

Co-inoculation and single-species inoculation with arbuscular mycorrhizal fungi are associated with different growth and survival patterns in olive seedlings

Arthur Joanello Cemin

Ceminarthur@gmail.com

Universidade Federal de Pelotas

Vagner Luiz Graeff-Filho

Universidade Federal de Pelotas, Programa de Pós-Graduação em Fitossanidade

José Pedro Spies Nolibos

Universidade Federal de Pelotas

Luana Vanessa Peretti Minello

Universidade Federal de Pelotas

Ezequiel Cesar Carvalho Miola

Universidade Federal de Pelotas

Paulo Mello-Farias

Universidade Federal de Pelotas

Vanessa Sacramento Cerqueira

Universidade Federal de Pelotas

Research Article

Keywords: AMF, Acaulospora longula, Gigaspora rosea, Mycorrhizal Inoculation, Seedling Production, Olea europaea L

Posted Date: August 7th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9418173/v1>

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Additional Declarations: No competing interests reported.

Abstract

Olive (*Olea europaea* L.) production is growing in Brazil. However, it is still limited by difficulties in seedling production, as it exhibits slow initial growth and high dependence on inputs, highlighting the need for sustainable alternatives. Therefore, this study evaluated the effects of inoculation with arbuscular mycorrhizal fungi of the species *Acaulospora longula*, *Gigaspora rosea*, and co-inoculation of these species on the initial development of olive seedlings in a nursery. The experiment was conducted in a completely randomized design with four treatments and 16 replicates, totaling 64 plants, over six months. Survival, growth, and biomass parameters were evaluated. Inoculation with *A. longula* was associated with greater seedling survival, while inoculation with *G. rosea* had its greatest effect on shoot growth over time. Co-inoculation showed intermediate responses, suggesting potential interactions between the fungal species. Mycorrhizal colonization was qualitatively confirmed by the presence of fungal structures in the roots; however, quantitative assessment was not possible due to limitations in root visualization. These results indicate that different AMF species may be associated with different performance patterns in nursery plants. However, due to the lack of quantitative colonization data, the responses could not be fully elucidated. The results support the potential use of targeted AMF inoculation in olive seedling production, but further studies integrating quantitative and physiological measures of colonization are still needed.

Introduction

The olive tree (*Olea europaea* L.) is a species of growing importance in Brazil, due to its economic potential and the health benefits associated with the consumption of olive oil [1]. In the state of Rio Grande do Sul, olive cultivation has also expanded, due to the edaphoclimatic conditions developed for its production [2]. The expansion of olive growing in the state is supported by initiatives such as the State Program for the Development of Olive Growing (PRÓ-OLIVA), which encourages the adoption of emerging technologies for olive oil production, with the aim of increasing productivity and improving producers' income [3].

Thus, for the successful establishment and productivity of new orchards, the production of high-quality and economically viable seedlings is necessary. This process remains a limiting factor, since olive trees exhibit slow initial growth in the nursery phase, which increases the time required for transplanting and production costs, as the crop demands a high quantity of fertilizers both in seedling production and in overcoming the juvenile period and establishing itself in the field [4; 5; 6]. Brazil imports approximately 85% of the mineral fertilizers used in national agriculture; this dependence directly impacts the economic sustainability of production systems [7]. In this scenario, the development of strategies that reduce the use of external inputs and increase efficiency in seedling production becomes essential.

A promising alternative is the use of bio-inputs, such as arbuscular mycorrhizal fungi (AMF). These fungi belong to the phylum Glomeromycota and are obligate symbionts, meaning they only survive in the presence of host plants, forming mutualistic associations with their roots [8; 9]. This association occurs

naturally in approximately 72% of terrestrial plant species [10], including crops such as tomato (*Solanum lycopersicum*), citrus (*Citrus × sinensis*), and olive (*Olea europaea*) [11; 12; 13].

Arbuscular mycorrhizal fungi (AMF) assist in the transfer of nutrients from the soil to primary trophic levels, utilizing photoassimilates provided by the host plant [14]. These fungi form networks of extraradicular hyphae that extend beyond the depletion zone explored by root hairs, increasing the contact area with the soil and improving the supply of water and nutrients, such as phosphorus and nitrogen, to the plants [15; 16]. This association also contributes to adaptation to water stress, promoting growth and biomass production in olive trees [17]. In addition, AMF activate antioxidant mechanisms in plants, reducing oxidative stress and increasing tolerance to heavy metals [18; 19]. These fungi can also be used in seedling production in nurseries, as they increase plant vigor and improve survival rates after transplanting [20; 21; 22].

In addition to the general effects of symbiosis, arbuscular mycorrhizal fungal species exhibit distinct functional traits, including differences in the ability to exploit edaphic niches, in the extent and architecture of hyphal growth, and in the efficiency of nutrient mobilization and absorption [23]. These variations reflect contrasting ecological strategies among the fungi, which can directly influence the dynamics of the interaction with the host plant [24]. In this context, simultaneous association with multiple species can favor the occurrence of functional complementarity, characterized by the joint action of organisms with distinct ecological and nutritional functions.

This complementarity can result in greater efficiency in resource acquisition, especially in heterogeneous environments, in addition to promoting improvements in vegetative development, vigor, and plant adaptability [25; 26]. Thus, co-inoculation of different species of arbuscular mycorrhizal fungi emerges as a strategy with the potential to optimize seedling performance in production systems.

Given this, we hypothesized that different arbuscular mycorrhizal fungal species are associated with distinct patterns of host plant performance, and that co-inoculation may be associated with enhanced seedling establishment compared to single-species inoculation. Accordingly, this study aimed to evaluate the effects of inoculation with *Acaulospora longula*, *Gigaspora rosea*, and their co-inoculation on the early development of olive seedlings under nursery conditions.

Material and Methods

Study area, Plant material and inoculum

The experiment was conducted in a polyethylene-covered greenhouse measuring 18 m in length, 9 m in width and 4 m in height, at the Universidade Federal de Pelotas (UFPe), in Pelotas (RS), Brazil (31°38'12.4"S, 52°32'54.0"W). The predominant climate is humid subtropical (Cfa) according to the Köppen classification [27]. The monitoring of seedling development occurred from October 11, 2024, to April 11, 2025.

The Koroneiki cultivar was chosen due to its presence in olive cultivation within the state of Rio Grande do Sul. Cuttings were collected in the municipality of Pelotas for seedling production. Collections were carried out at the end of autumn, using vigorous basal branches with a minimum diameter of 3 mm. Pruning shears sterilized with 70% ethanol were used for each cutting. After harvesting, the material was immediately stored in polypropylene bags to reduce moisture loss and preserve physiological viability until planting. In the Fruticultura de Clima Temperado Laboratory, the cuttings were standardized to 7 cm in length and prepared with one pair of leaves cut in half. Treatment with indole-3-butyric acid (IBA) was performed by immersing 2 cm of the basal end of the cuttings for five seconds in a solution of 3000 mg·L⁻¹ IBA [28] diluted in 90% ethanol.

The arbuscular mycorrhizal fungi (AMF) used in this study, *Acaulospora longula* and *Gigaspora rosea*, were selected based on two main criteria: (i) the recurrent presence of these genera in studies characterizing AMF communities associated with olive trees and in inoculation experiments with olive seedlings [29; 30]; and (ii) the availability of the inocula from the International Culture Collection of Glomeromycota (CICG), at the Regional University of Blumenau (FURB), Santa Catarina, Brazil

Experimental design

The experiment was arranged in a completely randomized design (CRD) with four treatments: without inoculation (control – T1); inoculation with *Gigaspora rosea* (T2); inoculation with *Acaulospora longula* (T3); and inoculation with a consortium of *Acaulospora longula* and *Gigaspora rosea* (T4). Sixteen replicates were used per treatment, totaling 64 plants.

Olive seedlings with root and callus formation, standardized to 7 cm in length and with one pair of leaves, were transplanted into 1.7 L polypropylene vase filled with a 1:1 (v:v) mixture of soil and sand. The substrate was autoclaved twice for 1 hour at 121 °C, with a 24-hour interval between sterilizations. The soil used was collected from the Centro agropecuário da Palma of UFPel (31°48'11.8"S 52°30'48.4"W), and classified as a Red-Yellow Argisol. AMF inoculation was performed at the time of transplanting by applying the inoculated soil directly into the planting holes. For single-species treatments, 0.5 g of soil inoculated with *Acaulospora longula* and 3.33 g of soil inoculated with *Gigaspora rosea* were applied per hole. These quantities contained an average of 100 viable spores of each species. In the co-inoculation treatment, the total amount of inoculum was proportionally divided between the species, with 0.25 g of soil inoculated with *A. longula* and 1.66 g of soil inoculated with *G. rosea*, also totaling approximately 100 spores per hole, with 50 spores of each species.

Analyzed parameters

The parameters evaluated in this study were divided into two groups: developmental analyses and destructive analyses. Developmental measurements, such as survival rate, branch length (cm), and branch and stem diameter (mm), were carried out weekly throughout the experiment. Branch length was

measured with a millimetre tape, recording the distance from the point of insertion of the branch on the stem to its apical tip. Stem diameter was determined with a digital caliper (± 0.01 mm) positioned at the base of the stem

Destructive measurements were performed at the end of the project on April 11, 2025, after 6 months of development, they included: root length (cm), fresh and dry mass of the shoot system (g), and fresh and dry mass of the root system (g). Root length was measured with a flexible tape after carefully washing the roots to remove adhering soil. Shoot and root fresh mass were determined immediately after harvest and washing, using an analytical balance (± 0.001 g). Subsequently, the material was dried in a forced-air circulation oven at 60 °C for 72 hours, and dry mass was obtained by reweighing the samples.

Determination of arbuscular mycorrhizal colonization

Root samples from seedlings were collected to evaluate mycorrhizal colonization and verify the purity of the control treatment. Colonization was verified solely based on the presence of fungal structures, without quantifying the percentage of colonization or the inoculation level.

Roots were clarified in 2.5% potassium hydroxide (KOH) and 20% ammonium hydroxide (NH₄OH), according to the method of Koske and Gemma (1989). To enhance clarification and improve dye penetration, samples were acidified in 1% hydrochloric acid (HCl) and subsequently stained with 0.05% trypan blue. Cut root segments were mounted on slides and detailed using an optical microscope.

Statistical analysis

The data were analyzed using generalized linear mixed models (GLMM), utilizing the glmmTMB package (Brooks et al. 2017) in the R/RStudio environment (R Core Team, 2024). Treatments, consisting of isolated and consortium inoculations of arbuscular mycorrhizal fungi, were considered fixed effects, while pots and experimental replicates were included as random effects. The proven response variables were survival changes and growth intervals, with emphasis on branch length. The adequacy and selection of the models was evaluated based on the Akaike Information Criterion corrected for small sample sizes (AICc), using the MuMIn package in the R environment, through the model.sel function.

Results

Analysis using generalized linear mixed models (GLMM) showed that the survival of olive seedlings was significantly influenced by mycorrhizal treatments ($p < 0.05$). For average survival (Fig. 1), the isolated *Acaulospora longula* treatment had a significantly higher survival chance of 93.5% when compared to the control (CI: 0.360–0.742; $p < 0.0001$). Similarly, the *Acaulospora longula* + *Gigaspora rosea* consortium showed significantly superior performance to the control, with approximately 86% probability of seedling survival (CI: 0.375–0.771; $p < 0.0001$). In contrast, inoculation with *Gigaspora rosea* alone did not show

significant differences when compared to the control treatment (CI: 0.753–1.558; $p = 0.9422$). *Acaulospora longula* alone showed a significant 110% increase in survival chances when compared to the isolated treatment with *Gigaspora rosea* (CI: 1.458–3.012; $p < 0.0001$). No significant difference was observed between treatment with *Acaulospora longula* alone and the *A. longula* + *G. rosea* consortium ($p = 0.9923$). However, the consortium showed a 102% higher probability of survival when compared to the treatment with *Gigaspora* alone ($p < 0,0001$)

Fig. 1 Survival of olive seedlings under different mycorrhizal treatments. Survival percentage was evaluated at the end of the experimental period in control plants and in plants inoculated with *Acaulospora longula*, *Gigaspora rosea*, and co-inoculated with both species. Data are presented as mean $\pm$ standard error ($n = 16$). Different letters indicate significant differences among treatments according to Tukey's test ($P < 0.05$).

Branch length (fig. 2) was significantly influenced by time after transplantation and by the interaction between time and mycorrhizal treatments. Time showed a highly significant effect (estimate = 0.047; $p < 2 \times 10^{-16}$), corresponding to an average increase of approximately 4.83% per day. At the initial time, the *Gigaspora rosea* treatment showed a marginal reduction of approximately 87.6% (estimate = -2.085; $p = 0.0557$) and the combined inoculation (*A. longula* + *G. rosea*) a non-significant reduction of approximately 48.3% (estimate = -0.659; $p = 0.3431$). Over time, isolated treatment of *Acaulospora longula* significantly reduced the growth rate by approximately 1.79% per day (estimate = -0.018; $p = 3.66 \times 10^{-8}$), while *G. rosea* significantly increased growth by approximately 2.29% per day (estimate = 0.023; $p = 0.0022$). Furthermore, the combined treatment significantly increased growth by 1.15% per day (estimate = 0.011; $p = 0.0032$).

Fig. 2 Branch length growth of olive seedlings over six months under different mycorrhizal treatments. Branch length (cm) was measured weekly. Treatments included non-inoculated plants (control), inoculation with *Acaulospora longula*, *Gigaspora rosea*, and co-inoculation with both species. Data points represent mean values. Significant differences among treatments were determined at $P < 0.05$.

Regarding stem diameter (Fig. 3), inoculation in consortium with *Acaulospora longula* + *Gigaspora rosea* promoted a significant 55% increase in stem diameter compared to the control (estimated difference = 0.43519; $p < 0.001$). However, inoculation with *A. longula* alone resulted in a significant reduction of approximately 18% in stem diameter compared to the control (estimated difference = -0.19439; $p = 0.0169$). Treatment with *G. rosea* alone also showed a reduction of 4%, without a statistically significant difference compared to the control ($p = 0.6836$), indicating no relevant effect on this variable.

Fig. 3 Stem diameter growth of olive seedlings during the initial development phase under different mycorrhizal treatments. Stem diameter (cm) was measured weekly over three months. Treatments included non-inoculated plants (control), inoculation with *Acaulospora longula*, *Gigaspora rosea*, and co-inoculation with both species. Data points represent mean values. Significant differences among treatments were determined at $P < 0.05$.

For the mean root length (Fig. 4). Isolated inoculation with *Acaulospora longula* resulted in an approximate increase of 18% compared to the control, but a non-significant effect was observed (estimated difference = 0.813; $p = 0.7855$). The isolated treatment of *Gigaspora rosea* showed no statistical differences when compared to the control (estimated difference = -0.094; $p = 0.9750$). The *A. longula* + *G. rosea* consortium promoted a marginal increase of 113% in the average root length compared to the control (estimated difference = 5.094; $p = 0.0879$).

Fig. 4 Root length of olive seedlings under different mycorrhizal treatments. Root length (cm) was measured at the end of the experimental period in control plants and in plants inoculated with *Acaulospora longula*, *Gigaspora rosea*, and co-inoculated with both species. Data are presented as mean values $\pm$ standard error. Significant differences among treatments were determined at $P < 0.05$.

Regarding biomass accumulation (fig. 5), no statistically significant differences were observed in the fresh or dry weights of shoots and roots between the treatments and the control group.

Fig. 5 Column graphs, which present the mean values for; (a) fresh weight of the aerial part, (b) dry weight of the aerial part, (c) fresh weight of the root and (d) dry weight of the root, obtained at the end of the experiment. The seedlings were grown under four conditions: control (no inoculation), inoculation with *Acaulospora longula*, inoculation with *Gigaspora rosea* and combined inoculation (*A. longula* + *G. rosea*).

Discussion

This study indicates that arbuscular mycorrhizal fungi (AMF) promote the initial adaptation of olive seedlings to the nursery environment, differentially modulating the performance of host plants through distinct functional strategies [24], resulting in a trade-off between initial establishment and subsequent growth. This pattern reinforces that AMF species are not functionally identical and that plant responses result from the balance between carbon investment and nutritional return within the symbiosis [31; 32].

Inoculation with *Acaulospora longula*, applied alone or in combination with *Gigaspora rosea*, resulted in a higher seedling survival rate, confirming the effectiveness of these mycorrhizal associations in promoting the initial adaptation of plants to the nursery environment. As observed, inoculation with *Gigaspora rosea* alone did not increase survival compared to the control, but had significantly lower survival when compared to the intercropping and the isolated *A. longula*. This suggests that the addition of *G. rosea* to the intercropping did not confer any additional benefit to the survival of olive seedlings in the intercropping treatment.

The observed differences between the effects of *Acaulospora* and *Gigaspora* on seedling survival can be explained by the distinct functional and reproductive strategies adopted by these two genera within the phylum Glomeromycota [33; 34]. These differences are directly reflected in the processes of germination and root colonization. The literature indicates that *Acaulospora* species exhibit faster germination and produce highly infectious extraradicular mycelium, which accelerates the establishment of symbiosis,

while *Gigaspora* species require more time for spore germination and maturation, resulting in slower initial colonization [35; 36]. Therefore, the time it took for the symbiosis to establish itself may have been crucial for the seedlings' survival, since the maturation and germination of *Acaulospora* spores may have accelerated the colonization process and thus reduced the plants' vulnerability period to transplant stress. On the other hand, the slower development of *Gigaspora* may have limited the initial protection of the seedlings, as processes such as oxidation and root degradation may have occurred before the fungus was effectively established in the roots.

However, despite the initial advantage over seedling survival, *Acaulospora longula* did not have an effect on increasing plant growth over time, evidencing the occurrence of a functional trade-off. This pattern indicates that, although this species favors the initial establishment of seedlings, its association may impose relatively high metabolic costs on the host plant, without a proportional return in nutrient acquisition.

This apparent compensation may be related to the carbon demand associated with the establishment and maintenance of mycorrhizal symbiosis, since arbuscular mycorrhizal fungi depend on carbon derived from plant photosynthesis to sustain their growth and the development of their structures, such as the extraradical mycelium [37; 38; 39]. During the initial stages of the interaction, when the plant still has low photosynthetic capacity and limited reserves, this cost can be proportionally high, making the symbiosis metabolically costly [40; 41; 42]. As the mycelial network develops, there is an increase in the efficiency of resource exchange between fungus and plant, with a greater supply of nutrients, which tends to compensate for the initial carbon investment [43; 44; 45]. Thus, since the inoculation was performed at a very young stage of the plant, still underdeveloped and with only two leaves, this may have made the symbiosis metabolically costly at this initial stage [41; 42], and thus the symbiosis probably did not reach a fully efficient functional stage throughout the experimental period.

In this sense, the absence of growth promotion in plants inoculated with *A. longula* may reflect a more conservative functional strategy, in which the demand for carbon is not accompanied by a proportional nutritional return. According to the biological market model, plants tend to preferentially allocate carbon to fungal partners that offer a greater return in nutrients, while less efficient partners may act as carbon sinks [31]. Thus, the cost-benefit balance of the symbiosis, which varies between species of arbuscular mycorrhizal fungi and plant species, may have favored the initial survival of the seedlings, but limited biomass accumulation and seedling growth [32].

In contrast, *Gigaspora rosea* showed a more evident effect on plant height growth over time, although without a significant impact on initial survival. This pattern becomes even more evident when compared to the behavior of the control treatment plants, which were the first to show increased growth, corroborating the findings of [42], who highlight the initial delay in the development of plants inoculated with AMF. However, with time and the stabilization of the symbiosis in this study, both the isolated treatment with *Gigaspora rosea* and the treatment with the consortium showed better performance in shoot growth compared to the control. This behavior suggests a change in the symbiotic balance,

shifting from a high initial cost of maintaining the symbiosis to a beneficial interaction, especially due to the increased soil exploration and nutrient absorption promoted by the fungi [46; 16].

This transition can be explained by the progressive expansion of extensive extraradicular mycelium that Gigasporaceae exhibit [47; 48; 49]. This fungal strategy increases soil exploration and favors the absorption of water and nutrients, especially phosphorus [14; 50; 34]. Thus, although slower initial colonization and initial carbon costs may delay plant growth, the subsequent development of the mycelial network results in greater efficiency in resource acquisition, promoting plant growth over time.

The results suggest a possible complementary interaction for the development of the host plant between *A. longula* and *G. rosea*, where we observed that the intercropping treatment showed greater stability in the survival and structural development of the seedlings. This may be attributed to differences in ecological strategies or in the use of resources made available by the plant, resulting in functional complementarity between the fungal species [24; 26]. Since each species presents distinct strategies in hyphal exploration and the mobilization of different nutrients, these differences may provide an increase in the benefits generated by AMF for the plant and a more efficient use of resources when present together in the host [23; 36; 26]. In this context, the interaction between the two species in the intercropping system likely favored greater physiological balance and nutritional support for the plants, with *Acaulospora longula* contributing more significantly in the initial phases, increasing survival and establishment, while *Gigaspora rosea* contributed to vegetative growth, especially when intercropped. This complementarity also resulted in improvements in stem diameter, which are consistent with studies that investigated AMF consortia, demonstrating that the combination of different species increases vigor and stem diameter in mycorrhizal plants, as observed in olive trees by [29] and [46]. This synergistic effect, reflected in the increase in stem diameter, is associated with improved vascular transport, photoassimilate storage, and structural support of seedlings [51].

However, no significant differences were observed for variables such as fresh mass and dry mass, which may be explained by limitations inherent to the experimental design and the nature of the analyses performed. As these were destructive evaluations conducted at the end of the experiment, only surviving seedlings were considered, which may have reduced the representativeness of certain treatments, especially the control, which showed a low survival rate; thus, the reduced final sample size may have compromised the robustness of the statistical comparisons. Even so, this pattern is not uncommon, since other studies have also reported neutral or negative responses of inoculated treatments compared to the control regarding biomass accumulation and growth [52; 53; 42]. Furthermore, arbuscular mycorrhizal fungi impose variable carbon and metabolic costs on host plants, and their benefits do not always translate immediately into increased biomass [40; 39; 45], being more evident in later stages of development, when the symbiosis is fully established [43; 44], thus, the absence of effects on biomass in the present study does not imply a lack of biological relevance, and may reflect the relatively short duration of the experiment, since, over the six-month period, it is possible that the fungus did not fully develop its symbiotic structure, limiting the full expression of benefits for the host plant.

A significant limitation of this study is the absence of a quantitative assessment of mycorrhizal colonization. Although fungal structures were observed in root samples from the inoculated treatments, the inability to determine the intensity of colonization prevents a deeper interpretation of the fungus-host relationship. Therefore, the observed associations between inoculation treatments and plant performance should not be interpreted as direct evidence of functional symbiotic efficiency, but rather as indicative associations that require further validation.

In the present study, it was not possible to conduct the experiment for a longer evaluation period, nor to perform physiological and nutritional analyses of the seedlings, which constitutes a relevant limitation for the comprehensive interpretation of the effects of mycorrhizal inoculation. In this sense, an important perspective opens up for the development of future studies that include long-term trials, ideally between one and two years, allowing for a more consistent capture of growth, nutrition, and physiological functioning responses throughout the establishment of the symbiosis. Additionally, the incorporation of physiological assessments throughout the cycle, such as measurements of gas exchange, photosynthetic efficiency, and leaf nutrient content, can deepen the understanding of the mechanisms involved, enabling a more integrated analysis of the effects of mycorrhizal fungi beyond morphological variables.

In general, the results indicate that arbuscular mycorrhizal fungi are not functionally equivalent and that their contributions to plant performance depend on the interaction between their ecological strategies and the temporal dynamics of the symbiosis. These observations reinforce the importance of considering the functional diversity of AMF in the development of bioinoculants, particularly under nursery conditions, where the initial establishment of plants is critical for successful adaptation to field transplantation.

Conclusions

The results of this study suggest that inoculation with arbuscular mycorrhizal fungi is associated with differences in the initial survival and growth of olive seedlings in the nursery. The distinct patterns observed among the treatments indicate that AMF species may play different roles during the initial development of the plant. The use of the species *Acaulospora longula* and *Gigaspora rosea* in the production of olive seedlings is particularly relevant, since *A. longula* can contribute essentially to increasing the survival and initial establishment of the seedlings, while *G. rosea* may exert its greatest effect on vegetative growth.

However, due to the absence of quantitative colonization data and physiological measurements, the mechanisms underlying these associations remain unclear. Further studies integrating colonization intensity, nutrient dynamics, and plant physiological responses are necessary to better understand the functional role of AMF in olive seedling production systems.

Declarations

Acknowledgement

I thank my research colleagues for sharing their knowledge and for the technical support provided during the development of this work. I also acknowledge the Graduate Program in Family Farming Agricultural Production for its solid academic training, the stimulating scholarly environment, and the institutional support offered throughout the entire course of this research.

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, through the granting of a postgraduate scholarship.

Authors' contribution statement

AJC; Conceptualization, Methodology, Project administration, formal analysis, Investigation, Writing - original draft, Formal analysis; VLGf; Writing - review & editing, formal analysis, Visualization; JPSN; Investigation, Writing - review & editing, Visualization; LVPM; Investigation, Formal analysis, Writing - review & editing; ECCM: Resources, Writing - review & editing, Validation, Methodology; ECCM; Writing - review & editing, ; PMF; Writing - review & editing, Visualization; VSC; Conceptualization, Supervision, Writing - review & editing, Resources, Validation, Methodology. All authors read, critically reviewed, and approved the final version of the manuscript, agreeing to its submission for publication.

Competing interests

The authors have no relevant financial or nonfinancial interests to disclose

Data availability

The datasets generated and analyzed during the current study are available from the author, Arthur Joanello Cemin, upon reasonable request.

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Figures

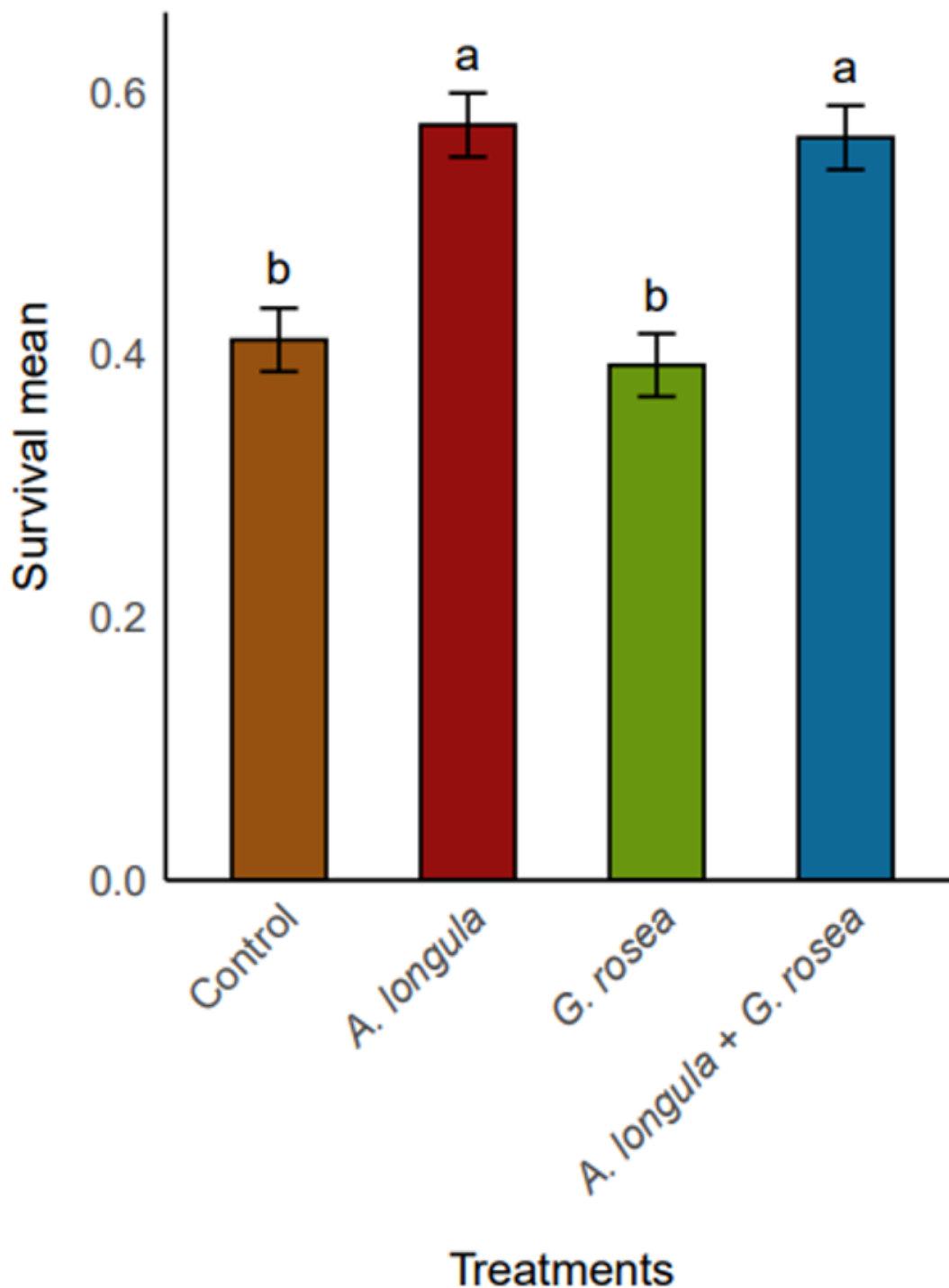


Figure 1

Survival of olive seedlings under different mycorrhizal treatments. Survival percentage was evaluated at the end of the experimental period in control plants and in plants inoculated with *Acaulospora longula*, *Gigaspora rosea*, and co-inoculated with both species. Data are presented as mean $\pm$ standard error (n = 16). Different letters indicate significant differences among treatments according to Tukey's test (P < 0.05).

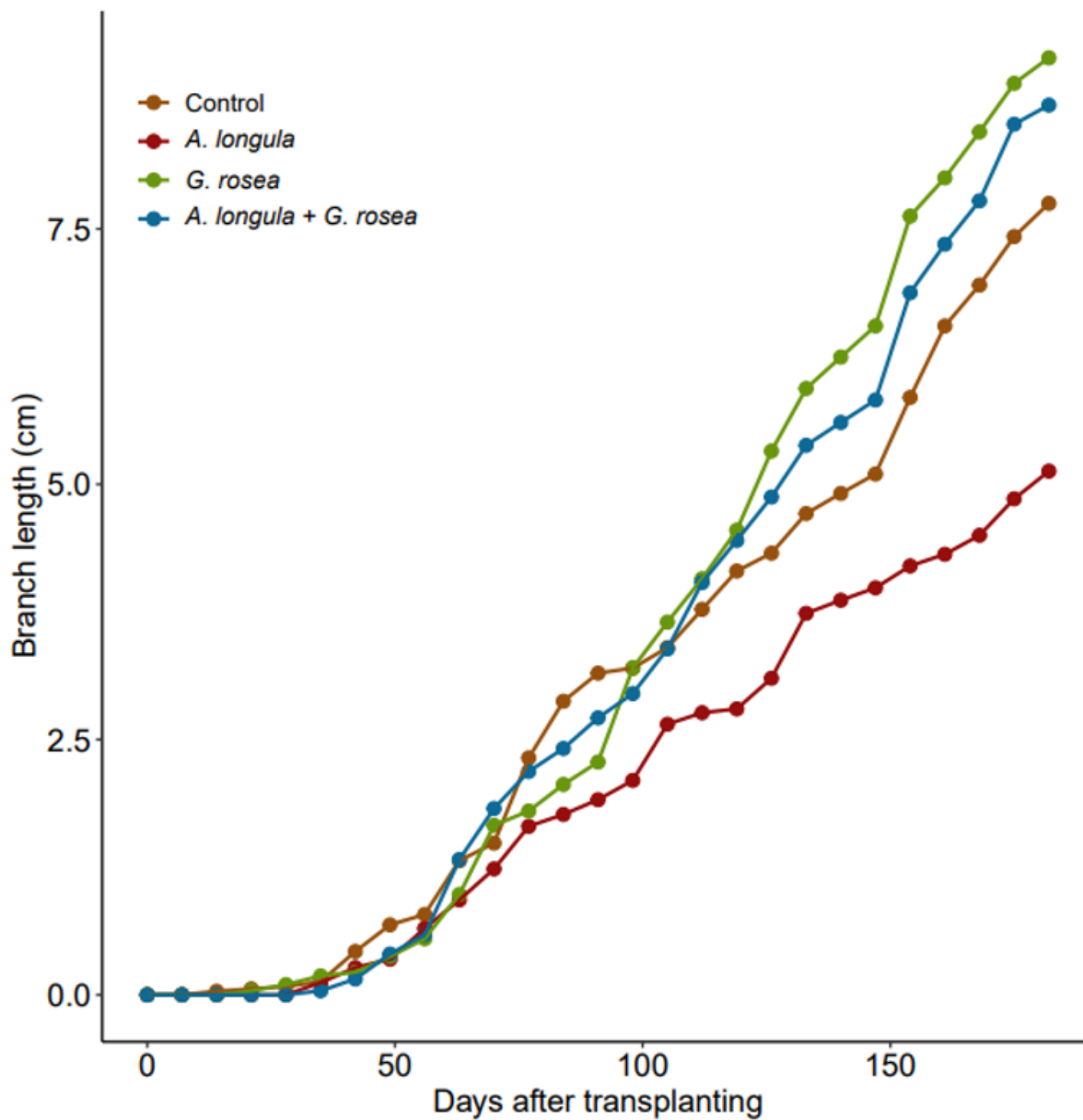


Figure 2

Branch length growth of olive seedlings over six months under different mycorrhizal treatments. Branch length (cm) was measured weekly. Treatments included non-inoculated plants (control), inoculation with *Acaulospora longula*, *Gigaspora rosea*, and co-inoculation with both species. Data points represent mean values. Significant differences among treatments were determined at $P < 0.05$.

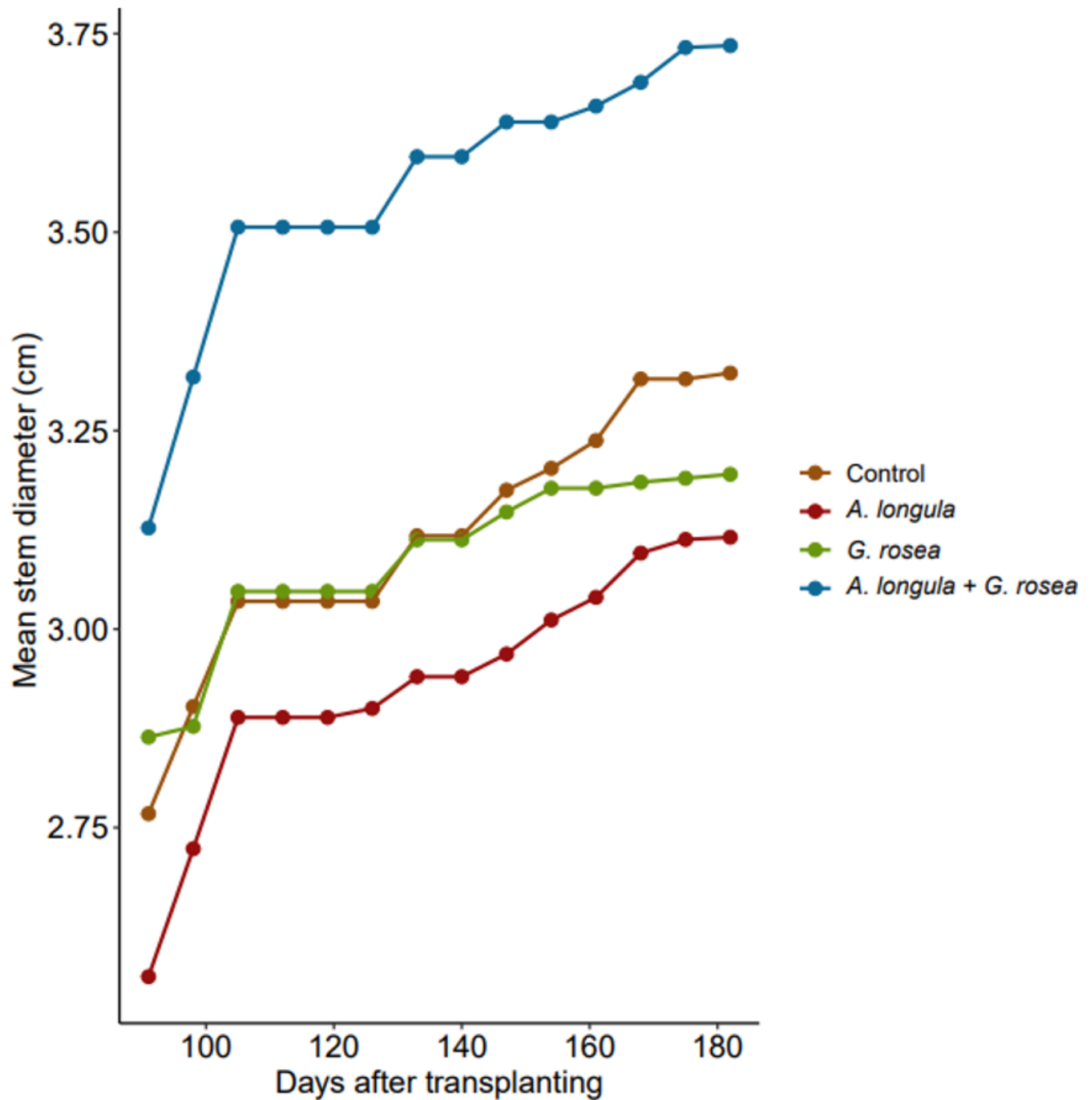


Figure 3

Stem diameter growth of olive seedlings during the initial development phase under different mycorrhizal treatments. Stem diameter (cm) was measured weekly over three months. Treatments included non-inoculated plants (control), inoculation with *Acaulospora longula*, *Gigaspora rosea*, and co-inoculation with both species. Data points represent mean values. Significant differences among treatments were determined at $P < 0.05$.

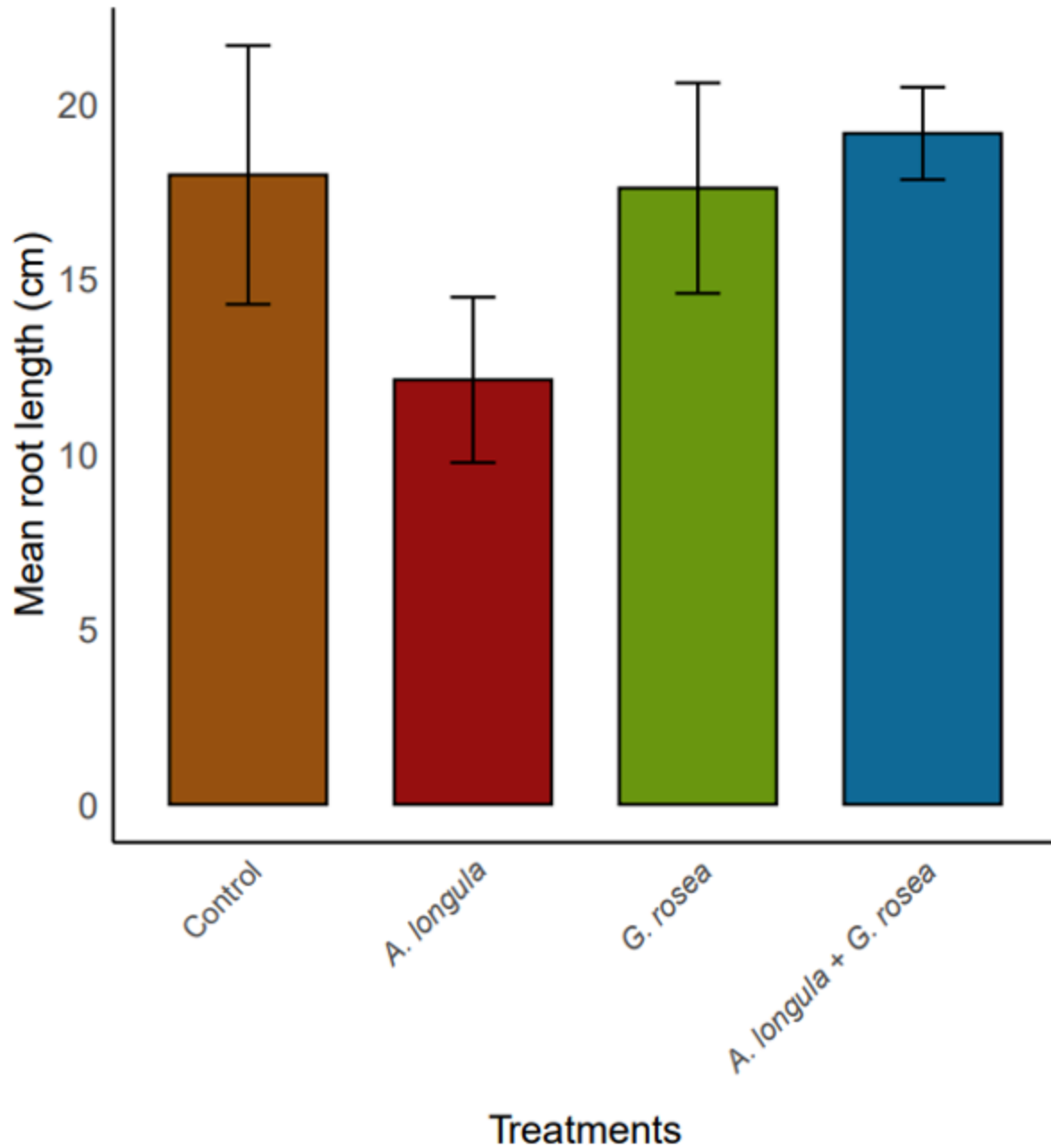


Figure 4

Root length of olive seedlings under different mycorrhizal treatments. Root length (cm) was measured at the end of the experimental period in control plants and in plants inoculated with *Acaulospora longula*, *Gigaspora rosea*, and co-inoculated with both species. Data are presented as mean values $\pm$ standard error. Significant differences among treatments were determined at $P < 0.05$.

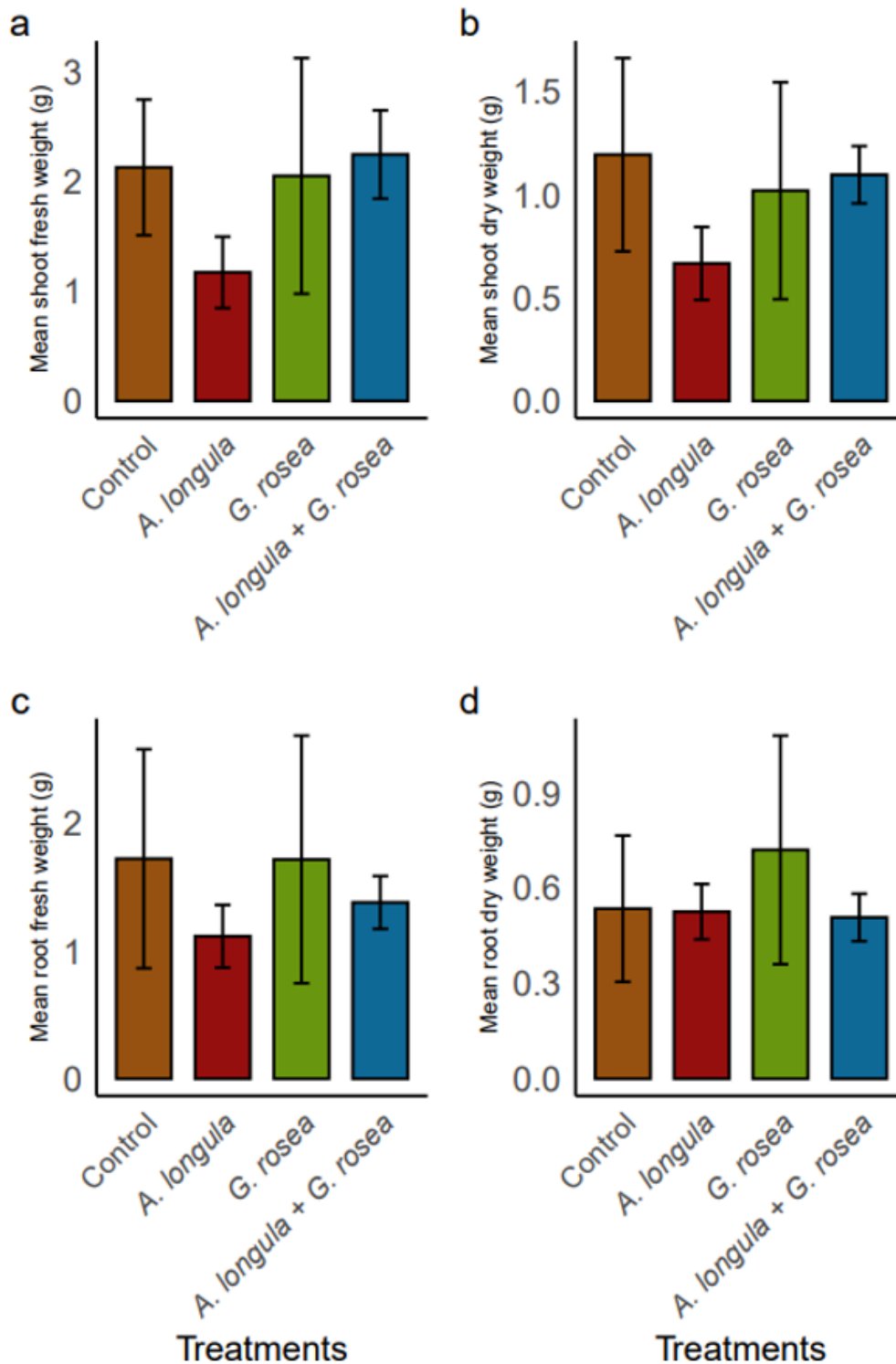


Figure 5

Column graphs, which present the mean values for; (a) fresh weight of the aerial part, (b) dry weight of the aerial part, (c) fresh weight of the root and (d) dry weight of the root, obtained at the end of the experiment. The seedlings were grown under four conditions: control (no inoculation), inoculation with *Acaulospora longula*, inoculation with *Gigaspora rosea* and combined inoculation (*A. longula + G. rosea*).

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