**Gallesia integrifolia** (Spreng.) Harms. Growth Under Different Shade and Water Availability Conditions

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**Abstract**

Our study sought to evaluate parameters of mycorrhizal growth and colonization of *Gallesia integrifolia* under different light and water availability conditions. The experiment was conducted in a greenhouse, with the following treatments: three light levels (0%, 50% and 80% shading), and two water regimes (watering daily and twice a week), in a completely randomized experimental design. The results showed that the plants kept under 50% shading and the ones kept under full light, both watered daily, had the best performance. Mycorrhizal association was observed in all treatments, which favored the establishment of seedlings even in environments with less water availability. *G. integrifolia* showed acclimation to higher levels of shading and lower availability of water, which may be related to the species plasticity and its wide distribution.

**Keywords:** leaf area, mycorrhizal association, dry mass, water stress, shading.

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1. **INTRODUCTION AND OBJECTIVES**

*Gallesia integrifolia* (Spreng.) Harms. (Phytolaccaceae) is a Brazilian native tree known as “pau d’alho” (“garlic wood”, due to its strong garlic smell), occurring from Bahia to Paraná and characteristic of the Semideciduous Forest and the Paraná basin. It is a perennial, heliophile, selective hygrophyte species that occurs in deep, humid and highly fertile soils (Lorenzi, 2014).

Forest species have severe limitations to propagation, since information on their ecophysiological characteristics is scarce (Borges et al., 2014). Identifying the factors that affect survival and early growth of plants in the field is one of the major problems of seedling producers (Lima et al., 2008). Water availability and light are important environmental factors that influence plant growth.

Luminosity is a determinant factor for the emergence and growth of plants due to its influence on photosynthesis (Cavatte et al., 2009). Light is an indicator of the environment where the plant grows, which can be measured by its intensity, quality, and direction. It’s an essential energy source for photosynthesis and consequential sugar production (Kozuka et al., 2005). Plants perceive changes in light quality through photoreceptors, such as phytochrome. These photoreceptors modulate seedling growth according to light quality. Light quality and intensity are important for the production of seedlings, since they control plant metabolism, growth, and development. These factors can also cause morphophysiologial changes in the plant (Costa et al., 2018; Meira et al., 2012). In addition to light intensity, water deficiency can also change plant behavior depending on the duration, the severity of the deficit, and the plant genotype (Santos & Carlesso, 1998).

Plant growth efficiency is related to its ability to acclimate to different environmental conditions, especially as seedlings (phase that covers the vegetable from the germination of the seed until the formation of the first leaf or eophyll) (Caron et al., 2010; Souza, 2009). Many growth variables can be used to evaluate the behavior of forest seedlings regarding the availability of light and water. These variables include the dry mass of the aerial, root and leaf areas, as well as plant height.

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Mycorrhizal fungi are known to influence plant growth by participating in nutrient cycling and soil carbon storage (Terrer et al., 2016). Plants provide carbohydrates, obtained by photosynthesis, from the leaves to the colonized roots through the phloem and, in turn, the fungus transfers water and nutrients from the soil to the plants (Garcia et al., 2016). Such interactions, frequent in tropical soils (Smith & Read, 2008), give the host plant greater tolerance to environmental stresses and, therefore, to water stress (Beltrano & Ronco, 2008). This higher tolerance may be related to a greater contact of the root with the soil, considering that the mycorrhizal association changes root architecture, water, and nutrient absorption, which enhance tolerance to water stress (Pozo et al., 2015).

The association of the mycorrhizal fungus with the roots also improves growth and nutrition of the seedlings due to the greater absorption of nutrients, mainly phosphorus (Rodrigues et al., 2018), favoring its establishment in the field.

The relationship of abiotic factors such as water availability and light with mycorrhizal colonization remains little studied. Our research group has found that Gallesia integrifolia depends on the mycorrhizal association (unpublished data). Considering the importance of this association in the establishment of forest species (Rodrigues et al., 2018), our study sought to evaluate growth and mycorrhizal colonization of plants kept in different conditions of light and water availability.

2. MATERIALS AND METHODS

We collected brownish samaras from five parent trees of Gallesia integrifolia (Spreng.) Harms located at the Caiuá Ecological Station, in August 2014. This conservation unit is located in the municipality of Diamante do Norte, north west of Paraná State (PR), between coordinates 52° 49’ to 52° 53’ W and 22° 34’ to 22° 37’ S and altitude ranging from 240 to 380 m (IAP, 1997). The native vegetation belongs to the Atlantic Forest Biome and the Semidecidual Seasonal Forest.

The samaras were taken to the Laboratory of Plant Physiology of the Universidade Estadual de Maringá and processed by removing the wings to obtain the seeds.

Latosol soil collected from the B horizon of the campus of the Universidade Estadual de Maringá was used for the substrate. It was initially dried outdoors before being sieved to remove particulate organic matter, then fertilized with P300 (mg.dm\(^{-3}\) soil). Subsequently, 180 pots were prepared. Each pot received 1 kg of substrate and five seeds. After emergence, only one seedling per pot was maintained.

The experiment was conducted in greenhouse with the treatments: 3 light intensities (0%, 50% and 80% shading) and 2 water regimes (plants irrigated daily and plants irrigated twice a week). Light was measured with a luxmeter standardized for all conditions, over three days at 10 a.m., 12 p.m., and 4 p.m. The measurements were repeated 3 times at intervals of 30 seconds, on a 70-cm-high bench. The mean light intensity data were 2207.0 lux for the full light environment, 762.2 lux for 50% shading, and 2.4 lux for 80% shading.

Sixty days after differentiation of the first pair of true leaves, the plants were divided into plants watered daily and plants watered twice a week, with 50 mL of tap water. Thus, the G. integrifolia plants were maintained in the following treatments: 0% shade and irrigated daily (0% ID); 0% shade and irrigated twice a week (0% IT); 50% shade and irrigated daily (50% ID); 50% shade and irrigated twice a week (50% IT); 80% shade and irrigated daily (80% ID); and 80% shade and irrigated twice a week (80% IT). The shading levels were obtained by using a shade cloth according to the manufacturer’s recommendations. For water availability, the plants watered daily were kept at the field capacity and plants watered twice a week were kept at 45% of water compared to the control. The pots filled with the soil were watered to the complete saturation and, after drainage, each pot was weighed to obtain the weight at total water capacity of soil.

Growth evaluation was conducted at three times: 30, 60, and 90 days after the beginning of the water treatment (DAWT). We randomly selected 10 plants from each treatment (five were used to assess mycorrhizal colonization and five to determine the dry matter and other growth parameters), totaling 60 plants evaluated for each time. To remove the plants without damage, the bags were vertically cut down and the soil was removed with running water to avoid the loss of root fragments. Measurements were taken for the following morphological variables: height (H), primary root length (RL), collar diameter (CD), leaf dry mass (LDM), root dry mass (RDM), stem dry mass (STDM), shoot dry mass (SDM), total dry mass (TDM), leaf number (LN), and leaf area (LA) at 30 and 90 days. Root dry mass/shoot dry mass ratio (RDM/SDM), root dry mass/total dry mass (RDM/TDM), leaf dry mass/total dry mass (LDM/TDM), stem dry mass/total dry mass (STDM/TDM), shoot dry mass/total dry mass (SDM/TDM).

Height and root length were measured with a ruler graduated in millimeters and collar diameter with a digital caliper. Plant height was measured from the root collar to the apex of the plant. To evaluate the mass, the plants were separated into roots, stem and leaves, placed in labeled paper bags, and incubated in an oven at 60 °C for 7 days. The dry mass of the different organs was weighed on a MARC/M214AI analytical balance. Leaf number was determined by counting the fully expanded leaves in each plant.
Leaf area (LA) was determined from five plants of each treatment. Fully expanded leaves of each plant were scanned and the leaf areas were measured using the Image-Pro PLUS® software. The leaf area ratio (LAR) was determined from leaf area (LA) values expressed in cm² and total dry mass (TDM) expressed in g. The height/dry mass ratio of the shoot was obtained from the plant height (H) and shoot dry mass (SDM) values.

We randomly collected five plants of each treatment at each time and separated the roots to assess root colonization by arbuscular mycorrhizal fungi. The roots were washed in tap water to remove substrate and were preserved in 50% ethanol. Then, roots were washed in fresh water and incubated in a water bath in tubes filled with 10% KOH for clearing of the cortex. Roots were washed again in tap water and acidified with 5% HCl. Afterwards, they were stained with trypan blue in a water bath according to Phillips & Hayman (1970) and stored in glass jars containing preservative solution until analysis. Root colonization was evaluated using a stereoscopic microscope. In the samples in which clearing was insufficient to observe the root cortex, the roots were mounted on slides and observed in an optical microscope, and the quantification of colonization followed the criteria of Trouvelot et al. (1986). The percentage of roots colonized by arbuscular mycorrhizal fungi was determined using a stereoscopic microscope, based on records of presence or absence of characteristic intra-radicular structures such as vesicles, hyphae, and arbuscules by the gridline intersect method (Giovannetti & Mosse, 1980).

The experiment was arranged in a (3 × 2) factorial design with three levels of light intensity (0%; 50% and 80% of shading) and two levels of water availability (plants watered daily and plants watered twice a week). All growth parameters were subjected to analysis of variance, and the means were compared by Tukey's test at 5% probability, using the Statistica 7.0 software.

3. RESULTS AND DISCUSSION

The seedlings of *Gallesia integrifolia* showed variation in growth due to different levels of light intensity and water availability. For most of the parameters analyzed, light was the factor that most influenced plant growth over the experimental period. At 30 days, the analysis showed interaction between light and water availability for the parameters RDM, TDM, RDM/SDM, RDM/TDM, LDM/TDM, STD/TDM and SDM/TDM. At 90 days, interaction was observed for the parameters STD/TDM, SDM, CD, STD/TDM, LA and LAR (Table 1). At 60 days, no interaction was found for the factors, therefore, they were not described.

![Table 1. Result of the factorial analysis regarding to the effect of light and water availability and their interaction on the growth parameters of *Gallesia integrifolia* at 30 and 90 days.](image)

* p < 0.05 indicates that the results are significantly different. L: light; W: water availability; ns: non-significant; SD: standard deviation.

Seeding emergence started seven days after sowing. In general, at 30 and 90 days after the start of the water treatment, there was no significant difference of shoot height (H), root length (RL), leaf number (LN), and leaf dry mass (LDM) for the seedlings maintained in the different treatments (Figures 1 and 2). Schwantes et al. (2013) also observed that the leaf number in seedlings of *Gallesia integrifolia* at different light levels showed no significant differences.

Concerning the root dry mass (RDM), interaction was recorded between the light effect and water availability (Table 1) after 30 days. The plants maintained in conditions under stress and without shade cloth (0% IT) presented higher masses when compared with the other treatments (Table 2) due to plant growth alterations and resulted in a greater investment in the root mass. This allowed greater success in obtaining water. The plants kept under higher light availability had greater photosynthetic capacity (Nascimento et al., 2015; Wu et al., 2017). This promoted the transport of photosynthetic products to the roots and enabled larger root system areas. Increased photosynthetic capacity results from greater stomatal conductance, which increases CO₂ concentration for carboxylation. Greater stomatal conductance also favors the increase of transpiration, and, consequently, greater root mass is needed for water absorption due to the evaporative demand.
Table 2. Dry mass at 30 days of young *Gallesia integrifolia* plants kept in a greenhouse under 0%, 50% and 80% of shading, irrigated daily and irrigated twice a week.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>50% ID</th>
<th>50% IT</th>
<th>80% ID</th>
<th>80% IT</th>
<th>0% ID</th>
<th>0% IT</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDM (g)</td>
<td>0.43 Ab*</td>
<td>0.39 Ab</td>
<td>0.09 Ac</td>
<td>0.08 Ac</td>
<td>0.34 Bb</td>
<td>1.31 Aa</td>
</tr>
<tr>
<td>TDM (g)</td>
<td>1.16 Aa</td>
<td>0.92 Ab</td>
<td>0.41 Ac</td>
<td>0.40 Ac</td>
<td>1.03 Ba</td>
<td>2.09 Aa</td>
</tr>
<tr>
<td>RDM/SDM</td>
<td>0.60 Aa</td>
<td>0.74 Ab</td>
<td>0.28 Ab</td>
<td>0.27 Ab</td>
<td>0.38 Bb</td>
<td>1.68 Aa</td>
</tr>
<tr>
<td>RDM/TDM</td>
<td>0.37 Aa</td>
<td>0.42 Ab</td>
<td>0.21 Ab</td>
<td>0.20 Ac</td>
<td>0.26 Ba</td>
<td>0.60 Aa</td>
</tr>
<tr>
<td>LDM/TDM</td>
<td>0.34 Ab</td>
<td>0.33 Ab</td>
<td>0.55 Aa</td>
<td>0.58 Aa</td>
<td>0.39 Ab</td>
<td>0.25 Bb</td>
</tr>
<tr>
<td>STDM/TDM</td>
<td>0.28 Ab</td>
<td>0.24 Aa</td>
<td>0.23 Ab</td>
<td>0.21 Ab</td>
<td>0.35 Aa</td>
<td>0.15 Bc</td>
</tr>
<tr>
<td>SDM/TDM</td>
<td>0.63 Ab</td>
<td>0.58 Ab</td>
<td>0.79 Aa</td>
<td>0.80 Aa</td>
<td>0.74 Aa</td>
<td>0.40 Bc</td>
</tr>
</tbody>
</table>

* Data followed by the same letters are not significantly different by the Tukey's test at 5%. Small letters compare light treatment in the same water condition. Capital letters compare plants ID and IT, in the same light condition. ID: irrigated daily; IT: irrigated twice a week; RDM: root dry mass; TDM: total dry mass; RDM/SDM: root dry mass/shoot dry mass ratio; RDM/TDM: root dry mass/total dry mass ratio; LDM/TDM: leaf dry mass/total dry mass ratio; STDM/TDM: stem dry mass/total dry mass ratio; SDM/TDM: shoot dry mass/total dry mass ratio.

At 90 days, no interaction was found between the sources of variation, occurring only the effect of light. The plants under 0% and 50% shading, in both water conditions, had RDM 300% higher than the plants under 80% shading (Table 3, Figure 2). This increase in root mass can allow a greater absorption of water and nutrients, a strategy used by the plant to cope with high rates of transpiration and photosynthesis (Silva et al., 2007). Moreover, seedling exposure to high radiation suggests the allocation of photoassimilates preferentially to the root system, promoting greater mass gain of roots (Câmara & Endres, 2008).

Total dry mass (TDM), at 30 days, showed interaction between the factors light and water availability, in which the plants in 0% and 50% ID showed greater TDM (Table 2). Considering only water availability, a difference was observed between the treatments 0% ID and 0% IT, with the largest TDM found for the treatment 0% IT (Table 2). At 90 days, no interaction was
observed between the factors of variation (Table 1). The results of our study indicate that, at 30 days, *G. integrifolia* has greater mass accumulation in environments with higher light intensity, considering that the plants kept in the 0% shading received light intensity up to 99% higher than the plants under shading, according to the luxmeter readings. In general, plants maintained at higher luminous intensities have higher photosynthetic rates and higher photoassimilate production (Nascimento et al., 2015; Wu et al., 2017), which may contribute to the greater accumulation of dry biomass.

Stem dry mass (STDM) showed interaction between light and water availability only at 90 days (Table 1). The highest STDM was recorded for the treatment 50% ID and the lowest for 80% ID (Table 3). For plants watered twice a week, the highest STDM was found for the treatments 50% IT and 0% IT. When the plants under the same light condition and different water availability were compared, the highest STDM was found for the plants watered daily. Thus, negative effect of lower water availability and the effect of light on STDM was observed, with a negative influence of the treatment 80% shading on this parameter. According to Souza et al. (2017), plants under higher light intensities have high-capacity machinery for photosynthesis, contributing to the increase of the photosynthetic rate. Increase in photosynthesis results in more carbohydrate content in leaves, stem, and root, and, consequently, influences the increase in dry mass.

At 90 days, we found that *G. integrifolia* seedlings under 80% shading had the lowest means of leaf dry mass (LDM), stem dry mass (STDM), and root dry mass (RDM) and, consequently, the lowest total dry mass (TDM) at both evaluation times. Feijó et al. (2009) reported higher growth of *G. integrifolia* seedlings when kept under 25% and 50% shading. However, the authors observed a significant reduction in growth when the seedlings were under 75% shading, which agrees with our findings for 80% shading, in which we found the lowest values for the growth variables dry mass of stem and shoot and the smallest collar diameter at 90 days (Table 3).

The factorial analysis showed no interaction of the variation factors on collar diameter of *G. integrifolia* seedlings at 30 days, with only the light effect occurring at this time. At 90 days after the beginning of the water treatment (DAWT), an interaction was observed between light and water availability on CD (Table 1), with the smallest diameter recorded in plants under 80% shading, regardless of water availability (Table 3). Reduction in the collar diameter of plants under 80% shading, regardless of water availability, was also found by Ferreira et al. (2012) and, according to Mota et al. (2012), a high correlation was observed between collar diameter and seedling survival after planting. Thus, the highest collar diameter found in plants kept under 50% ID, 0% ID, and 0% IT (Table 3) may indicate a higher survival rate after planting.

The shoot dry mass (SDM), at 90 DAWT, was significantly higher for the plants under 50% ID, followed by the plants under 0% ID (Table 3), with significant interaction between light and water availability (Table 1). At 30 DAWT, no interaction was found between the sources of variation. The highest shoot dry masses correspond to more lignified and tough seedlings, capable of establishing with greater success.

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**Table 3.** Leaf dry mass, root dry mass, stem dry mass, total dry mass, total dry mass, shoot dry mass, collar diameter, leaf area, leaf area ratio, height/shoot dry mass ratio of young *Gallesia integrifolia* plants kept in greenhouse conditions under 0%, 50% and 80% of shading and irrigated daily and irrigated twice a week.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>50% ID</th>
<th>50% IT</th>
<th>80% ID</th>
<th>80% IT</th>
<th>0% ID</th>
<th>0% IT</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDM (g)</td>
<td>0.87 Aa*</td>
<td>0.72 Aa</td>
<td>0.49 Ab</td>
<td>0.27 Ab</td>
<td>0.80 Aa</td>
<td>0.62 Aa</td>
</tr>
<tr>
<td>RDM (g)</td>
<td>1.10 Aa</td>
<td>0.84 Aa</td>
<td>0.27 Ab</td>
<td>0.22 Ab</td>
<td>1.24 Aa</td>
<td>0.90 Aa</td>
</tr>
<tr>
<td>STDM (g)</td>
<td>1.11 Aa</td>
<td>0.66 Ba</td>
<td>0.29 Ac</td>
<td>0.31 Ab</td>
<td>0.82 Ab</td>
<td>0.57 Ba</td>
</tr>
<tr>
<td>STDM/TDM</td>
<td>0.36 Aa</td>
<td>0.26 Ba</td>
<td>0.28 Ab</td>
<td>0.33 Aa</td>
<td>0.30 Ab</td>
<td>0.27 Ab</td>
</tr>
<tr>
<td>TDM (g)</td>
<td>3.09 Aa</td>
<td>2.22 Aa</td>
<td>1.06 Ab</td>
<td>0.94 Ab</td>
<td>2.86 Aa</td>
<td>2.09 Aa</td>
</tr>
<tr>
<td>SDM (g)</td>
<td>1.98 Aa</td>
<td>1.38 Ba</td>
<td>0.79 Ac</td>
<td>0.72 Ab</td>
<td>1.61 Ab</td>
<td>1.20 Ba</td>
</tr>
<tr>
<td>CD (mm)</td>
<td>6.00 Aa</td>
<td>4.50 Bb</td>
<td>3.80 Ac</td>
<td>3.40 Ac</td>
<td>5.60 Aa</td>
<td>5.60 Aa</td>
</tr>
<tr>
<td>LA (cm²)</td>
<td>19.96 Aa</td>
<td>21.36 Aa</td>
<td>19.77 Aa</td>
<td>13.13 Ba</td>
<td>11.04 Ab</td>
<td>10.46 Ab</td>
</tr>
<tr>
<td>LAR (cm² g⁻¹)</td>
<td>6.63 bA</td>
<td>8.56 bA</td>
<td>19.04 aA</td>
<td>14.08 aB</td>
<td>3.99 cA</td>
<td>5.02 cA</td>
</tr>
<tr>
<td>H/SDM (cm g⁻¹)</td>
<td>17.24 Ab</td>
<td>17.38 Ab</td>
<td>37.87 Aa</td>
<td>34.41 Ab</td>
<td>15.65 Ab</td>
<td>16.30 Ab</td>
</tr>
</tbody>
</table>

* Data followed by the same letters are not significantly different by the Tukey's test at 5%. Small letters compare light treatment in the same water condition. Capital letters compare plants ID and IT, in the same light condition. ID: irrigated daily; IT: irrigated twice a week; LDM: leaf dry mass; RDM: root dry mass; STDM: stem dry mass; STDM/TDM: stem dry mass/total dry mass ratio; TDM: total dry mass; SDM: shoot dry mass; CD: collar diameter; LA: leaf area; LAR: leaf area ratio; H/SDM: height/shoot dry mass ratio.
in unfavorable environments, considering that the SDM indicates the seedling robustness (Gomes & Paiva, 2006).

At 30 days DAWT, the highest means for the SDM/TDM ratio were observed in plants under 80% shading. The lowest SDM/TDM ratio was found in plants exposed to the highest light intensity with lower water availability (Table 2), which may be related to the greater investment in RDM. The highest SDM/TDM ratio found in G. integrifolia seedlings under 80% shading reflects the greater investment of plants in height growth, with lower mass of leaves and roots at the growth beginning. At 90 DAWT, no interaction was observed between light and water availability on SDM/TDM (Table 1).

At 30 days DAWT, the RDM/SDM ratio was higher in the plants under 50% ID when compared with the plants under 80% shading and 0%, in the same water condition. However, the plants watered twice a week had the highest ratio in the treatment 0% IT (Table 2). Plants kept under 80% shading had the lowest RDM/TDM ratio. The highest RDM/TDM ratio in plants from environments more exposed to sunlight indicates a greater allocation of photosynthates to the root system (Siebeneichler et al., 2008). Carvalho et al. (2006) reported that the highest root/shoot ratio and the lowest leaf area ratio in plants from environments more exposed to sunlight indicate that the biomass was distributed more preferentially to the roots than to the photosynthesizing organs. This allocation allows a greater absorption of water and nutrients, which is a strategy that would guarantee greater capacity to withstand the higher rates of photosynthesis and transpiration in environments more exposed to sunlight.

The highest LDM/TDM ratio was recorded for plants under 80% ID and 80% IT, at 30 days DAWT (Table 2). The other treatments were not significantly different. These results may be related to the lowest total dry mass found in the plants under 80% shading, reflecting a higher leaf investment, although no interaction of the factors light and water availability was observed on LDM at 30 days. However, this interaction was significant when assessing LDM regarding TDM (Tables 1 and 2).

A significant interaction was observed between light and water availability for the SDM/TDM ratio, at 30 and 90 days DAWT (Table 1). Considering, at 30 days, the plants watered daily, the highest SDM/TDM was found in plants under 0% ID, whereas the plants with lower water availability had the highest SDM/TDM in the treatment 50% IT and lowest in the treatment 0% IT (Table 2). At 90 days, when comparing the plants watered daily, the lowest SDM/TDM was found in the plants under 80% shading, which is related to the lowest SDM found for these plants (Table 3).

Plants that grow in shaded environments usually have longer stems (Souza et al., 2017). Longer stems compensate for light deficiency and is an important mechanism of adaptation of the species. Stem growth is a valuable strategy plants use to escape conditions of low luminosity (Moraes Neto et al., 2000). In competition for light, plants adjust their architecture to allow their leaves to reach places with more sunlight availability for the lower strata. These architectural responses include accelerated stretching (of the hypocotyl, internodes, and petioles), upward leaf movement, and reduced shoot branching. These strategies are collectively named as “shadow avoidance syndrome”. Shaded environments have a greater proportion of distant red light (FR). This involves a reduced red light / distant red light ratio (R: FR), which is perceived by phytochromes (Pierik & De Wit, 2014). The reduction of the R: FR ratio decreases active phytochrome (phyB) levels. This promotes the expression of genes for auxin and gibberellin synthesis, as well as the consequential stem elongation (Casal, 2012).

However, G. integrifolia seedlings kept in the most shaded environment had the lowest SDM, which may indicate that these plants had thinner stems, providing lower stem mass for plants kept in more shaded environments, since no significant difference for the height was observed between plants kept in the different treatments (Table 1). This can also be confirmed by the higher H/SDM ratio observed in the plants kept under 80% shading, independent of water availability (Table 3), indicating a lower investment in shoot mass (stem and leaves) than in height.

At 30 days DAWT, we found no interaction of the sources of variation on LA. On the other hand, at 90 days, greater LA was observed for the plants under 50% ID, 50% IT, and 80% ID (Table 3). This indicates the effect of light on the increase of leaf area, increasing the chances of light capture and photosynthetic area (Siebeneichler et al., 2008).

The leaf area ratio (LAR) at 90 DAWT was highest for plants under 80% shading, which was established by the significant interaction between light and water availability (Table 3). LAR indicates the plant's investment in leaf area considering the total dry mass of the plant, which was determined by the highest LA in the plants under 80% ID and reduction in TDM. Souza et al. (2017) found that young plants of Enterolobium contortisiliquum growing under shade had larger leaf size and greater leaf area ratio (cm$^2$ g$^{-1}$), indicating investment in larger leaves with thinner mesophyll, which makes the light capture in a shaded environment more efficient. This response was also recorded for G. integrifolia seedlings under 80% shading, with the lowest LDM and TDM and the highest LAR at 90 days (Table 3).

The arbuscular mycorrhizal association in young plants of G. integrifolia was observed in all treatments, and at 30 DAWT, the highest colonization percentage was found for plants kept under 0% ID and 0% IT, and in plants under the treatment 50% ID (Figure 3).
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Figure 3. Mycorrhizal colonization (MC) (%) at 30, 60, and 90 days after the beginning of the water treatment.

At 60 days DAWT, there was a decrease in the colonized segments, and the plants kept in 0% IT and 50% IT showed the highest colonization rates, and at 90 days the highest colonization percentages were observed in plants under 0% IT and 50% IT. The lowest values were found in plants kept under 80% shading, regardless of water availability (Figure 3), which may be associated with the reduction in root mass of plants kept under the highest level of shading.

The mycorrhizal association is characterized by nutritional changes between plant and fungi. The fungus provides the autotrophic host with mineral nutrients and water, promoting the growth of plants, in exchange, the host plant provides sugar from the photosynthesis (photoassimilates) to the heterotrophic symbiont (Garcia et al., 2016).

Braghirolli et al. (2012) reported that the carbon allocated to the roots is also maintained in the symbioses established by plants with soil microorganisms, mainly nitrogen-fixing bacteria and arbuscular mycorrhizal fungi (AMF). Thus, the higher level of shading may have led to lower allocation of photoassimilates to the roots, also reducing the mass of the root system, compromising the mycorrhizal association (Konvalinková & Jansa, 2016).

Mycorrhizal fungi are considered key components in the production of seedlings (Rodrigues et al., 2018). Moratelli et al. (2007) pointed out that plants under water restriction have a higher root/shoot ratio when colonized by native fungi. This was observed at 30 days, in which plants under 0% IT showed higher RDM/SDM ratio than plants under 0% ID (Table 2), which may have ensured the initial establishment of these plants.

In our study, different light intensities and water availability significantly affected the colonization percentage in the plants subjected to the highest shading level. Therefore, light intensity is one of the factors that affect the mycorrhizal association with the plant (Moratelli et al., 2007), as well as water availability (Yooyongwech et al., 2016). Thus, seedlings of G. integrifolia could adapt to variations in the availability of resources regarding the association with arbuscular mycorrhizal fungi (AMF), which may favor the establishment of plants in environments with limiting water and light (Konvalinková & Jansa, 2016; Pozo et al., 2015).

4. CONCLUSIONS

The mycorrhizal association of young garlic wood plants is affected by lower light availability, which is the factor that mostly affect plant growth. Despite showing favorable acclimatization capacity in conditions of higher levels of shading and water restriction, the recommended conditions for the cultivation of the G. integrifolia seedlings were 50% ID and 0% ID, since they presented the best performance. This tendency may be related to the plant plasticity, which would justify its wide distribution, considering that the plant can be both pioneer and secondary.

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