

Effects of arbuscular mycorrhizal fungi on early development of persimmon seedlings

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ABSTRACT

The present study evaluated the effects of five species of arbuscular mycorrhizal fungi (AMF) on vegetative development parameters and nutrient uptake of persimmon (*Diospyros kaki* L.) seedlings. The experiment was carried out in a completely randomized experimental design with six treatments involving AMF inoculation (non-inoculated; *Dentiscutata heterogama*, *Claroideoglossum etunicatum*, *Rhizophagus clarus*, *Acaulospora scrobiculata* and *A. morrowiae*), in sterile soil (Oxisol – Dystrophic Red Latosol) under greenhouse conditions. Persimmon seeds were germinated in sterile sand and the seedlings were transplanted to the sterile soil and received AMF inocula. After 360 days, the following vegetative parameters were analyzed: plant height, number of leaves, leaf surface area, stem diameter, shoot and root dry mass, percentage of root colonization and nutrient accumulation in shoot and root biomass. We observed different responses to the AMF species tested. Seedling height and leaf surface area were promoted by inoculation with *D. heterogama* and *A. morrowiae*, and these species also promoted a higher percentage of root colonization. Plants inoculated with *D. heterogama* and *C. etunicatum* presented a greater number of leaves, but only *D. heterogama* promoted significant increases in stem diameter. Shoot dry mass was improved by inoculation with *D. heterogama*, *A. morrowiae* and *C. etunicatum*, while the first two species and *R. clarus* increased root dry mass. Nutrient accumulation in shoot and root biomass was stimulated by AMF inoculation, especially by *D. heterogama* and *A. morrowiae*. Therefore, the effects on early vegetative development were more pronounced in the persimmon seedlings inoculated with *D. heterogama*.

Key words: *Diospyros kaki*, mycorrhizal inocula, nutrient uptake, seedling development

Abbreviations:

Al – aluminium, AMF – arbuscular mycorrhizal fungi, Ca – calcium, C – carbon, H+Al – saturation with aluminium, K – potassium, Mg – magnesium, P – phosphorus

INTRODUCTION

It is widely recognized that most of terrestrial plants form a mutualistic association with arbuscular mycorrhizal fungi (AMF) that can promote plant

growth by increasing the uptake of limiting nutrients (e.g. P), reducing drought stress (i.e. by increasing water absorption), and reducing root diseases (Davies and Call, 1990). Thus, AMF may

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increase the survivability of seedlings following transplantation.

Different AMF species may have distinct levels of benefits for the host plants, particularly fruit trees. These benefits for seedling development have been reported in several studies for different plants (Saggin-Júnior et al., 1994; Andrade et al., 2009; Balota et al., 2011). Inoculation of sweet passion fruit (*Passiflora alata*) seedlings with *Gigaspora albida* promoted increases in shoot biomass (Silva et al., 2004). In yellow passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* Deg.), seedlings inoculated with *G. albida* in sterile soil showed significant increases in height, stem diameter, number of leaves and tendrils (Cavalcante et al., 2002b). Inoculation of avocado (*Persea* sp.) rootstocks with AMF species promoted improvements in the nutritional status of avocado seedlings (Silveira et al., 2002).

Among the genera of AMF, those which have shown promising results in the inoculation of fruit plants are *Glomus*, *Gigaspora*, *Scutellospora* and *Acaulospora* (Trindade et al., 2010). In this context, inoculation of seedlings of fruit plants with AMF can contribute to the economy of fertilizer use, reduce the period of seedling development, increase productivity in commercial nurseries and improve survival and growth rates following transplantation and during acclimatization (Sbrana et al., 1994; Trindade et al., 2001; Silveira et al., 2003; St-Denis et al., 2017; Viera et al., 2017).

The cultivation of the fruit species *Diospyros kaki* L. (persimmon) in Brazil is becoming important as evidenced by the increase in the area planted and in the yield and supply of the fruit for the domestic market and exportation (IBGE, 2015). Persimmon originated from Asia and has spread to other tropical and subtropical regions (Simão, 1971). China is the largest producer of persimmon, followed by the Republic of Korea, Japan and Brazil, according to FAO – Food and Agriculture Organization of the United Nations (2014). Persimmons belong to the family Ebenaceae (Woolf and Ben-Arie, 2011) and their importance as a horticultural crop is due to the composition of the fruit, comprising carbohydrates, fibre, pectin and antioxidant compounds such as vitamins A and C (Giordani et al., 2011), which are essential nutrients for human health.

The propagation of persimmon can be by seed or root suckers. However, to our knowledge, there is little information on consolidated technologies for the seedling development stage. The occurrence of AMF association in persimmon cultivation has

been reported on (Gai et al., 2006), but studies on the effect of AMF inoculation are scarce. The first report on the inoculation of *D. kaki* seedlings with AMF was in Japan, and the AMF used were found to enhance the initial growth in the acclimation of seedlings (Matsubara and Hosokawa, 1999). Later, AMF (*G. intraradices*) were also found to enhance the growth of micropropagated *D. kaki* seedlings in Spain (Marin et al., 2003). Inoculation with *Glomus etunicatum* also promoted the growth and development of *Diospyros virginiana* seedlings used as rootstock for persimmon production in Turkey (Incesu et al., 2015). No studies regarding the efficiency and specificity of AMF in persimmon have been conducted in Brazil.

Propagated persimmon seedlings are often irregular in size (Pêche et al., 2016) and exhibit low efficiency of phosphorus acquisition (Matsubara and Hosokawa, 1999). Inoculation with AMF may contribute to better plant nutrition and reduce development time, and also increase the resistance to stress during transplant acclimation. In this context, the aim of this study was to evaluate the effects of inoculation with different AMF species on the early vegetative development of persimmon seedlings.

MATERIAL AND METHODS

AMF inocula

Mycorrhizal inocula were obtained from the AMF Species Collection maintained in the Instituto Agrônômico do Paraná, IAPAR, in sterile soil, using *Brachiaria decumbens* as host, as described by Saggin-Júnior et al. (2011). The AMF species used were: *Dentiscutata heterogama* (T.H. Nicolson & Gerd.), *Claroideoglomus etunicatum* (W.N. Becker & Gerd.), *Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck), *Acaulospora scrobiculata* (Trappe) and *A. morrowiae* (Spain and Schenck), with the species names reviewed according to Redecker et al. (2013) and Schüßler and Walker (2010). Samples were extracted from the soil by wet sieving and decanting (Gerdemann and Nicolson, 1963), followed by centrifugal flotation in 50% sucrose (Jenkins, 1961). Approximately 150 spores from each species were isolated to compose mycorrhizal inocula for each plant. Spores were stored in water (1 mL) until inoculation.

Experimental conditions

For the production of persimmon seedlings, sterile sandy soil (Dystrophic Red Latosol, an Oxisol) from Miraselva-Paraná, Brazil, was used as substrate in

pots at 7 kg of soil per pot. The following chemical properties were determined: P (Mehlich) 4.8 mg kg⁻¹ soil, C (Walkley-Black) 6.32 g kg⁻¹ soil, pH-CaCl₂ 5.1, Al 0.0 mg kg⁻¹, H+Al 4.42 cmol_c dm⁻³, Ca 350.7 mg kg⁻¹ soil, Mg 70.5 mg kg⁻¹ soil, and K 39.1 mg kg⁻¹ soil. Soil base saturation was corrected to 60% using incubation with limestone for 60 days, and the phosphorus (P) level was raised to 20 mg kg⁻¹. The experiment was conducted under greenhouse conditions at 28°C ± 4°C, at an experimental station of IAPAR at Londrina-PR (23°21'23" S, 51°09'40" W). The climate is humid subtropical, Cfa – Köppen classification, altitude of 570 m (IAPAR, 2017).

Seeds of the persimmon (*D. kaki*) cultivar Chocolate were germinated in carbonized rice husks, and seedlings without abnormalities, after reaching 5 cm in height, were transplanted into pots containing the sandy soil and divided into 6 treatments: five with AMF species (1. *Dentiscutata heterogama*, 2. *Claroideoglomus etunicatum*, 3. *Rhizophagus clarus*, 4. *Acaulospora scrobiculata*, and 5. *A. morrowiae*), and one non-inoculated treatment. The inoculation procedure was carried out during the transplantation stage by placing AMF inocula (AMF spores in water) as close as possible to the radicular system. The non-inoculated treatment received water without AMF spores. A completely randomized design consisting of 5 treatments, with three replicates of one plant each, was used in the experiment. Every 60 days, we applied a nutritive solution containing: 0.6 mM KNO₃, 0.4 mM Ca(NO₃)₂, 0.2 mM MgSO₄, 0.1 mM of micronutrient solution (2.86 g L⁻¹ H₃BO₃, 1.81 g L⁻¹ MnCl₂ · 4H₂O, 0.22 g L⁻¹ ZnSO₄ + 7H₂O, 0.08 g L⁻¹ CuSO₄ + 5H₂O and 0.02 g L⁻¹ H₂MoO₄ + H₂O), and 0.05% of iron solution (26.1 g L⁻¹ EDTA and 24.9 g L⁻¹ FeSO₄ + 7H₂O), without a P source.

Early vegetative development parameters

After 1 year of growth, we measured the following: seedling height, stem diameter, number of leaves, leaf surface area, shoot and root dry biomass. The seedling height was the distance between the apical shoot bud and the root tip. The leaves were counted and leaf surface area was measured with a LAI-3000 leaf area meter (Li-Cor Corporate, Lincoln, Nebraska, USA). Shoot and root dry biomass were determined after drying at 65°C in an oven operating with forced air circulation, until reaching a constant mass.

We measured the percentage of root colonization by AMF in persimmon seedlings. The roots were cleared in 10% KOH, acidified with 1% HCl, washed

and stained with a 0.05% lactoglycerol solution containing trypan blue (Phillips and Hayman, 1970). The presence of mycorrhizal structures in the roots was counted by the grid-line intersect method described by Giovannetti and Mosse (1980).

To determine whether the AMF affected plant nutrition, we measured shoot and root nutrients (i.e. P, K, Ca, Mg, Cu, Zn, B and Mn) according to Miyazawa et al. (1992), where P was extracted by sulphuric digestion and determined by colorimetry; K, Mg, Cu, Zn, B and Mn were extracted with 1 M HCl, and K was determined by flame photometry; Cu, Zn and Mn by atomic absorption spectrophotometry and B by Azometine H colorimetric method; Ca and Mg were determined by atomic absorption spectrophotometry with lanthanum. To estimate total plant nutrition, we multiplied the nutrient concentrations by the root and shoot dry weights.

Statistical analysis

To test whether the response variables were varied by the AMF treatments, we used analysis of variance (ANOVA) followed by Tukey's test ($p \leq 0.05$) to compare the means, using SASM-Agri software (Canteri et al. 2001). Root colonization percentage data were transformed to $(\arcsin \sqrt{P\%/100})$ for homogenization of variance.

RESULTS

Persimmon seedlings inoculated with AMF tended to show increased plant growth (i.e. greater seedling height, stem diameter, number of leaves, leaf surface area, shoot and root dry biomass). However, different responses were observed to the AMF species tested (Fig. 1). Persimmon seedlings inoculated with *D. heterogama* and *A. morrowiae* had a greater plant height ($p \leq 0.05$) and leaf surface area ($p \leq 0.05$) compared to the non-inoculated treatment. Inoculation with *D. heterogama* and *C. etunicatum* increased the number of leaves ($p \leq 0.05$), and the inoculation with *D. heterogama* also increased the stem diameter of persimmon seedlings ($p \leq 0.05$) compared with non-inoculated plants.

Persimmon seedlings inoculated with *D. heterogama*, *A. morrowiae* and *C. etunicatum* had a greater shoot biomass compared to the non-inoculated treatment ($p \leq 0.05$), and *D. heterogama*, *A. morrowiae* and *R. clarus* increased root biomass ($p \leq 0.05$) (Tab. 1). Furthermore, the highest percentage of root colonization in persimmon

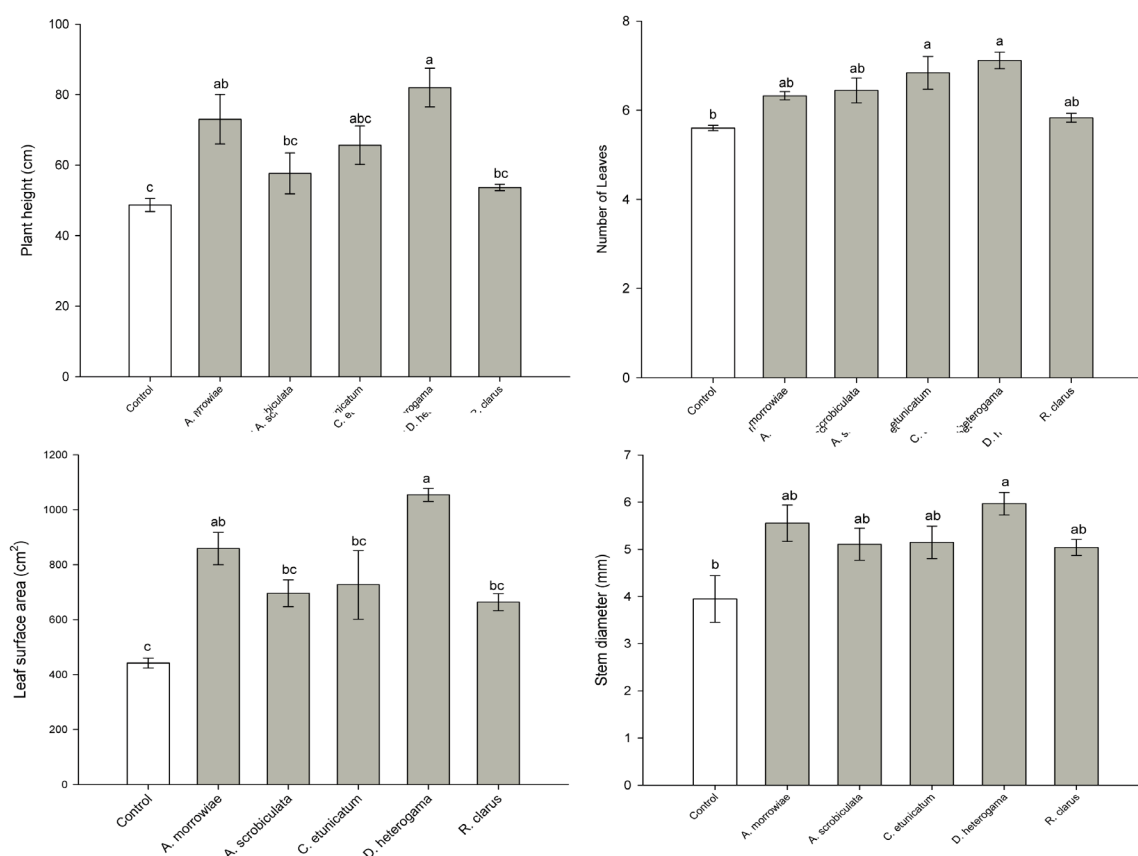


Figure 1. Plant height, number of leaves, leaf surface area and stem diameter of persimmon seedlings (*Diospyros kaki* L.) inoculated with species of arbuscular mycorrhizal fungi. Means compared by Tukey's test ($p \leq 0.05$)

seedlings was reached by the species *D. heterogama* and *A. morrowiae* ($p \leq 0.05$).

In general, inoculation with the AMF species increased nutrient uptake and accumulation in the biomass compared to the non-inoculated treatment (Tab. 2). *D. heterogama*, followed by *A. morrowiae*, promoted increased nutrient accumulation in dry shoot and root biomass, above all of P, K and Ca. These fungal species were able to increase the

growth of persimmon seedlings by maximizing nutrient uptake.

DISCUSSION

Seedling production is important for establishing orchards (Franzon et al., 2010). Inoculation with AM fungi has been found to enhance the growth of seedlings of several fruit crops and has helped to achieve high production levels (Rodrigues and

Table 1. Dry mass of shoots (SDM) and roots (RDM), and percentage colonization of the roots of persimmon seedlings inoculated with different species of arbuscular mycorrhizal fungi

Treatment	SDM (g)	RDM (g)	% Root Colonization transformed to arc sin $\sqrt{P\%/100}$
Non-inoculated	6.96 ± 0.47 c	5.88 ± 1.00 c	0.00 ± 0.0 c
<i>D. heterogama</i>	17.33 ± 0.51 a	15.53 ± 2.09 a	36.67 ± 3.3 a
<i>C. etunicatum</i>	12.30 ± 1.93 b	9.16 ± 1.14 bc	16.67 ± 3.3 b
<i>A. scrobiculata</i>	11.19 ± 0.67 bc	8.45 ± 1.38 bc	13.33 ± 3.3 b
<i>R. clarus</i>	10.17 ± 0.50 bc	12.18 ± 0.22 ab	20.0 ± 5.7 ab
<i>A. morrowiae</i>	14.29 ± 1.24 ab	11.96 ± 0.80 ab	26.67 ± 3.3 ab
CV (%)	14.94	20.42	19.71

*Mean values ± standard deviations with the same letters are not significantly different by Tukey's test ($p \leq 0.05$), CV = coefficient of variation

Table 2. Nutrients accumulated in shoot and root dry biomass of persimmon seedlings inoculated with different species of arbuscular mycorrhizal fungi

	P	K	Ca	Mg	Cu	Zn	B	Mn
Treatment	accumulated mg per plant				accumulated µg per plant			
Root dry matter								
Non-inoculated	8.3 b*	78.3 c	51.0 c	16.2 c	15.3 b	58.9 c	311.0 b	823.0 b
<i>D. heterogama</i>	20.6 a	182.1 a	140.3 a	48.4 a	40.5 a	136.2 a	694.9 a	2393.3 a
<i>C. etunicatum</i>	12.3 b	113.2 bc	84.1 bc	27.2 bc	23.9 b	94.3 abc	460.8 ab	1379.7 ab
<i>A. scrobiculata</i>	13.3 b	121.3 bc	76.3 bc	26.7 bc	17.8 b	84.5 bc	502.5 ab	1472.3 ab
<i>R. clarus</i>	11.1 b	117.3 bc	73.8 bc	23.3 bc	22.2 b	76.4 bc	465.6 ab	1950.2 a
<i>A. morrowiea</i>	14.0 ab	134.8 ab	98.3 b	35.5 ab	25.8 b	120.5 ab	691.1 a	2017.5 a
CV (%)	18.8	14.8	16.1	17.1	17.9	19.3	18.9	23.3
Shoot dry matter								
Non-inoculated	19.2 b	55.3 ns	26.7 b	33.0 b	26.7 c	132.3 c	71.6 b	663.6 ab
<i>D. heterogama</i>	29.4 b	127.6	48.6 a	72.4 a	97.9 abc	365.4 a	203.5 a	893.0 ab
<i>C. etunicatum</i>	26.0 b	82.9	34.0 ab	53.7 ab	55.1 abc	237.0 abc	122.2 ab	538.8 b
<i>A. scrobiculata</i>	19.0 b	75.3	30.9 ab	52.5 ab	36.3 bc	197.3 bc	101.7 ab	1014.7 ab
<i>R. clarus</i>	42.8 a	95.0	47.9 ab	73.9 a	129.4 ab	308.3 ab	148.1 ab	1937.0 a
<i>A. morrowiea</i>	31.2 ab	112.8	43.8 ab	70.3 a	140.6 a	294.2 abc	162.6 ab	1565.3 ab
CV (%)	17.1	15.8*	20.6	22.0	23.6*	23.6	27.9	21.6*

Means of three replicates, when followed by the same letter in a column, do not differ significantly by the Tukey test ($p \leq 0.05$), CV = coefficient of variation, ns = not significant

*Transformed data $(x+1)^{1/2}$

Rodrigues, 2014). In our study, we tested the effects of inoculation with five different AMF species on the early vegetative development of persimmon seedlings under sterile sandy soil conditions of Brazil, this being the first report on persimmon inoculation in that country.

Inoculation with the different AMF species increased the growth and vegetative development of persimmon seedlings. Furthermore, we found that the effects varied depending on the AMF species. The growth of the seedlings was enhanced most by *D. heterogama* and *A. morrowiea*. Although in our findings the inoculation with the genera *Rhizophagus* and *Claroideoglossum* did not significantly promote increases in seedling growth, in previous studies the two genera had increased the growth of persimmon seedlings. Incesu et al. (2015) had observed that inoculation with the species *G. etunicatum* (*Claroideoglossum etunicatum* W.N. Becker & Gerd), *G. clarum* (*Rhizophagus clarus* T.H. Nicolson & N.C. Schenck) and *G. mosseae* (*Funneliformis mosseae* T.H. Nicolson & Gerd.) resulted in the tallest seedlings of *Diospyros virginiana*, a persimmon species from North America. *Diospyros kaki* seedlings were taller when inoculated with the AMF species *Glomus aggregatum* (*Rhizophagus aggregatus* N.C. Schenck & G.S. Sm.), *Glomus* sp. R-10 and

Gigaspora margarita (Matsubara and Hosokawa, 1999). Marin et al. (2003) also found an increase in the growth of *D. kaki* seedlings colonized by *G. intraradices* (*Rhizophagus intraradices* N.C. Schenck & G.S. Sm.).

Although there are no reports of *D. heterogama* and *A. morrowiea* affecting the height of persimmon seedlings, their effects on other agronomic fruit species have been reported. For example, Silveira et al. (2002) evaluated the use of six AMF species for the inoculation of rootstocks for the production of avocado (*Persea* sp.) seedlings and observed that *D. heterogama* increased plant height. This same fungal species was mentioned by Anjos et al. (2005) as one that contributed to the growth of passion fruit (*Passiflora edulis* f. *flavicarpa*) plants. Additionally, Ambrosini et al. (2015) observed that the species *A. morrowiea* with the species *Gigaspora gigantea*, *Rhizophagus clarus* and *R. irregularis* promoted the growth of young vines (*Vitis* sp.) cultivated in a copper-contaminated soil.

Inoculation with either *D. heterogama* or *C. etunicatum* increased the number of leaves compared to non-inoculated plants, and *D. heterogama* and *A. morrowiea* increased leaf surface area of persimmon seedlings. Increases in leaf surface area and in the number of leaves are likely to affect the overall plant performance and

fruit production because the leaves are essential for photosynthesis and growth (Favarin et al., 2002). Similar results have been observed for leaf surface area in avocado inoculated with *D. heterogama* (Silveira et al., 2002), as well as in other species such as: *Passiflora edulis* Sims. f. *flavicarpa* (Anjos et al., 2005; Cavalcante et al., 2002a), *Cecropia pachystachya* (Carneiro et al., 2004) and *Citrus reticulata* (Sena et al., 2004).

In our study, *D. heterogama* was the only AMF species to increase the stem diameter of persimmon seedlings. To date, no study has reported AMF effects on this growth parameter, including that by Matsubara and Hosokawa (1999). However, some AMF species, such as *Gigaspora albida*, *Gigaspora margarita* and *C. etunicatum*, have already been reported to increase the stem diameter of yellow passion fruit (Cavalcante et al., 2002a). Stem diameter of seedlings in nursery conditions is widely used as an indicator of seedling quality because stems accumulate starch reserves, which is correlated with a well-developed root system, strong resistance to desiccation, and better survival and growth after transplantation (ITTO, 2006).

Although the beneficial effects of AMF on plant growth have often been related to increased phosphorus uptake (Bolan, 1991), the inoculation with AMF in our study increased the levels of all the nutrients estimated in the root and shoot dry mass. Since AMF affect the size and nutrient content of seedlings, AMF inoculants may improve nursery production, especially as fertilizer costs rise.

Here we have shown that all the tested species of AMF improved the early vegetative development of persimmon seedlings compared to the non-inoculated ones. Also, the species *D. heterogama* and *A. morrowiae* had the most beneficial effects on seedling growth and may have the most positive effects on agricultural production, as they show potential for use in seedling production on a commercial scale. The AMF species included in this study, which preferentially colonized persimmon seedlings, differ from the species studied by Matsubara and Hosokawa (1999), Marin et al. (2003) and Incesu et al. (2015). Several studies have reported that soil characteristics such as moisture, structure, and fertility (Lekberg et al., 2007; Egerton-Warburton et al., 2007), as well as plant species (Alguacil et al., 2011; Torrecillas et al., 2012) influence the composition of AMF communities and their colonization potential. In this context, it can be inferred that a plant species can associate with different AMF species depending

on the specific conditions. Therefore, it is important to test several AMF isolates, since different fungal species may present different levels of benefits to plants.

CONCLUSIONS

Inoculation with arbuscular mycorrhizal fungi improves the early development of persimmon seedlings cultivar Chocolate. The effects obtained varied depending on the AMF species. Two species, *Dentiscutata heterogama* and *Acaulospora morrowiae*, had the most consistent beneficial effects on the growth of and nutrient uptake by persimmon seedlings.

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AUTHOR CONTRIBUTIONS

G.S.M. – performed statistical analysis and participated in drafting the article; C.A.G.V. and C.H. – carried out the experiment and performed analytical measurements and data computations; O.M. and E.L.B. – designed the experiment and supervised the findings of this work; M.F.G. – revised the manuscript critically and made important contributions to the Discussion; G.S.M. – wrote the manuscript with support from O.M. and M.F.G., and all the authors contributed to the final version of the manuscript.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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