Application of arbuscular mycorrhizal fungi on the production of cut flower roses under commercial-like conditions

I. Garmendia* and V. J. Mangas
Department of Environment and Earth Sciences, University of Alicante, Ap. 99, 03080 Alicante, Spain

Abstract
The objective of this work was to study the influence of two arbuscular mycorrhizal fungi (AMF) —*Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, and *G. intraradices* (Schenck & Smith) — on cut flower yield of rose (*Rosa hybrida* L. cv. Grand Gala) under commercial-like greenhouse conditions. Flower production was positively influenced by *G. mosseae* inoculation. Both inocula tested caused low levels of mycorrhizal root colonization, with higher percentages in *Rosa* associated with *G. mosseae*. Significant improvement of plant biomass, leaf nutritional status or flower quality was not detected in inoculated plants probably due to the low symbiosis establishment. However, *G. mosseae* reduced by one month the time needed for 80% of the plants to flower and slightly increased number of cut flowers relative to non-mycorrhizal controls on the fourth, sixth and eighth months after transplanting. It is suggested that an altered carbohydrate metabolism could contribute to this positive effect. Low colonization of rose roots supports the idea that more effort is required to ensure successful application of AMF in ornamental production systems.

Additional key words: Floriculture; *Glomus intraradices*; *Glomus mosseae*; phosphorus; *Rosa hybrida*.

Resumen
Aplicación de hongos micorrícico-arbusculares en el cultivo de rosas para corte bajo condiciones comerciales
El objetivo de este trabajo fue evaluar el efecto de los hongos micorrícico-arbusculares (MA) —*Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, y *G. intraradices* (Schenck & Smith) — en la producción comercial de rosas para corte (*Rosa hybrida* L. cv. Grand Gala) en invernadero. La inoculación con *G. mosseae* influyó positivamente en la producción de flores. Los resultados mostraron bajos niveles de colonización micorrícica de las raíces de rosa independientemente de la especie empleada, si bien las plantas tratadas con *G. mosseae* presentaron mayores porcentajes de colonización. El reducido establecimiento de la simbiosis muy probablemente fue la causa de que no se observara un aumento significativo de la biomasa vegetal o una mejora del estado nutricional y calidad de las flores en las plantas inoculadas. No obstante, la aplicación de *G. mosseae* redujo en un mes el tiempo requerido para que el 80% de las plantas estuviesen en floración y provocó un ligero aumento de la producción de flores en comparación con las plantas no micorrizadas, en el cuarto, sexto y octavo mes tras el trasplante. El metabolismo del carbono de estas plantas pudo haber contribuido al efecto positivo sobre la producción. La baja colonización micorrícica alcanzada por las raíces de rosa sugiere ajustar las condiciones de cultivo con el fin de mejorar la aplicación de las MA en los sistemas de producción ornamental.

Palabras clave adicionales: Floricultura; fósforo; *Glomus intraradices*; *Glomus mosseae*; *Rosa hybrida*.

Introduction
The mycorrhizal symbiosis is a natural relationship between plant roots and fungi that can enhance plant growth, reduce plant nutrient requirements, increase survival rate and development of micropropagated plants, improve plant resistance to abiotic and biotic stresses, enhance crop uniformity and rooting of cuttings, and increase fruit production (Azcón-Aguilar & Barea, 1997; Garmendia *et al.*, 2004, 2005). Moreover, related to

*Corresponding author: idoia.garmendia@ua.es
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Abbreviations used: AMF (arbuscular mycorrhizal fungi); DM (dry matter); Gi (*Glomus intraradices*); Gm (*Glomus mosseae*); MPN (most probable number); NM (non-mycorrhizal); TSS (total soluble sugars).
flower production, arbuscular mycorrhizal fungi (AMF) can reduce the days to flower bud emergence (Aboul-Nasr, 1996; Scagel, 2003, 2004a,b; Scagel & Schreiner, 2006), increase number of flowers per plant (Aboul-Nasr, 1996; Scagel, 2003, 2004a,b; Perner et al., 2007; Meir et al., 2010) and/or enhance flower longevity (Besmer & Koide, 1999; Scagel, 2004b). Application of AMF in rose production system could play a key role in a sustainable management of flower yield that includes reduction of chemicals and water use. Mycorrhizal symbiosis can improve water status and photosynthetic yield of rose plants under water stress (Augé et al., 1986a, 1987), increase rooting of miniature rose cuttings (Scagel, 2001, 2004c) and enhance concentrations of proteins and amino acids in miniature rose cuttings (Scagel, 2004c).

Greenhouse cut flower roses, such as *Rosa hybrida* L. cv. Grand Gala, are grown in intensive production systems with high fertilization and irrigation rates and tend to undergo multiple flushes of growth and flowering (Cabrera et al., 1995). Rose flower yield depends on induction of lateral shoots after flowers are cut, production of basal shoots, and time between flowerings (Cáballero et al., 1996). On the other hand, the vase-life of cut flowers is a commercially important trait. AMF can increase flower longevity (Scagel, 2004b), induce early flowering (Gaur et al., 2000) or raise weight, width and height of flowers (Sohn et al., 2003). Higher nutrient uptake as N, K and Mn, and increased carbohydrates concentration due to AMF symbiosis seems to be crucial (Sohn et al., 2003; Scagel, 2004b).

Recently, Koltai (2010) reviewed the difficulties in and opportunities for using AMF in floriculture, considering the prerequisite of availability of high quantities of good quality inocula, the need to use different types and dosages of AMF inocula and the agricultural crop growth practices that are best-suited for integration with mycorrhizal symbiosis. Therefore, the objective was to study the influence of AMF on the quantity and quality of cut flower roses under commercial-like greenhouse conditions.

**Material and methods**

**Biological material, growth conditions and experimental design**

One-year-old bareroot *Rosa hybrida* L. cv. Grand Gala on *Rosa manetti* rootstock (Universal Plantas, Spain) were transplanted to 5 L plastic pots filled with a mixture of perlite-coconut fibre (1:1 v/v). When transplanted to pots, plants were divided into three groups (24 plants per treatment): (a) non-inoculated plants (NM), (b) plants inoculated with *Glomus intraradices* (Schenck and Smith) (Gi), and (c) plants inoculated with *Glomus mosseae* (Nicol. and Gerdt.) Gerdt. and Trappe (Gm). Mycorrhizal inocula were supplied by Plant Biology Department of Navarra University (Spain) which were multiplied over three months using leek (*Allium porrum*) and alfalfa (*Medicago sativa*) grown in a mixture of perlite-coconut fibre (1:1 v/v). Infectivity of these inocula was evaluated by Most Probable Number (MPN) assay (Schenck, 1982) with *Sorghum bicolor* as the host plant. The bioassay was performed with five replicates per treatment. Sorghum was sown and grown for 4 weeks after germination in 200 mL pots under greenhouse conditions. Results for infectivity assay showed that *G. intraradices* and *G. mosseae* inocula had 228 and 223 propagules per 100 mL of substrate, respectively. Each AMF was applied as substrate-based inocula at a rate of 100 mL per pot containing root fragments, spores and hyphae of *Glomus*. The inocula were added to the planting hole and mixed with the surrounding growing substrate ensuring that good contact was achieved with runner roots.

Plants (including NM plants) were drip irrigated weekly with 1 L of Long Ashton Nutrient Solution (LANS) (Hewitt, 1966) at one-quarter phosphorus strength (12 mg P L\(^{-1}\)) to contribute to the establishment of mycorrhizal symbiosis (Azcón-Aguilar & Barea, 1997). The experiment was carried out in a greenhouse at 25/20°C day/night and plants received natural daylight supplemented with irradiation from sodium lamps Son-T Plus (Philips Nederland B.V., Eindhoven) during a photoperiod of 14 h. The experiment was carried out at the research greenhouse of University of Alicante (southeast of Spain) during the months between February and November of 2009. This research was done in a completely randomized design with a mean of 16 replicates per treatment to assess the effect of AMF on yield of rose plants, and 50 replicates per treatment to study the effect on flower quality. Pots were rotated regularly to avoid any positional effect under the semi-controlled conditions.

Plants (8 per treatment) were harvested 2, 5 and 9 months after transplanting and application of AMF inocula treatments.

**Estimation of AMF colonization**

Fresh samples from the middle root system were cleared and stained (Phillips & Hayman, 1970) and the
percentage of AMF root colonization was assessed by examining a minimum of 100 1-cm root segments for each treatment (Hayman et al., 1976).

**Plant growth and nutrients in leaves**

In each harvest, to assess growth of rose plants, total fresh weight was quantified before subsamples were taken, and dry matter (DM) of leaves, stems and roots per plant was determined after drying at 80°C for 2 days.

For mineral analysis, youngest five-leaflet leaves from vegetative shoots were used. Samples (0.5 g leaf dry weight) were dry-ashed and dissolved in HCl according to Duque (1971). Phosphorus, potassium, magnesium, calcium, manganese and iron were determined using a Perkin Elmer Optima 4300 inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer, USA). The operating parameters of the ICP-OES were: radio frequency power, 1300 W; nebulizer flow, 0.85 L min⁻¹; auxiliary gas flow, 0.2 L min⁻¹; sample introduction, 1 mL min⁻¹ and three replicates. Total nitrogen was quantified after combustion (950°C) of leaf dry matter with pure oxygen by an elemental analyser (TruSpec CN, Leco, USA) provided with a thermal conductivity detector.

**Flower quantity and quality**

Two times per week flowering was assessed and when at least one sepal had reflexed horizontally, flowers were deemed harvestable and flower yield was quantified (cumulative number of flowers per plant). Stem length of cut flowers or flowering shoots was measured from the shoot base to the base of the flower bud, and flower post-harvest longevity was assessed as the days between when flowers were harvested to when there was necrosis of at least the 50% of petals. When flowers were harvested quality features were also measured, including flower length and basal shoot diameter that give information about the flower size and shoot consistency respectively.

To determine flower longevity, flowering shoots were maintained in a growth chamber (Sanyo Gallenkamp PLC, SGC066.PHX.F, UK) with 20°C day/night, 60% of relative humidity and 15 μmol m⁻² s⁻¹ photosynthetic photon flux during a 12 h photoperiod (Muller et al., 2001). Each shoot was immersed in a graduated cylinder containing 100 mL of water with aluminium sulphate (up to pH = 5) in order to avoid bacterial proliferation.

First five-leaflet leaves from flowering shoots were used to determine chlorophyll concentration according to Séstak et al. (1971). Samples (20 mg of fresh leaves) were immersed in 5 mL of 96% ethanol at 80°C for 10 min to extract the pigments. The absorbance of extracts was spectrophotometrically measured and the equations reported by Lichtenthaler (1987) were used to calculate pigment concentrations.

**Biochemical analysis**

These determinations were performed on fresh subsamples of the middle root system and youngest five-leaflet leaves from vegetative shoots collected at midday in each harvest, frozen in liquid nitrogen and stored at −20°C for later analysis. Carbon balance of plants was analysed by total soluble sugars (TSS) and starch quantification in roots and leaves in potassium phosphate buffer (KPB) (50 mmol L⁻¹, pH = 7.5) extracts of fresh tissue (0.1 g). These extracts were filtered through four layers of cheese-cloth and centrifuged at 28,710 g for 15 min at 4°C. The pellet was used for starch determinations (Jarvis & Walker, 1993). Total soluble sugars were analysed spectrophotometrically in the supernatant with the anthrone reagent (Yemm & Willis, 1954).

**Statistics**

Statistical analyses were performed using SPSS software. Except for the percentage of plants showing flowers, all variables were analysed with one-way analysis of variance (ANOVA); means ± SD were calculated and, when the F-ratio was significant, least significance differences were evaluated by the Tukey-b test. Frequencies of rose plants showing flowers were analysed by χ² test. These frequencies were subjected to arc-sin transformation before applying χ² test. Significance levels were always set at 5%.

**Results**

The percentage of root colonization was low in rose inoculated with mycorrhizal fungi at the first harvest (2 months after transplanting and AMF inoculation) (Table 1), although plants inoculated with G. mosseae showed significantly greater values. This tendency was also observed in the second harvest (5 months after
transplanting). However, by the final harvest, plants inoculated with *G. mosseae* and *G. intraradices* reached to similar percentages of root colonization. There were no differences in plant biomass and DM partitioning between non-inoculated and inoculated rose at any harvest (Table 1). Rose plants non-inoculated with mycorrhiza remained uncolonized.

Although all plants were irrigated with LANS solution containing one-quarter strength P, foliar P concentration at the third harvest was normal even in NM plants (Table 2). Moreover, plants inoculated with AMF had similar concentrations in leaves of the analysed nutrients in comparison with NM plants 9 months after mycorrhizal inoculation.

Flower production was positively influenced by *G. mosseae* inoculation relative to non-mycorrhizal controls (Fig. 1). Induction of flowering took place 2 months after AMF inoculation and on the fourth, plants associated with *G. mosseae* exhibited the 80% of plants with flowers in comparison with the 50% in NM plants (Fig. 1a). In addition, the highest flower yield corresponded to Gm plants on the fourth, sixth and eighth months after transplanting relative to NM plants (Fig. 1b). Rose plants inoculated with *G. intraradices* did not show a significant greater flowering in comparison with NM plants, although they do not differ with Gm plants’ response.

In general, flowering shoots showed a mean length of 55 cm of shoot and 3.2 cm of flower, green leaves and 13 days of longevity (Table 3). Mycorrhizal inoculation did not produce significant differences in flowering characteristics (length of flowering shoots, basal diameter of flowering shoots, flower length and flower longevity). However, cut flowers of Gi plants had a lower chlorophyll concentration in leaves than that of non-mycorrhizal controls.

Plants inoculated with *G. mosseae* had similar starch concentrations in leaves as NM plants at the beginning of flowering (2 months after inoculation) (Fig. 2a1). Five months after AMF inoculation, starch concentration in leaves and roots was similar between treatments. And by the end of the experiment, Gm plants had lower foliar starch concentrations than NM plants, while AMF inoculation had no influence on starch concentration in roots (Fig. 2b1). Starch reductions in leaves during plant culture seemed to be concomitant with increases in roots (Fig. 2a1, b1). In reference to TSS, leaf concentration was no influenced by mycorrhizal inoculation (Fig. 2a2). In contrast, the influence of *Glomus* on TSS concentration in roots varied during the experiment (Fig. 2b2). Five months after mycorrhizal inoculation, Gi plants had the lowest TSS concentration in roots. In fact, only Gm plants had similar root TSS concentration to NM plants at the end of the experiment.

### Table 1. Plant dry matter (DM), root to shoot DM ratio and mycorrhizal colonization in non-inoculated plants and rose plants inoculated with *Glomus intraradices* or *Glomus mosseae* measured 2, 5 and 9 months after AMF inoculation

| Treatment          | Plant DM (g plant⁻¹) | Root/ Shoot | Mycorrhizal colonization (%) | Plant DM (g plant⁻¹) | Root/ Shoot | Mycorrhizal colonization (%) | Plant DM (g plant⁻¹) | Root/ Shoot | Mycorrhizal colonization (%) |
|--------------------|-----------------------|-------------|------------------------------|-----------------------|-------------|------------------------------|-----------------------|-------------|------------------------------|
|                    | 2                     | 5           | 9                            | 2                     | 5           | 9                            | 2                     | 5           | 9                            |
| Non-mycorrhizal    | 57.05 a               | 0.30 a      | 0 c                          | 95.89 a               | 0.25 a      | 0 c                          | 86.01 a               | 0.34 a      | 0 c                          |
| *G. intraradices*  | 52.98 a               | 0.40 a      | 3.02 b                       | 95.95 a               | 0.29 a      | 3.46 b                       | 92.74 a               | 0.32 a      | 10.53 a                      |
| *G. mosseae*       | 53.87 a               | 0.40 a      | 8.29 a                       | 89.20 a               | 0.26 a      | 12.69 a                      | 86.85 a               | 0.35 a      | 12.35 a                      |

Means (*n = 5-8 plants*) were analysed with one-way ANOVA, and least significant differences were evaluated by the Tukey-b test. Within each column values followed by a common letter are not significantly different (*p* ≤ 0.05).

### Table 2. Foliar concentration of nutrients in non-inoculated plants and rose plants inoculated with *Glomus intraradices* or *Glomus mosseae* after 9 months of AMF inoculation

| Treatment          | N (mg g⁻¹) | P (mg g⁻¹) | K (mg g⁻¹) | Ca (mg g⁻¹) | Mg (mg g⁻¹) | Mn (mg kg⁻¹) | Fe (mg kg⁻¹) |
|--------------------|------------|------------|------------|-------------|-------------|--------------|--------------|
| Non-mycorrhizal    | 30.1 a     | 2.3 a      | 21.5 a     | 24.8 a      | 2.0 a       | 194.34 a     | 204.49 a     |
| *G. intraradices*  | 30.9 a     | 2.4 a      | 20.0 a     | 23.4 a      | 1.9 a       | 204.63 a     | 205.33 a     |
| *G. mosseae*       | 32.0 a     | 2.6 a      | 22.4 a     | 22.6 a      | 1.9 a       | 202.92 a     | 202.65 a     |

Means (*n = 8 plants*) were analysed with one-way ANOVA, and least significant differences were evaluated by the Tukey-b test. Within each column values followed by a common letter are not significantly different (*p* ≤ 0.05).
The experimental system used, similar to commercial conditions, resulted in normal P concentration in leaves (Eymar et al., 2000) even in non-mycorrhizal plants. The rate of nutrient solution application, even with P at one-quarter strength, could negatively affect mycorrhizal association of rose plants (Azcón-Aguilar & Barea, 1997). Koltai (2010), in his recent review, discusses the need to change the use of fertilizers and pesticides in floriculture to improve the potential benefits from AMF. In fact, by the second month after inoculation, mycorrhizal colonization of the rootstock *R. manetti* only achieved to a mean of 6%, although it slightly increased until the final of the experiment to a mean of 11%. Several authors (Augé et al., 1986b; Green et al., 1998; Pinior et al., 2005) describe high colonization percentages of *R. hybrida* roots by *G. intraradices*. According to Augé et al. (1987), *R. hybrida* and *R. manetti* produce coarse, rather scanty root system and so, may be more reliant upon AMF symbiosis than more profusely rooting plants. However, high P fertilization rates can cause the depression of colonization levels of rose plants (Augé et al., 1986b).

The addition of mycorrhizal inoculum did not promote plant biomass of *Rosa cv. Grand Gala*. The percentage of mycorrhizal colonization achieved by rose roots probably resulted insufficient to influence growth of plants. On the other hand, Parke & Kaeppler (2000) caution that modern plant breeding indirectly and inadvertently may be selecting plants with less genetic capacity to respond positively to the mycorrhizal association, at least in terms of biomass production, suggesting low mycorrhizal dependency of new cultivars.

In rose, uptake of nutrients is low when shoot elongation rate is at its maximum and it increases as the...
flowering shoots reach commercial maturity (Cabrera et al., 1995). Nine months after growing, when nearly 100% of plants had produced harvestable flowering shoots, plants exhibited normal nutrient concentration in leaves, with the exception of quite high Ca and Fe levels (Eymar et al., 2000). Disagreeing with the general idea of enhanced nutrition of mycorrhizal hosts (Estaún et al., 2002), mineral analysis of rose plants did not indicate a nutritional effect of mycorrhizal inoculation in leaves, probably due to the low root colonization percentages reached. Similarly, Aboul-Nasr (1996) found that the effect of mycorrhizal symbiosis in Tagetes and Zinnia was independent of changes in nutrient contents of plants, despite the average percentage of mycorrhizal root colonization was 50%.

The strongest effect of AMF was found in flower yield. Plants associated with *G. mosseae* inoculum exhibited early flowering and increased number of cutflowers of *R. hybrida* comparing with non-mycorrhizal ones. This trait of AMF is of particular interest to flower farming. *Glomus mosseae* reduced by one month the time needed for 80% of the plants to flower and could

![Starch concentrations in leaves and roots](image1)

![Total soluble sugars concentrations in leaves and roots](image2)

**Figure 2.** Starch (mg g⁻¹ DM) (a1, a2) and total soluble sugars (TSS) (mg g⁻¹ DM) (b1, b2) concentrations in leaves (a1, b1) and roots (a2, b2) of non-inoculated plants (NM) and rose plants inoculated with *Glomus intraradices* (Gi) or *Glomus mosseae* (Gm). Means ± SD (n = 5–8) were analysed with one-way ANOVA, and least significant differences were evaluated by the Tukey-b test. Within each variable and month after arbuscular mycorrhizal fungi (AMF) inoculation, columns with the same letter do not differ significantly (p ≤ 0.05).
increase flower yield between 30 and 50%. Similar results have been reported in number of ornamental plants in response to mycorrhizal inoculation (Aboul-Nasr, 1996; Gaur et al., 2000; Sohn et al., 2003; Scagel, 2003, 2004a,b; Nowak, 2004; Meir et al., 2010) even with commercial formulations (Perner et al., 2007). The enhancement of flowering in plants inoculated with G. mosseae was independent of foliar nutrient concentration, suggesting a non-nutritional basis of the effect of mycorrhizal association, as Aboul-Nasr (1996) explained in flower production of Tagetes and Zinnia after their inoculation with G. etunicatum. Also, the presence of some microorganisms in the rhizosphere of Gm plants could contribute to the beneficial effect of inoculum of G. mosseae (Azcón-Aguilar & Barea, 1997).

In general, flowering shoots of Grand Gala showed normal to low marketable quality. Marketable quality features in rose include high length of flowering shoots (50-70 cm), long conservation vase life, flower length and diameter, flowering shoot consistence (stem diameter) and green and bright leaves (Eymar et al., 2000; Infoagro, 2010). Mycorrhizal inoculation did not improve rose quality and/or longevity probably due to low root colonization percentages, even flowering shoots from Gi plants showed lower leaf chlorophyll concentration than NM ones. According to Sohn et al. (2003) AMF improved fresh weight, width and/or height of chrysanthemum (with 40% of root mycorrhizal colonization). Similarly, mycorrhizal symbiosis significantly increased flower vase-life of snapdragon (with 71% of root mycorrhizal colonization) related in part to a decrease of flower ethylene production (Besmer & Koide, 1999). In rose, Green et al. (1998) described a reduction in transpiration of detached leaves from plants associated with Glomus, which could suggest higher longevity for harvested flowering shoots.

Mycorrhizal root systems influence the source to sink balance by utilizing recent photosynthesize supplied by photosynthesis in leaves and a considerable proportion of the assimilated C (Douds et al., 2000; Smith & Read, 2008). Nevertheless, AMF can increase the ability of plant to fix CO₂ and, consequently, the C expense of the fungus remained offset (Smith & Read, 2008). According to Scagel (2003), AMF inoculation increased carbohydrate production and storage in bulbs of Zephyranthes, playing a mayor role for growth and flowering during the following growing cycle. Similarly, corms produced by Brodiaea inoculated plants had higher concentration of nonreducing sugars than non-inoculated plants (Scagel, 2004b). In rose, higher leaf starch and lower total soluble carbohydrate content has been described due to mycorrhizal association (Augé et al., 1987). The carbohydrate data of the present study indicate that only plants associated with G. mosseae, which showed an earlier and greater flower production relative to non-mycorrhizal plants, were able to reach similar leaf starch concentration to non-mycorrhizal plants at the beginning of flowering, assuring the counterbalance of the C cost of AMF association. Despite starch concentration in roots colonized by G. mosseae was high and root TSS concentrations were similar to controls till the end of the experiment, leaf starch levels of Gm plants were significantly lower than non-mycorrhizal plants by the final harvest. The influence of G. mosseae on leaf starch may be related to the plants’ metabolic requirements to sustain the higher flower yield. The enhance of flowering of rose plants associated with G. mosseae may be the consequence of higher carbohydrate production, especially at the beginning of flower production, and/or more efficient carbohydrate use of these plants during the reproductive phase.

Large-scale application of mycorrhizal inocula may guarantee to be cost effective. These results document the potential variation in responsiveness of mycorrhizal symbiosis depending on the AMF endophyte used and support that the host plant genotype is crucial to assure its efficacy. Glomus mosseae contributed to faster flowering and to some extent, increased flower yield of Grand Gala roses, which could translate to higher profits for farmers. Carbohydrate metabolism seems to be the responsible of this physiological effect. Nevertheless, mycorrhizal establishment in rose roots was low and therefore, neither growth enhancement, nor foliar nutritional influence or effect on quality of roses was observed due to AMF inoculation. More research concerning AMF-friendly agricultural practices together with the best AMF-rose variety interaction could help to achieve a sustainable floriculture with flower yield benefits.

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References

Aboul-Nasr A, 1996. Effects of vesicular-arbuscular mycorrhiza on *Tagetes erecta* and *Zinnia elegans*. Mycorrhiza 6: 61-64.

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

Augé RM, Schekel KA, Wample RL, 1986b. Greater leaf conductance of well-watered VA mycorrhizal rose plants is not related to phosphorus nutrition. New Phytol 103: 107-116.

Augé RM, Schekel KA, Wample RL, 1987. Leaf water and carbohydrate status of VA mycorrhizal rose exposed to drought stress. Plant Soil 99: 291-302.

Azcón-Aguilar C, Barea JM, 1997. Applying mycorrhiza biotechnology to horticulture: significance and potentials. Sci Hortic 68: 1-24.

Besmer YL, Koide RT, 1999. Effect of mycorrhizal colonization and phosphorus on ethylene production by snapdragon (*Antirrhinum majus*) flowers. Mycorrhiza 9: 161-166.

Cabrera RI, Evans RY, Paul JL, 1995. Cyclic nitrogen uptake by greenhouse roses. Sci Hortic 63: 57-66.

Douds DD, Pfeffer PE, Shachar-Hill Y, 2000. Carbon partitioning, cost, and metabolism of arbuscular mycorrhizas. In: *Arbuscular mycorrhizas: physiology and function* (Capulnik Y, Douds DD, eds). Kluwer Acad Publ, Boston, USA. pp: 107-129.

Duque F, 1971. Determinación conjunta de fósforo, potasio, calcio, hierro, manganeso, cobre y zinc en plantas. Ann Edafol Agrobiol 30: 207-229. [In Spanish].

Estaun V, Camprubi A, Poner EJ, 2002. Selecting arbuscular mycorrhizal fungi for field application. In: *Mycorrhizal technology in agriculture. From genes to bioproducts* (Gianinazzi S, Schüepp H, Barea JM, Haselwandter K, eds). Birkhäuser Verlag, Berlin, Germany. pp: 249-259.

Eymar E, López-Vela D, Cadahia C, 2000. Fertilirrigación de coníferas y rosal. In: *Fertilirrigación: cultivos hortícolas y ornamentales* (Cadahia C, ed.). Ediciones Mundi-Prensa, Madrid, Spain. pp: 419-463. [In Spanish].

Garmendia I, Goicoechea N, Aguirreolea J, 2004. Effectiveness of three *Gnomus* species in protecting pepper (*Capsicum annuum* L.) against verticillium wilt. Biol Control 31: 296-305.

Garmendia I, Goicoechea N, Aguirreolea J, 2005. Moderate drought influences the effect of arbuscular mycorrhizal fungi as biocontrol agents against *Verticillium*-induced wilt in pepper. Mycorrhiza 15: 345-356.

Gaur A, Gaur A, Adholeya A, 2000. Growth and flowering in *Petunia hybrida*, *Callistephus chinensis* and *Impatiens balsamina* inoculated with mixed AM inocula or chemical fertilizers in a soil of low P fertility. Sci Hortic 84: 151-162.

Green CD, Stodola A, Augé RM, 1998. Transpiration of detached leaves from mycorrhizal and nonmycorrhizal cowpea and rose plants given varying abscisic acid, pH, calcium, and phosphorus. Mycorrhiza 8: 93-99.

Hayman DS, Barea JM, Azcón R, 1976. Vesicular-arbuscular mycorrhiza in southern Spain: its distribution in crops growing in soil of different fertility. Phytopathol Mediterr 15: 1-6.

Hewitt EJ, 1966. Sand and water culture methods used in the study of plant nutrition. Commonwealth Agricultural Bureaux, London, Technical Communication No. 22, 2nd ed. rev.

INFOAGRO, 2010. El cultivo de rosas para corte. Available in [http://www.infoagro.com/flores/flores/rosas.htm](http://www.infoagro.com/flores/flores/rosas.htm) [10 December 2010]. [In Spanish].

Jarvis CE, Walker JRL, 1993. Simultaneous, rapid, spectrophotometric determination of total starch, amylose and amylopectin. J Sci Food Agric 63: 53-57.

Koltai H, 2010. Mycorrhiza in floriculture: difficulties and opportunities. Symbiosis 53: 55-63.

Lichtenhaler HK, 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148: 350-382.

Meir D, Pivonia S, Levita R, Dori I, Ganot L, 2010. Application of mycorrhizae to ornamental horticultural crops: *lisianthus* (*Eustoma gradiflorum*) as a test case. Span J Agric Res 8(S1): 5-10.

Muller R, Stummann BM, Andersen AS, 2001. Comparison of postharvest properties of closely related miniature rose cultivars (*Rosa hybrida* L.). Sci Hortic 91: 325-338.

Nowak J, 2004. Effects of arbuscular mycorrhizal fungi and organic fertilization on growth, flowering, nutrient uptake, photosynthesis and transpiration of geranium (*Pelargonium hortorum* L. H. Bailey ‘Tango Orange’). Symbiosis 37: 259-266.

Parke JL, Kaeppler SA, 2000. Effects of genetic differences among crop species and cultivars upon the arbuscular mycorrhizal symbiosis. In: *Arbuscular mycorrhiza: physiology and function* (Capulnik Y, Douds DD, eds). Kluwer Acad Publ, Boston, USA. pp:131-146.

Perner H, Schwarz D, Bruns C, Mäder P, George E, 2007. Effect of arbuscular mycorrhizal colonization and two levels of compost supply on nutrient uptake and flowering of pelargonium plants. Mycorrhiza 17: 469-474.

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

Pinior A, Grunewald-Stöcker G, Von Alten H, Strasser RJ, 2005. Mycorrhizal impact on drought stress tolerance of rose plants probed by chlorophyll a fluorescence, proline content and visual scoring. Mycorrhiza 15: 596-605.
Scagel CF, 2001. Cultivar specific effect of mycorrhizal fungi on the rooting of miniature rose cuttings. J Environ Hort 19: 15-20.

Scagel CF, 2003. Inoculation with arbuscular mycorrhizal fungi alters nutrient allocation and flowering of Freesia × hybrida. J Environ Hort 21: 196-205.

Scagel CF, 2004a. Inoculation with vesicular-arbuscular mycorrhizal fungi and rhizobacteria alters nutrient allocation and flowering of harlequin flower. HortTecnology 14: 39-48.

Scagel CF, 2004b. Soil pasteurization and mycorrhizal inoculation alter flower production and corm composition of Brodiaea laxa ‘Queen Fabiola’. HortScience 39: 1432-1497.

Scagel CF, 2004c. Changes in cutting composition during early stages of adventitious rooting of miniature rose altered by inoculation with arbuscular mycorrhizal fungi. J Am Soc Hort Sci 129: 624-634.

Scagel CF, Schreiner RP, 2006. Phosphorus supply alters tuber composition, flower production, and mycorrhizal responsiveness of container-grown hybrid Zantedeschia. Plant Soil 283: 323-337.

Schenck NC, 1982. Methods and principles of mycorrhizal research. The American Phytopathological Society, St. Paul, MN, USA.

Séstak Z, Cátsky J, Jarvis P, 1971. Plant phosynthetic production. Manual of methods. Dr Junk Publ, The Hague, The Netherlands.

Smith SE, Read DJ, 2008. Growth and carbon economy of arbuscular mycorrhizal symbionts. In: Mycorrhizal symbiosis (Smith SE, Read DJ, eds.). Academic Press, London, UK. pp: 117-144.

Sohn BK, Kim KY, Chung SJ, Kim WS, Park SM, Kang JG, Rim YS, Cho JS, Kim TH, Lee JH, 2003. Effect of the different timing of AMF inoculation on plant growth and flower quality of chrysanthemum. Sci Hortic 98: 173-183.

Yemm EW, Willis AJ, 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57: 508-514.