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ORIGINAL PAPER

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ARBUSCULAR MYCORRHIZAL INOCULATION AND SHADING ENHANCE CROP PERFORMANCE AND QUALITY OF GREENHOUSE *Begonia semperflorens*

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ABSTRACT

Mycorrhizal fungi are gaining interest in the floriculture sector due to the beneficial effects on a crop performance and ornamental quality. The aim of the current study was to assess the effect of inoculation with the arbuscular mycorrhizal (AM) fungi *Rhizophagus irregularis* on ornamental quality of *Begonia* × *semperflorens-cultorum* grown in two different protected cultivation systems: a shadehouse or glasshouse. The inoculated plants incurred a significant increase of plant height by 34.6%, lateral shoot length by 27.9%, number of lateral shoots by 41.2%, number of flowers per plant by 102.9%, flower diameter by 27.5%, and stems dry weight by 263.6%. High temperatures in the glasshouse negatively affected the AM root colonization. On the contrary, shading induced higher mycorrhizal colonization (48.6%) and increased plant height, number of lateral shoots, number of flowers per plant and flower diameter compared to the glasshouse environment. Taking all together, our results clearly demonstrated that mycorrhizal inoculation at transplanting and shading may be beneficial to floriculture growers wishing to produce high quality *B. semperflorens-cultorum* plants during the spring-summer season.

Key words: wax begonia, mycorrhizal inoculation, bedding plant, pot plant, ornamental quality

INTRODUCTION

Arbuscular mycorrhizal (AM) symbiosis is formed between the majority of terrestrial plants [Smith and Read 2008] and fungi of the monophyletic phylum *Glomeromycota* [Schüßler et al. 2001]. AM fungi are obligate symbionts and acquire carbon from their host plant to complete their life cycle [Bago et al. 2000]. In return, the fungus provides multiple benefits for the plant, including tolerance to abiotic and biotic stresses [Sawers et al. 2008, Smith and Read 2008]. Several authors confirmed that AM is a promising and environment friendly tool to increase ecological stresses tolerance [Cantrell and Linderman 2001] such as thermal stresses [Zhu et al. 2011] and stressful edaphic conditions, including soil salinity [Cantrell and Linderman 2001], water stress [Püschel et al. 2014] and soil toxicity produced by heavy metals [Ouziad et al. 2005, Shahabivand et al. 2012, Kumar et al. 2015, Rouphael et al. 2015]. Other authors found that mycorrhizal plants exhibit enhanced post-transplant survival and growth [Biermann and Linderman 1983a, Chang 1992, Vosátka 1995] and are considered more resistant to certain pathogens [Dugassa et al. 1996]. AM fungi are the predominant association on most cultivated plants. However, it is rarely present in soilless media without

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intentional inoculation and management by the growers. Nevertheless, if growth media have soil included, the soil could carry inoculum or it could be introduced as an airborne contaminant in dust [Linderman and Davis 2003]. The use of AM fungi is often promoted for a wide range of applications [Feldmann et al. 2008, Gianinazzi et al. 2010], including cultivation of ornamental plants such as *Dimorphoteca sinuata*, *Impatiens hawkerii*, *Pelargonium peltatum*, *Pelargonium zonale* and *Sanvitalia procumbens* [Vosátka and Albrechtová 2008, Koltai 2010, Püschel et al. 2014]. AM fungi were found to increase the length or number of branches in *Eustoma grandiflorum* [Meir et al. 2010], the number and size of flowers in *Tagetes erecta*, *Zinnia elegans* and *Pelargonium peltatum* [AboulNasr 1996, Perner et al. 2007, Long et al. 2010] and the shoot dry weight in *Verbena* sp., *Torenia fournieri* and *Diascia cordata* [Vosátka et al. 1999, Šrámek et al. 2000]. AM fungi can also induce early flowering [Hunter 1997, Gaur et al. 2000, Garmendia and Mangas 2012]. However, some authors reported neutral or negative effects of AM fungi on ornamental plants [Koide et al. 1999, Linderman and Davis 2004, Gaur and Adholeya 2005]. Furthermore, it was found that soil environment, cultivation substrates [Linderman and Davis 2003, Püschel et al. 2014] and growth environment conditions may affect the AM colonization capacity, spore germination and fungal hyphal growth [Jahromi et al. 2008, Miransari 2010]. For instance, Bowen [1987] and Heinemeyer et al. [2004] reported that AM fungi colonization of plant roots is reduced when the temperature exceeds 30°C. Moreover, Bendavid-Val et al. [1997] and Martin and Stutz [2004] found that temperature up to 40°C often becomes lethal to AM fungi. In this regard, some researchers have reported that mycorrhizal plants grow better than non-mycorrhizal plants under high temperature stress [Haugen and Smith 1992, Gavito et al. 2005]. Garden design in areas with Mediterranean climate nowadays relies mainly on native ornamental shrubs that are tolerant to the hot and dry local summer [Iapichino et al. 2006, Lopez et al. 2006, Cassaniti et al. 2009, Sabatino et al. 2014]. However, the use of tender herbaceous perennials in mixed borders and island beds is still common in Mediterranean-type environments as long as irrigation during the summer season is provided. The wax begonia (*Begonia* × *semperflorens-cultorum* Hort.) is commonly used as a bedding and pot plant adaptable for shade and sunny areas [Bailey and Bailey 1976]. Although adequate temperature, light and humidity are the most important environmental requirements for producing the high quality begonia plants, there are limited information on the influence of AM fungi on growth and plant quality of wax begonia in the Mediterranean environment.

Based on the above considerations, the aim of our study was to assess the growth, development, and ornamental quality response of *Begonia* × *semperflorens-cultorum* in relation to AM fungi inoculation under two different growing conditions (shadehouse and glasshouse) in a typical Mediterranean region such as Sicily.

MATERIALS AND METHODS

Plant material, growth conditions and treatments. The trial was conducted at the Department of Agriculture, Alimentary and Forest Sciences (SAAF) of the University of Palermo, at Palermo in the northern coast of Sicily (Italy) (longitude 13°19'E, latitude 38°9'N) during the spring-summer growing season in 2016. *Begonia* × *semperflorens-cultorum* plug-plants were transplanted (one plant per pot) into round plastic pots (15 cm in diameter and 13 cm in height) containing 1.0 L of peat-perlite mixture (VIGORPLANT, SER FS V10-18, Italy) 80:20 (v/v) (experimental pots). The substrate had the following properties: pH 5.5, electric conductivity 0.20 dSm^{-1}, (dry) bulk density 95 kg m^{-3}, total porosity 94%, NH_4 220 mg kg^{-1}, NO_3 833 mg kg^{-1}, P 40 mg kg^{-1}, K 631 mg kg^{-1}.

Prior to transplanting, half of the experimental pots received a mycorrhizal inoculum carrying 40 spores g^{-1} of *Rhizophagus irregularis* (formerly *Glomus intraradices*). Inoculum was applied in the substrate mix before transplant at a rate of 10 g $plant^{-1}$. Transplanting was performed on 9th May 2016. After transplanting, half of inoculated (AM) plants and not-inoculated (NM) plants were placed into an unheated glasshouse and the other half were moved into a shadehouse covered with a 80% black shade cloth (Retes srl, Milan, Italy). Air temperature in both growth environments was recorded during the night and during the day (Figs. 1, 2 and 3). Light level inside the two growth environments was recorded using a quantum light

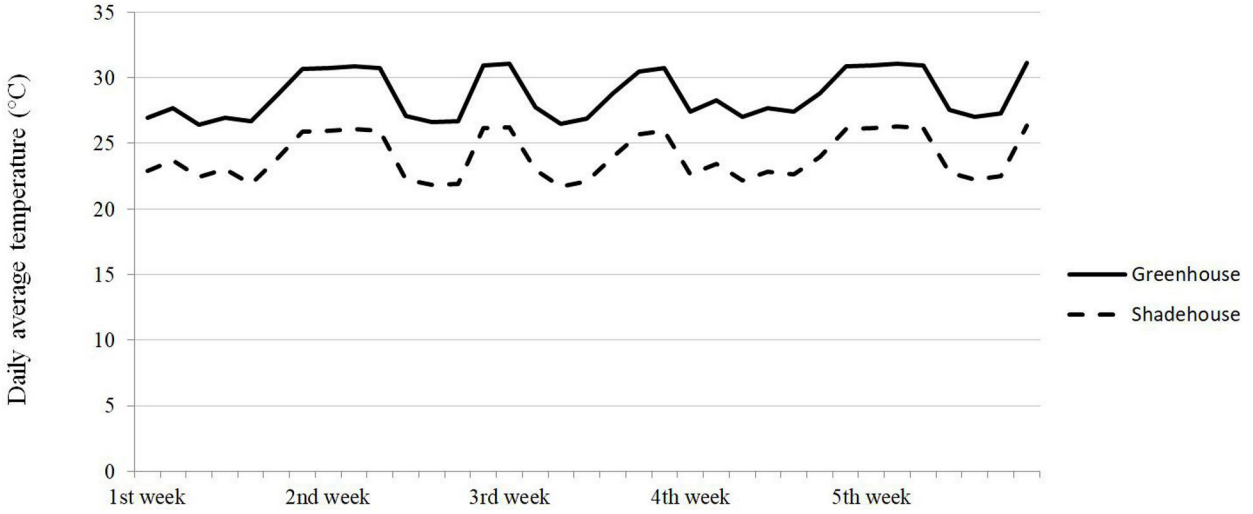

Fig. 1. Daily average temperature in Palermo, Sicily, during the experimental period in an unheated greenhouse covered with glass and in a shadehouse with a 80% shade cloth

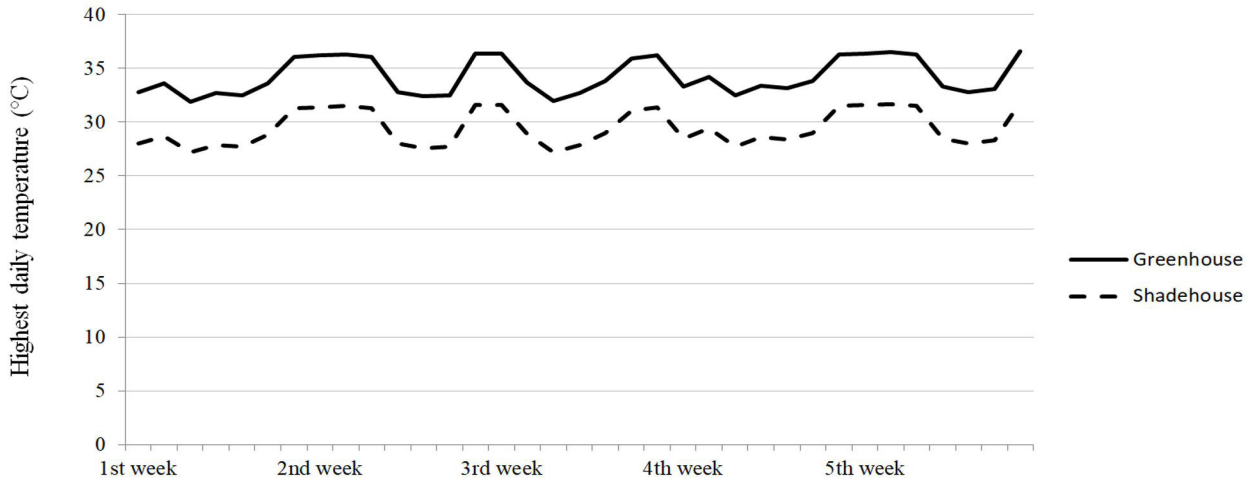

Fig. 2. Highest daily temperature in Palermo, Sicily, during the experimental period in an unheated greenhouse covered with glass and in a shadehouse with a 80% shade cloth

meter [LI-190 quantum sensor (Licor, Lincoln NE)] and the average daily photosynthetic light integral (DLI) was calculated (Fig. 4). The experiment was watered as necessary to ensure that the plants would not be exposed to drought stress. No additional fertilizer was applied throughout the experiment, because fertilization application to soil mixes has been documented to suppress AM association [Biermann and Linderman 1983].

Growth and ornamental quality measurements. In order to assess the ornamental quality of begonia plants, the measurements were recorded three times (on 20th July 2016, 4th August 2016 and 19th August 2016). The first and second sampling date were recorded 72 and 87 days after transplanting, respectively, for plant height, lateral shoot length, number of lateral shoots, number of leaves and number of flowers. The third sampling date set was taken after 102 days,

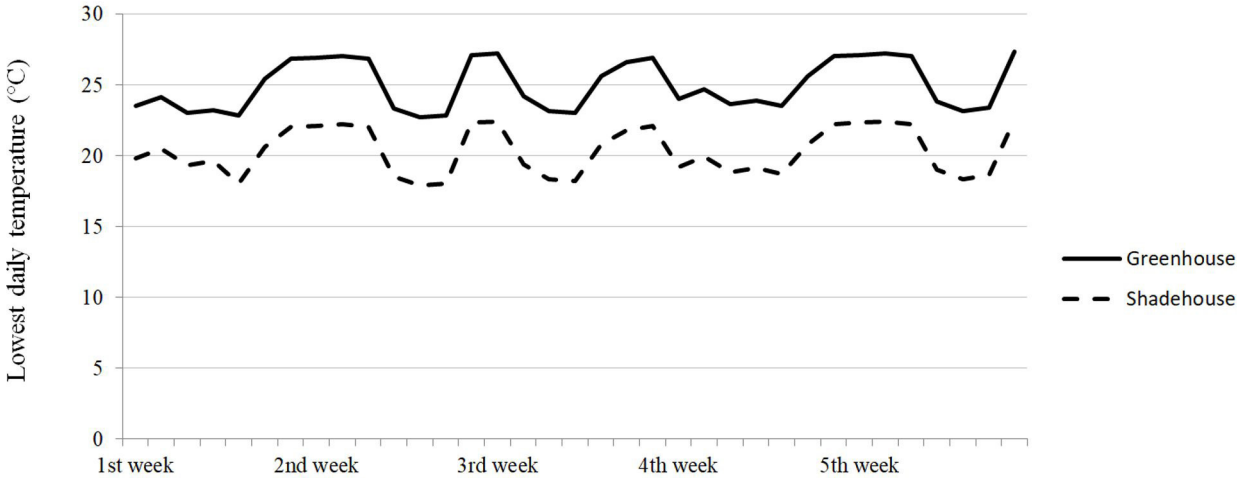

Fig. 3. Lowest daily temperature in Palermo, Sicily, during the experimental period in an unheated greenhouse covered with glass and in a shadehouse with a 80% shade cloth

for plant height, lateral shoot length, number of lateral shoots per plant, number of leaves per plant, number of flowers per plant as well as for flower diameter. Additionally, to compare the growth plant response to inoculation, mycorrhizal growth response (MGR) was calculated for shoot dry weight according to the equation MGR = $(M - NM_{mean})/NM_{mean} \times 100\%$, where M is the value of shoot dry weight recorded for a given inoculated plant and NM_{mean} is the mean value of shoot dry weight of plants in the corresponding not-inoculated treatment [Gange and Ayres 1999].

Biomass production and partitioning. At the end of the experiment (19th August 2016 − 102 days after transplanting), each plant was separated in leaves, stems, and roots. Fresh and dry weights of each organs were determined. Roots were washed to remove the substrate and then weighed. The dry weight was recorded by drying samples in a thermo-ventilated oven at 80°C for 72 h until constant weight was reached.

Root colonization. To visualize the mycorrhizal development, three samples of lateral roots from each AM plant were collected and stained by acid fuchsine. In particular, the Phillips and Haymann's technique [1970], modified by Torta et al. [2003], was applied. The mycorrhizal colonization (Mycorrhization Index: MI = % of stained tissue, with respect to hyaline portion, on the unit of length of the root) was determined on three fragments, obtaining the average value [Kormanik and McGraw 1991]. The weight of the root sub-sample used for the determination of mycorrhizal colonization was added to root dry weight after recalculation of its fresh weight to dry weight.

Statistical analysis. The experiment was designed as a two-factorial combination of two mycorrhizal treatments (I) − with AM, (AM) or without AM, (NM) control treatment; and two growth environments (G) − glasshouse or shadehouse with 80% shading. The treatments were arranged in a randomized complete block design with four replicates per treatment. Each experimental unit consisted of ten pot plants. All data were statistically analyzed using the SPSS software package version 14.0 (StatSoft, Inc., Chicago, USA). Data were subjected to a two-way ANOVA [inoculation (I) × growth environments (G)]. For MGR parameter, which shows only one factor (growth environments), one-way ANOVA was performed. Mean separation was assessed by Tukey HSD test. Percentage data were subjected to arcsin transformation before ANOVA analysis [$Ø = \arcsin (p/100)^{1/2}$]. The relationship between MGR and root colonization was assessed using linear regression performed on the means of the two parameters.

RESULTS

Climatic conditions inside the greenhouse

Average light intensity during the growing period ranged from 26.6 to 30.6 mol m^2 d^{-1} and from 18.8

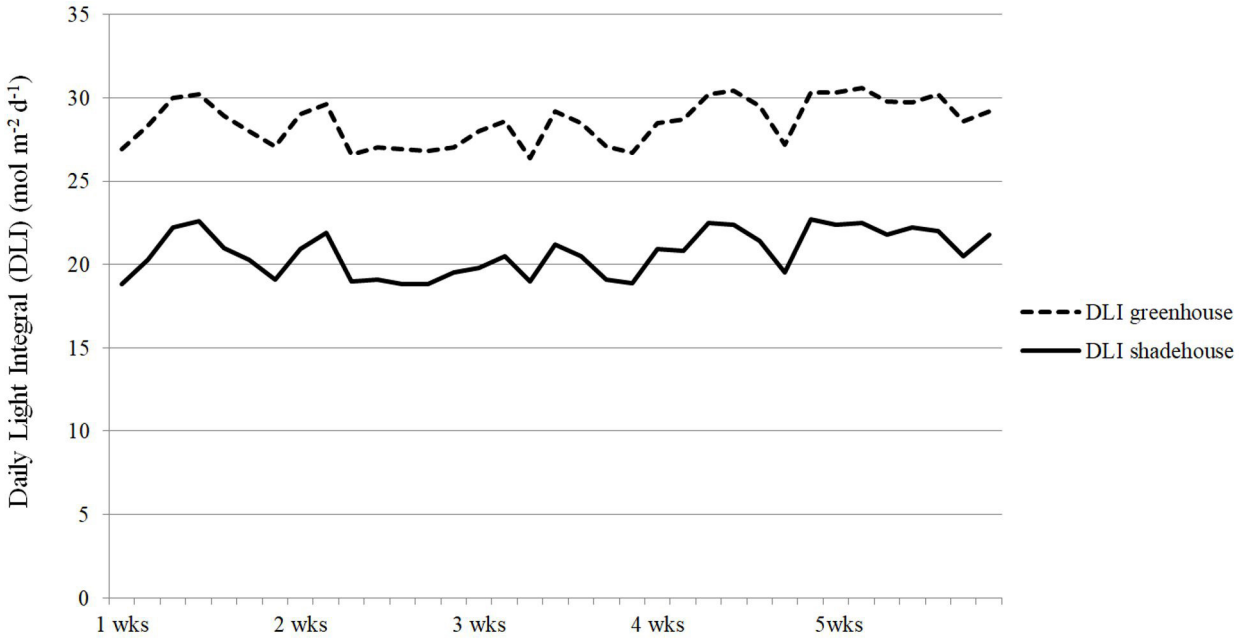

Fig. 4. Average daily light integrals during the experimental period in Palermo, Sicily, in an unheated greenhouse covered with glass and in a shadehouse covered with a 80% shade cloth

to 22.7 mol m^2 d^{-1} in the glasshouse and in the shadehouse, respectively (Fig. 4). According to Hamrick [2003], this range of light levels should produce high quality plants of wax begonia. Average temperatures during the growing period ranged from 26.4 to 31.2°C and from 21.7 to 26.4°C in the glasshouse and in the shadehouse, respectively (Fig. 1). Therefore, shading lowered air temperature compared to the glasshouse with an average daily decrease of 4.8°C.

Root colonization

Regardless of the inoculation, the shadehouse environment significantly increased the mycorrhizal colonization (48.6%) compared to the glasshouse environment (29%) (Tab. 1). Mycorrhizal inoculation reached 66.5% in AM plants and 11.1% in NM plants. ANOVA showed a significant effect of the interaction (I × G) (Tab. 1). The highest mycorrhizal colonization was recorded from AM plants grown into the shadehouse (81.3%), followed by AM plant cultivated into the glasshouse (51.8%), which in turn showed a higher mycorrhizal colonization than NM plants grown the shadehouse (16%). The lowest value, in terms of mycorrhizal colonization, was recorded from NM plants grown into the glasshouse (6.3%) (Fig. 5).

Plant quality and growth parameters

First sampling date. Regardless of the inoculation, the growth environment did not significantly affect the plant height. Conversely, inoculated (AM) plants were significantly higher than non-inoculated (NM) ones. No significant interaction was observed between I and G in terms of plant height (Tab. 1).

Irrespective of the inoculation, lateral shoot length value was significantly higher by 86.2% in plants grown in the shadehouse in comparison to the glasshouse (Tab. 1). Regardless of the growth environment, inoculation significantly increased the lateral shoot length by 27.1% compared with NM plants. ANOVA for lateral shoot length showed a significant effect of the interaction (I × G). The highest lateral shoot length was recorded in AM plants grown in the shadehouse (6.7 cm), whereas, the lowest value was found in AM and NM plants grown in the glasshouse (2.9 and 2.9 cm, respectively) (Fig. 6). Regardless of the inoculation, growth environment did not significantly affect

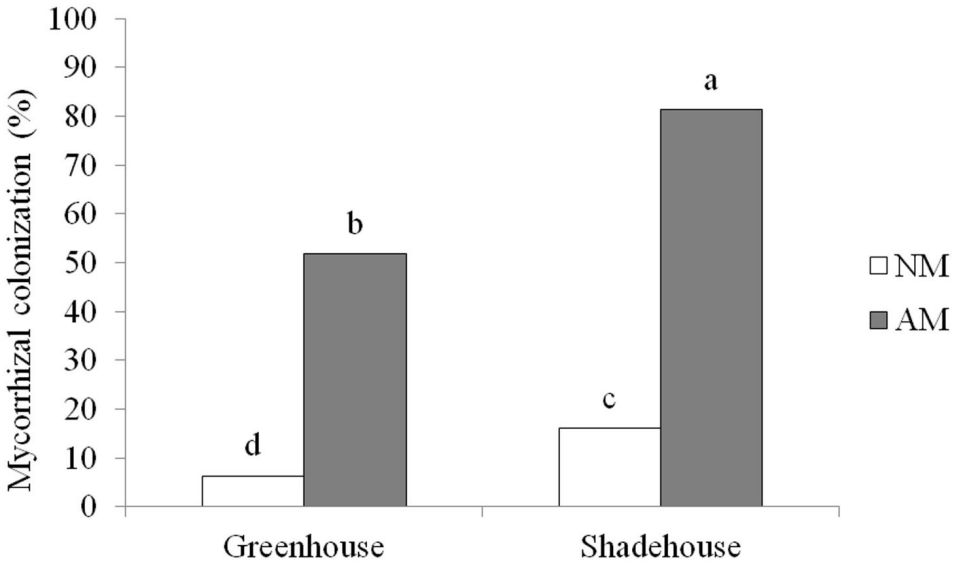

Fig. 5. Effect of growth environment (greenhouse or shadehouse) and inoculation (AM or NM) on *Begonia semperflorens* mycorrhizal colonization 102 days after planting. Bars with different letters are significant by Tukey HSD Test (P < 0.001)

Table 1. Effects of growth environment and AM inoculation on plant growth and ornamental quality parameters of potted begonia 72 days after transplanting

Treatments		Mycorrhizal colonization (%)		Plant height (cm)		Lateral shoot length (cm)		No. of lateral shoots (no. plant^{-1})		No. of leaves (leaves plant^{-1})		No. of flowers (flowers plant^{-1})	
Inoculation	AM	66.5	a	18.2	a	4.8	a	9.6	a	106.9	a	24.5	a
	NM	11.1	b	15.0	b	3.5	b	6.5	b	59.9	b	16.7	b
Growth environment	greenhouse	29.0	b	14.7	ns	2.9	b	7.8	ns	74.2	b	13.4	b
	ghadehouse	48.6	a	17.4		5.4	a	8.4		92.6	a	27.8	a
Effect of factors		*F* value		*F* value		*F* value		*F* value		*F* value		*F* value	
	inoculation	1159	***	8.72	**	5.6	*	14.8	**	34.4	***	8.3	**
	growth environment	167.5	***	2.41	ns	21.5	***	0.5	ns	5.3	*	28.9	***
	interaction	18.5	***	0.22	ns	5.7	*	25.7	***	3.8	ns	1.8	ns

In each column and for each fixed factor, values followed by same letters are not statistically different according to Tukey HSD Test (P ≤ 0.05). The significance is designated by asterisks as follows: *, statistically significant differences at P-value below 0.05; **, statistically significant differences at P-value below 0.01; ***, statistically significant differences at P-value below 0.001. ns, not significant. F value = F-test ANOVA

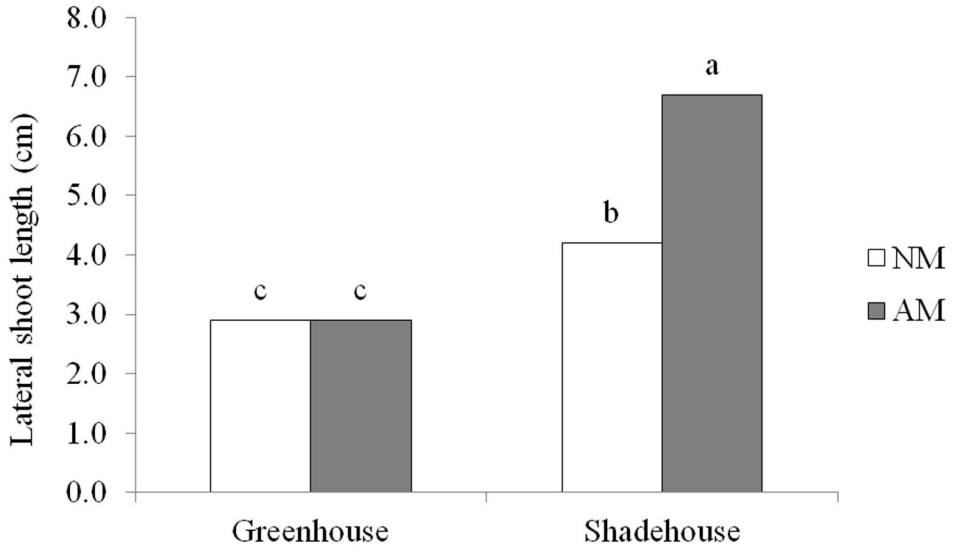

Fig. 6. Effect of growth environment (greenhouse or shadehouse) and inoculation (AM or NM) on *Begonia semperflorens* lateral shoot length 72 days after planting. Bars with different letters are significant by Tukey HSD Test ($P < 0.05$)

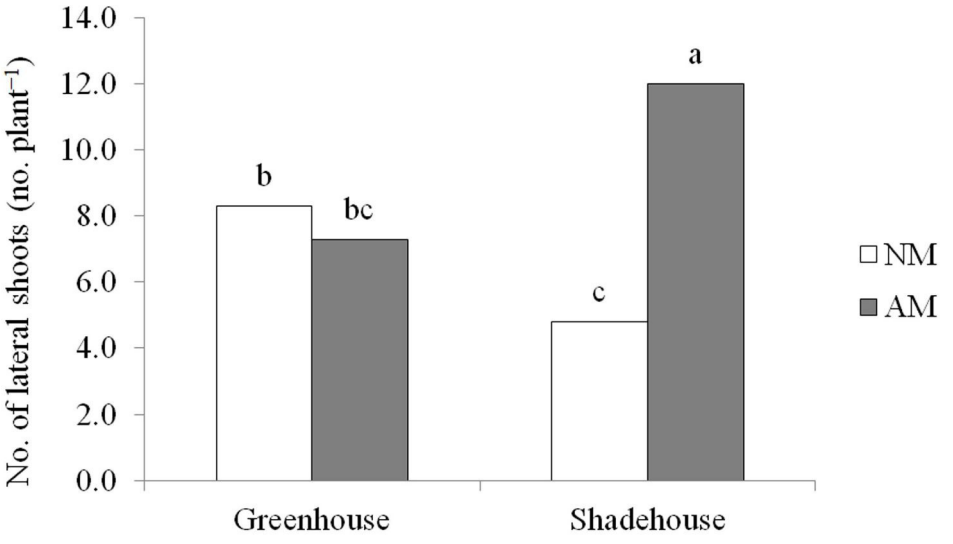

Fig. 7. Effect of growth environment (greenhouse or shadehouse) and inoculation (AM or NM) on *Begonia semperflorens* number of lateral shoots per plant 72 days after planting. Bars with different letters are significant by Tukey HSD Test ($P < 0.001$)

the number of lateral shoots. On the contrary, inoculation significantly influenced the above mentioned plant parameter (Tab. 1). AM plants produced a significantly greater number of lateral shoots (9.6) than NM ones (6.5). A significant interaction was found between I and G; the highest number of lateral shoots was identified in AM plants grown in the shadehouse, followed by NM plants grown in the glasshouse, which in turn showed a higher number of shoots per plant than AM plants grown in the glasshouse (7.3). The lowest value was recorded from NM plants grown in the shadehouse (4.8) (Fig. 7). Regardless of the inoculation, shading significantly increased the number of leaves per plant and number of flowers per plant by 19.9 and 51.8%, respectively compared with glasshouse growing conditions (Tab. 1). Irrespectively of the growth environment, AM plants showed higher values in terms of number of leaves per plant and number of flowers per plant (106.9 and 24.5, respectively). Finally, no significant interaction was recorded between G and I in terms of number of leaves and flowers per plant.

Second sampling date. Irrespectively of the inoculation, growth environment did not significantly affected the plant height, while, regardless of the growth environment, inoculation significantly influenced the above mentioned plant parameter. AM treated plants were significantly taller (23.4 cm) than NM plants (19.0 cm) (Tab. 2).

Irrespectively of the inoculation, lateral shoot length value was significantly higher in plants grown in the shadehouse (4.3 cm) (Tab. 2), whereas, regardless of the growth environment, AM fungi significantly increased lateral shoot length by 31.0%. Regardless of growth environment, inoculated plants, gave a greater number of lateral shoots per plant, number of leaves per plant and number of flowers per plant (14.7, 111.7 and 50.1, respectively) than NM plants (8.5, 65.6 and 29.5, respectively). Irrespective of the inoculation, number of lateral shoots, number of leaves and number of flowers per plant were significantly higher in plants grown in the shadehouse (12.8, 106.6 and 58.8, respectively) compared with those cultivated in the glasshouse (10.4, 70.7 and 25.7, respectively) (Tab. 2).

Table 2. Effects of growth environment and AM inoculation on plant growth and ornamental quality parameters of potted begonia 87 days after transplanting

Treatments		Plant height (cm)	Lateral shoot length (cm)	No. of lateral shoots (no. plant^{-1})	No. of leaves (leaves plant^{-1})	No. of flowers (flowers plant^{-1})
Inoculation	AM	23.4 a	4.2 a	14.7 a	111.7 a	50.1 a
	NM	19.0 b	2.9 b	8.5 b	65.6 b	29.5 b
Growth environment	greenhouse	20.4 ns	2.9 b	10.4 b	70.7 b	25.7 b
	shadehouse	22.0	4.3 a	12.8 a	106.6 a	58.8 a
		F value	*F* value	*F* value	*F* value	*F* value
Effect of factors	inoculation	16.9 ***	12.6 ***	43.5 ***	62.6 ***	15.2 ***
	growth environment	2.5 ns	13.3 ***	6.9 **	37.9 ***	28.3 ***
	interaction	0.2 ns	1.0 ns	0.1 ns	2.7 ns	1.3 ns

In each column and for each fixed factor, values followed by same letters are not statistically different according to Tukey HSD Test ($P \leq 0.05$). The significance is designated by asterisks as follows: *, statistically significant differences at P-value below 0.05; **, statistically significant differences at P-value below 0.01; ***, statistically significant differences at P-value below 0.001. ns, not significant. F value = F-test ANOVA

Table 3. Effects of growth environment and AM inoculation on plant growth and ornamental quality parameters of potted begonia 102 days after transplanting

Treatments		Plant height (cm)	Lateral shoot length (cm)	No. of lateral shoots (no. plant^{-1})	No. of leaves (leaves plant^{-1})	No. of flowers (flowers plant^{-1})	Flower diameter (mm)
Inoculation	AM	27.2 a	7.8 a	20.9 a	92.9 b	69.6 a	26.9 a
	NM	20.2 b	6.1 b	14.8 b	114.7 a	34.3 b	21.1 b
Growth environment	Greenhouse	21.3 b	7.0 ns	15.9 b	100.6 ns	38.9 b	19.2 b
	Shadehouse	26.2 a	7.0	19.8 a	107.1	65.1 a	28.6 a
		F value	*F* value	*F* value	*F* value	*F* value	*F* value
Effect of factors	Inoculation	30.4 ***	11.2 **	38.5 ***	14.6 ***	42.9 ***	22.2 ***
	Growth environment	14.9 ***	0.0 ns	15.4 ***	1.3 ns	23.7 ***	60.6 ***
	Interaction	0.6 ns	3.6 ns	5.3 *	26.5 ***	8.7 **	1.5 ns

In each column and for each fixed factor, values followed by same letters are not statistically different according to Tukey HSD Test ($P \leq 0.05$). The significance is designated by asterisks as follows: *, statistically significant differences at P-value below 0.05; **, statistically significant differences at P-value below 0.01; ***, statistically significant differences at P-value below 0.001. ns, not significant.
F value = F-test ANOVA

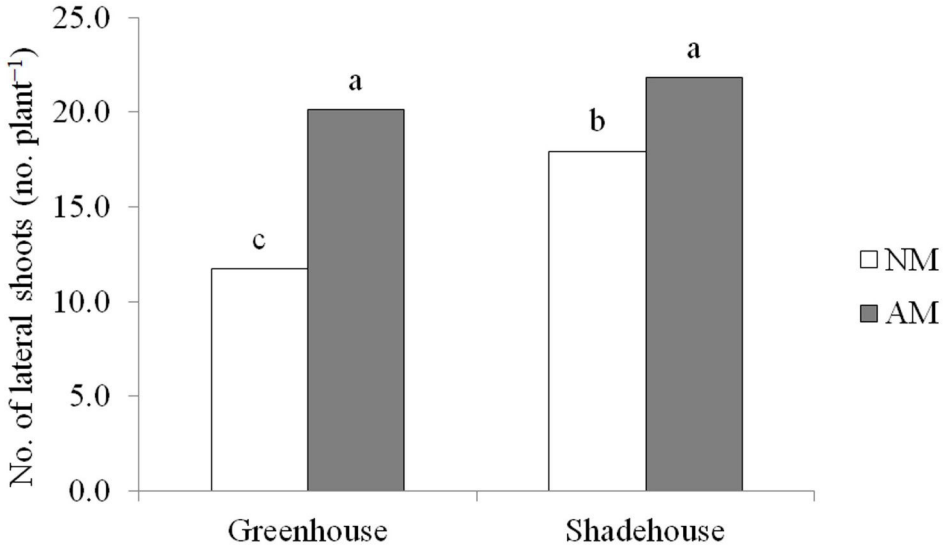

Fig. 8. Effect of growth environment (greenhouse or shadehouse) and inoculation (AM or NM) on *Begonia semperflorens* number of lateral shoots per plant 102 days after planting. Bars with different letters are significant by Tukey HSD Test ($P < 0.05$)

Third sapling date. Irrespectively of the inoculation, plant height was significantly higher in plants grown in the shadehouse (26.2 cm) (Tab. 3), whereas, regardless of the growth environment, AM fungi significantly increased plant height by 25.7%. No significant interaction was found between G and I in terms of plant height (Tab. 3). Regardless of the inoculation, the growth environment did not significantly affect lateral shoot length. Conversely, inoculation significantly affected the above mentioned plant parameter. A higher lateral shoot length value was observed from inoculated plants (AM) (7.8 cm). No significant interaction I × G was recorded (Table 3). Without regard of the inoculation, the shadehouse environment significantly increased the number of lateral shoots per plant (19.7%) (Tab. 3), whereas, irrespective of the growth environment AM plants showed a higher number of lateral shoots per plant (20.9) than NM plants (14.8). ANOVA for number of lateral shoots per plant showed a significant effect of the interaction I × G; plants inoculated and grown under shade had the highest number of lateral shoots per plant (21.8), followed by AM plant grown under shade (20.1), which in turn displayed a higher value than NM plants grown under shade (17.9). The lowest value in terms of number of lateral shoots per plant was recorded from NM plants grown into the glasshouse (11.7) (Fig. 8). Regardless of the inoculation, the growth environment did not significantly affect the number of leaves per plant. Moreover, inoculation significantly influenced the above mentioned plant parameter (Tab. 3). NM plants produced a higher number of leaves per plant (114.7) than AM plants (92.9). A significant interaction was found between G and I; the highest number of leaves per plant was identified in AM plants grown in the glasshouse (126.1), followed by those grown into the shadehouse (inoculated and non-inoculated) (103.3 and 110.9, respectively), which in turn exhibited a higher number of shoots per plant than NM plants grown into the glasshouse (75) (Fig. 9). Irrespective of the inoculation, the shadehouse environment significantly increased the number of flowers per plant (65.1) compared with the glasshouse environment (38.9) (Tab. 3), whereas AM fungi significantly increased the number of flowers per plant (50.7%). ANOVA showed a significant effect of the interaction (I × G) (Tab. 3). The highest number of flowers per plant was recorded from AM plants grown into the shadehouse (90.8),

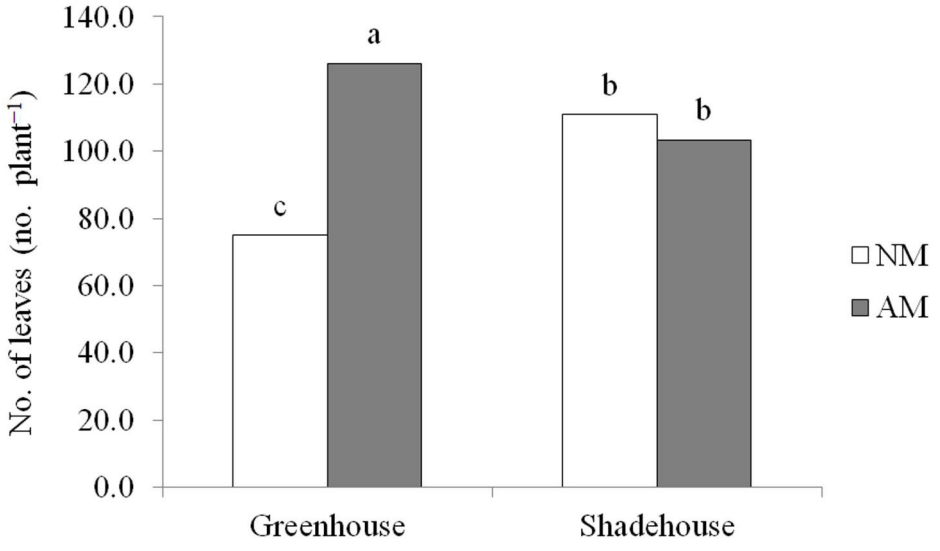

Fig. 9. Effect of growth environment (greenhouse or shadehouse) and inoculation (AM or NM) on *Begonia semperflorens* number of leaves per plant 102 days after planting. Bars with different letters are significant by Tukey HSD Test ($P < 0.001$)

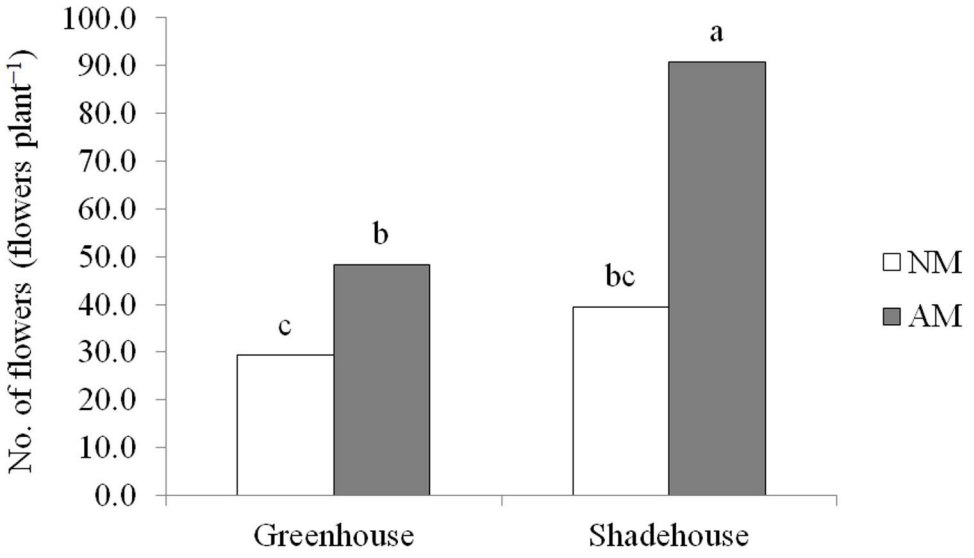

Fig. 10. Effect of growth environment (greenhouse or shadehouse) and inoculation (AM or NM) on *Begonia semperflorens* number of flowers per plant 102 days after planting. Bars with different letters are significant by Tukey HSD Test (P < 0.01)

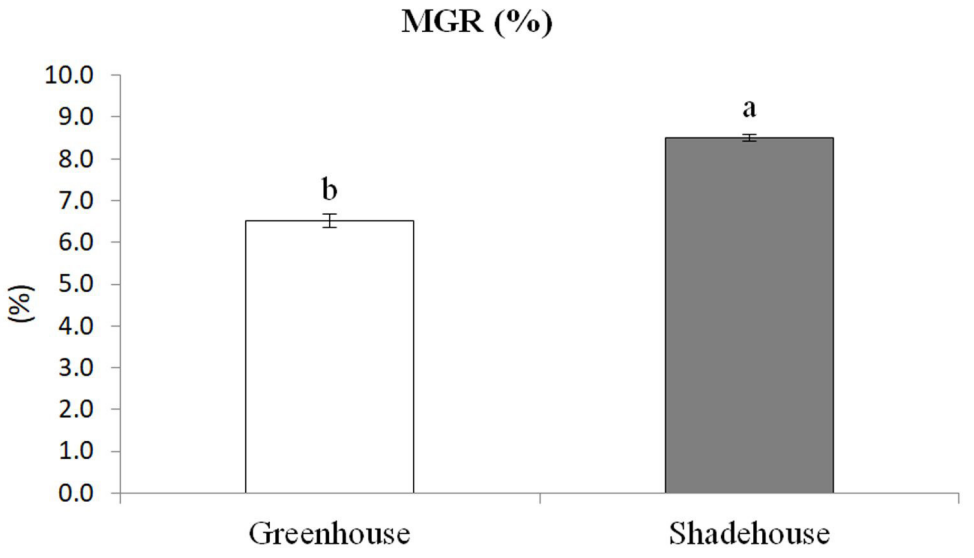

Fig. 11. Mycorrhizal growth response (MGR) of *Begonia semperflorens* plants expressed as percentage increases of shoot dry weight. The presented data are means of 4 replicates ± standard error of mean. Bars with different letters are significant by Tukey HSD Test (P < 0.01)

followed by AM plant cultivated into the glasshouse (48.4). The lowest number of flowers per plant was observed from NM plants grown into the glasshouse (29.3) (Fig. 10). AM plants, without regard of growth environment, gave a greater flower diameter (26.9 mm) than NM plants (21.1 mm). Irrespectively of the inoculation, shading significantly increased flower diameter (32.9%) (Tab. 3). No significant interaction was found between G and I in terms of flower diameter (Tab. 3).

ANOVA highlighted a significant interaction ($p < 0.05$) between the growth environments tested in terms of MGR calculated for shoots DW (Fig. 11). Plants in the shadehouse had a higher MGR than plants grown into the glasshouse. Our findings indicate a positive relationship ($p = 0.005$, $r = 0.870$) between the mean

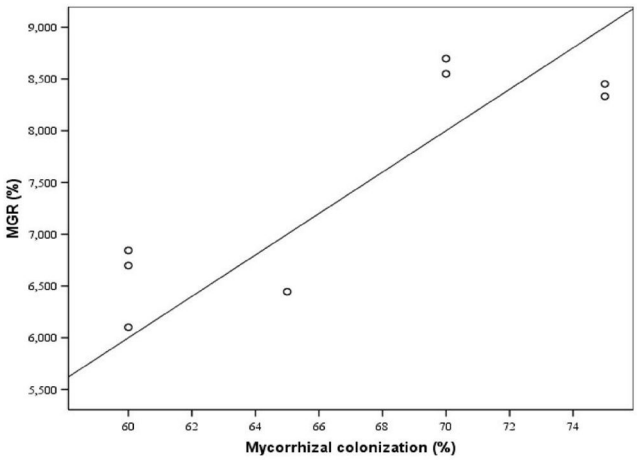

Fig. 12. Relationship between mean values of mycorrhizal colonization of roots and mycorrhizal growth response (MGR) of *Begonia semperflorens* plants

Table 4. Effects of growth environment and AM inoculation on leaves, stems and roots dry weight 102 days after transplanting

Treatments		Leaves DW (g plant^{-1})	Stems DW (g plant^{-1})	Root DW (g plant^{-1})
Inoculation	AM	1.1 ns	8.0 a	4.9 ns
	NM	1.1	2.2 b	4.6
Growth environment	greenhouse	0.9 b	4.0 ns	3.0 b
	shadehouse	1.4 a	6.1	6.6 a
Effect of factors		*F* value	*F* value	*F* value
	inoculation	0.1 ns	23.3 ***	0.0 ns
	growth environment	4.7 *	3.1 ns	5.3 *
	interaction	0.0 ns	1.1 ns	0.0 ns

In each column and for each fixed factor, values followed by same letters are not statistically different according to Tukey HSD Test ($P \leq 0.05$). The significance is designated by asterisks as follows: *, statistically significant differences at P-value below 0.05; **, statistically significant differences at P-value below 0.01; ***, statistically significant differences at P-value below 0.001. ns, not significant
F value = F-test ANOVA

level of root colonization and the observed mean MGR (Fig. 12).

Plant biomass

Irrespectively of the growth environment, inoculation did not significantly affected leaves DW, whereas, regardless of the inoculation, growth environment significantly influenced the above mentioned plant parameter. Higher values in terms of leaves DW were obtained from plants grown under shade (1.4 g plant^{-1}) than plants cultivated into the glasshouse (0.9 g plant^{-1}) (Tab. 4). No significant effect of the interaction (I × G) was found (Tab. 4). Regardless of the inoculation, the growth environment did not significantly affect stem DW. Irrespective of the growth environment, AM fungi significantly increased stem DW (72.5%). Higher stem DW was observed from AM plants (8.0 g plant^{-1}) than no-inoculate ones (2.2 g plant^{-1}) (Tab. 4). ANOVA did not show a significant effect of the interaction (I × G) (Tab. 4). Without regard of the growth environment, inoculation did not significantly affect root DW (Tab. 4). Conversely, shading condition significantly increased the above mentioned parameter by 54.5% as compared with glasshouse growing conditions. A higher root DW was obtained from plant grown under shade (6.6 g plant^{-1}) compared to those grown into the glasshouse (3.0 g plant^{-1}) (Tab. 4). No significant interaction I × G was found in terms of root DW (Tab. 4).

DISCUSSION

In our experiment, all AM begonia plants were successfully inoculated. This is a fundamental prerequisite for AM symbiosis enterprise-wide use. In addition, our results clearly demonstrated that mycorrhizal inoculation reached 11.1% in NM plants. As reported by Linderman and Davis [2003], the presence of mycorrhizae in soilless media, although rare, might be attributed to air born contamination in dust. Optimal growing conditions for wax begonia are night temperature of 16−18°C and day temperature of 18−21°C [Dole and Wilkins 2005]. In general, plants will become heat stressed at night temperature above 24°C and day temperature above 32°C. Average temperatures during the experiment were higher in the glasshouse compared to the shadehouse. According to our results, plants grown in the shadehouse performed better than those in the glasshouse, because shading lowered the air temperature by 4.8°C. Furthermore, shading appeared to be beneficial for AM inoculation and consequently positively influenced the AM crop performance, as compared to NM plants (Tabs. 1, 2 and 3). There are reports that increased temperatures (up to 30°C), which usually occur in glasshouses during the spring-summer season, may foster the development of AM fungi [Entry et al. 2002, Gavito et al. 2005]. Conversely, temperatures higher than 30°C, could be detrimental on mycorrhizal colonization and thus on plant productivity [Heinemeyer and Fitter 2004, Compant et al. 2010].

One of the most wished effect of AM fungi treatment on ornamental plants is the increase in the main plant quality traits such as flowering [Püschel et al. 2014]. We revealed a positive effect of the AM fungi on number of flowers per plant. Our results are in accord with those obtained for a wide range of species such as *P. peltatum* [Perner et al. 2007], *Verbena* sp. [Vosátka et al. 1999], *Antirrhinum majus* [Asrar et al. 2012], *Chrysanthemum morifolium* or *Tagetes erecta* [Vaingankar and Rodrigues 2012], *Capsicum annum* [Püschel et al. 2014], *Dimorphoteca sinuata* [Püschel et al. 2014], *Gazania splendens* [Püschel et al. 2014], *Sanvitalia procumbens* [Püschel et al. 2014] and *Verbena* × *hybrida* [Püschel et al. 2014]. On this regard, in the present experiment, plants grown into the shadehouse (low temperature and DLI level) showed the highest values in terms of number of flowers per plant and flower diameter. These findings are consistent with those of Sohn et al. [2003] and Asrar and Elhindi [2011], who reported an increase in terms of flower size due to AM fungi. In this respect, the beneficial effects on flowering-related parameters observed in our study could be attributed to greater AM fungi activities in the shadehouse and related advantages such as plant tolerance to abiotic stress, plant improvement in water and nutrient uptake and increase in plant biomass. Gaur et al. [2000] revealed that in many species, the quantity of flowers is proportional to plant size and nutrient content. Thus, the greater number of flowers of inoculated plants could be related to greater plant biomass produced. However, Püschel et al. [2014] found that the flowering of *Verbena* × *hybrida* and *I. hawkerii* was stimulated despite the fact that their

plant biomass was not increased by AM fungi. This may be due to the high carbon cost/benefit ratio of the AM fungus with the host plants and/or a high fungal activity [Martin and Stutz 2004]. Furthermore, different results in terms of plant biomass point to rather different mechanisms of mycorrhizal stimulation of flowering such as the effects on the plant hormonal profile and balance [Allen et al. 1982, Perner at al. 2007].

Our study revealed that AM fungi increased the plant height, lateral shoots length, number of lateral shoots, number of leaves and stems DW in wax begonia. This findings are consistent with those of Püschel et al. [2014], who reported that AM fungi [inoculum Symbivit® that consists of a mixture of zeolite and expanded clay that acts as a carrier of propagules (spores, mycelium and colonized root fragments) of six different *Glomus* species] increase plant length in *Capsicum annuum* and *Pelargonium zonale*, number of branches in *Pelargonium zonale* and *Sanvitalia procumbens*, the number of leaves in *Capsicum annuum*, *Gazania splensens*, *Pelargonium peltatum* and *Pelargonuium zonale*. According to Püschel et al. [2014], AM fungi also increase the shoot DW in *Pelargonium peltatum*, *Pelargonium zonale* and *Sanvitalia procumbens*. However, Koide et al. [1999] found growth depressions in all ornamental plant species studied (*Salvia splendens*, *Impatiens walleriana*, *Tagetes patula*, *Petunia* × *hybrida*, *Coleus* × *hybridus* and *Viola* × *wittrockiana*) when subjected to the inoculation both at plug seedlings stage and/or when transplanted into a containers contained mycorrhizal inoculum (*Glomus intraradices* Schenck & Smith). These negative effects of inoculation seem to suggest a level of incompatibility among genotype, AM fungi and their substrate, or a high carbon cost/benefit ratio of the AM fungus with the host plants [Zhu et al. 2011]. According to Jeong et al. [2009], begonia plants grown in full sun are visibly more stunted and compact than plants grown under shade. On this respect, our results are consistent with this finding, as in the present study, wax begonia plants grown under shade revealed a higher values of the biometric parameters and plant dry weight (DW) than plants grown into the glasshouse.

CONCLUSIONS

To summarize, our results demonstrated that AM inoculation significantly enhanced stem dry weight, growth and flowering-related parameters. High temperatures in the glasshouse negatively affected the AM root colonization. Due to the high temperature during the spring-summer season typical of the Mediterranean region, plant growth and ornamental quality parameters were limited in the glasshouse growth environment. On the contrary, shading lowered the air temperature, induced higher mycorrhizal colonization and increased the biometric and flowering-related parameters compared to the glasshouse environment. Therefore, our study suggests that in the Mediterranean region, mycorrhizal inoculation with *Rhizophagus irregularis* (formerly *Glomus intraradices*) and shading may be beneficial to growers wishing to produce high quality wax begonia plants during the spring-summer season.

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