinity of 'Cornicabra' for root colonisation by *Glomus* spp. The aerial/root compartment ratio was higher in the inoculated than in the control plants.

Mycorrhiza and the development of plants in substrate-filled 5 L pots

Figure 1 shows the development of the stem diameter in the colonised and control plants (now older).

Table 5 provides statistical comparisons of these diameters at the two measuring heights (10 and 60 cm).

All three AM fungi had a positive effect on stem diameter. The greatest diameters were obtained in plants inoculated with *G. intraradices* (significantly greater than those inoculated with *G. mosseae* and the controls, but not significantly different to the diameters obtained with *G. claroideum*).

Table 6 shows the percentage of plants colonised by the AM fungi and the dry weights of the aerial and root compartments (plus the ratio between these compartments) at the end of the second year. All plants remained colonised by the fungi with which they were inoculated. The control plants remained uncontaminated. The positive influence of the mycorrhizal systems on the development of the aerial and root compartments (and their dry weight ratio) was greater in the inoculated than in the control plants.

Discussion

The rooting percentage obtained with the present 'Cornicabra' cuttings raised under mist-propagation conditions was greater than that obtained by other authors in experiments designed to test the technique with a view to olive propagation (Caballero and del

Table 4. Percentage of plantlets colonised and dry weight of aerial and root compartments (in 2 L pots during first year of growth)

Treatment	% of plants colonised showing mycorrhizae	Dry weight (g)		
		Aerial comp.	Root comp.	Aerial comp./ root comp.
G. intraradices	100	32.3 a	18.4 a	1.75
Not inoculated	0	12.7 b	8.0 b	1.58
G. mosseae	100	31.7 a	17.8 a	1.78
Not inoculated	0	11.7 b	7.4 b	1.58
G. claroideum	100	38.1 a	22.5 a	1.69
Not inoculated	0	15.4 b	11.2 b	1.37

Values in columns with different letters indicate significant differences, as determined by Duncan's multiple range test ($p \leq 0.05$). Each value corresponds to the mean of 12 repetitions.

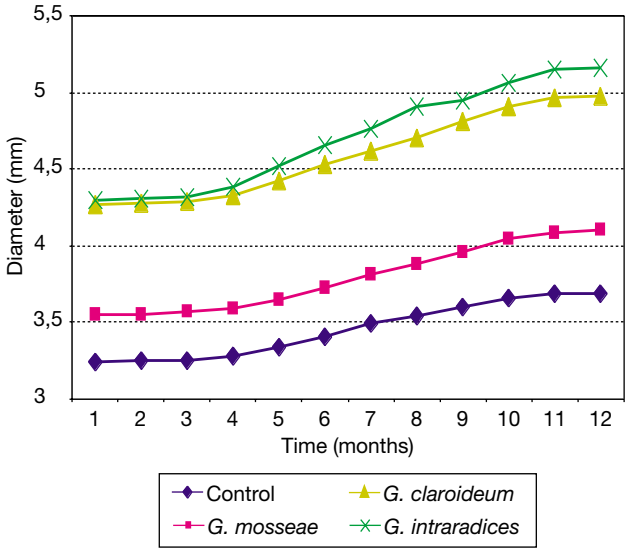

Figure 1. Mean monthly change in stem diameter of plants (pruned to a single stem) in 5 L pots measured 60 cm from the ground.

Río, 1994; Porras *et al.*, 2000). This shows that the plant material, the control systems and the climatic conditions imposed during the 75 days over which rooting took place were appropriate for obtaining plants of the necessary quality.

Mycorrhizal growth began when the AM fungi entered the young main and secondary roots before suberisation (Barea, 1998). The cuttings were inoculated when they had produced their first roots (at six months). All became colonised showing that inoculation is possible at this stage. These results agree with those reported by Jaizme-Vega and Rodríguez-Romero (1997) and Camprubí *et al*. (2000).

As reported by other authors (Barea *et al.*, 1987; Vidal *et al*., 1992), only 4% of the inoculated plants were lost in the compressed peat containers during preparation for the assay, while 7% of the non-inoculated plants died. The Pearson χ^2 test showed that inoculation significantly ($P \leq 0.05$) improved the resistance of the plants to the stress caused by transplantation.

Table 5. Stem diameter at 12 months of plants (5 L pots) pruned to a single stem, measured at 10 and 60 cm from the soil

Treatment	Diameter (cm)	
	Height above soil 10 cm	Height above soil 60 cm
Not inoculated	7.61 a*	3.38 a*
G. intraradices	9.61 c	4.58 c
G. mosseae	8.56 b	3.72 b
G. claroideum	9.43 c	4.49 c

* Values in columns with different letters indicate significant differences, as determined by Duncan's multiple range test ($p \leq 0.05$). Each value corresponds to the mean of 15 repetitions.

All three fungi had a positive effect on the development of the plants in the 2 L substrate-filled pots. All three provoked the growth of a greater number of shoots per plant (which were also longer than those of the controls) and led to the growth of taller plants. These results agree with those of Azcón and Barea (1997) and Barea *et al.* (1999).

At the beginning of the trial (during the winter) few differences were seen in the growth of the inoculated plants compared to the controls. This might have been due to the period of latency associated with the establishment of symbiosis. In addition, olive plants normally experience a halt in vegetative growth during the winter in Castilla-La Mancha. At the beginning of spring, however, significant differences ($P \leq 0.05$) in growth were seen (as determined by ANOVA and the Duncan multiple range test). This agrees with that reported by Vidal *et al.* (1992), Ganz *et al.* (2002) and

Table 6. Percentage of plants colonised and dry weight of the aerial and root compartments at 12 months in plants (pruned to a single stem) in 5 L pots

Treatment	% of plants colonised showing mycorrhizae	Dry weight (g)		
		Aerial comp.	Root comp.	Aerial comp./ root comp.
Not inoculated	0	75.85 a*	81.85 a	0.93
G. mosseae	100	119.42 b	111.42 b	1.07
G. claroideum	100	132.55 b	116.97 b	1.13
G. intraradices	100	189.85 c	153.90 c	1.23

* Values in columns with different letters indicate significant differences, as determined by Duncan's multiple range test ($p \leq 0.05$). Each value corresponds to the mean of 6 repetitions.

Estaún *et al.* (2003), who also found that mycorrhizas improve plant growth and development.

Eighteen months elapsed between the start of the rooting period and the time when the plantlets were removed from the 2 L pots, over which the growth of the inoculated cuttings (both aerial and root compartments) was significantly greater than that shown by the controls. This agrees with that reported for olive plantlets by Ganz *et al.* (2002) and Estaún *et al.* (2003), and by Vidal *et al*. (1992) for avocado plantlets. In the 5 L pots, significant differences ($P \leq 0.05$) were seen (as determined by the Duncan multiple range test) between the control and colonised plants. While the growth provoked by *G. intraradices* appeared to be greater than that induced by *G. claroideum*, no significant differences were seen. Significant differences were seen, however, between the growth provoked by these latter species and that induced by *G. mosseae*. These results show that not all AM fungi have the same effect on plants, as indicated by Barea *et al.* (1999) and Citernesi *et al.* (1998).

During winter, few differences were seen in the increase in stem diameter between colonised and control plants. Once again, this was probably due to the halt in vegetative growth that olive trees experience during winter in Castilla-La Mancha.

The aerial/root compartment ratios were greater in the plants in the 2 L pots (Table 4) than in the 5 L pots (Table 6). According to Pastor and Humanes (2000) and Ortega Nieto (2002), this may be an effect of pruning, which reduces aerial biomass. The length of time the plants were in these 5 L pots would have been insufficient for them to have reached their natural equilibrium. The development of both compartments, however, was always greater in the inoculated than in the control plants. These results confirm the benefits of using mycorrhiza-forming fungi referred to by Vidal *et al.* (1992), Ganz *et al.* (2002) and Estaún *et al.* (2003).

In conclusion it may be said that: i) early inoculation of the roots of 'Cornicabra' olive cuttings with AM fungi stimulates their growth during the nursery and early development stages; ii) the results confirm the possibility of using AM fungi habitually in olive nurseries; iii) the development obtained by such inoculation differs depending on the fungus used, although from an agronomic point of view all those used in the present work were helpful; iv) the economic and ecological interest in inoculating 'Cornicabra' with AM fungi requires the latters' role in the nutrition and health of mist-propagated olive plantlets be further studied.

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