

MORPHOPHYSIOLOGICAL CHANGES IN SEEDLINGS OF *Eugenia uniflora* L. AFTER CHEMICAL HARDENING

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Resumo

Alterações morfofisiológicas em mudas de Eugenia uniflora L. após a rustificação química. O uso de mudas de qualidade aumenta as chances de sobrevivência e desenvolvimento após o plantio. A rustificação almeja modificar a morfologia das mudas para aclimatá-las as condições estressantes de campo. Neste sentido, este estudo objetivou quantificar os efeitos da rustificação química com metil jasmonato na morfofisiologia em mudas de *Eugenia uniflora* L. submetidas a 12 dias de suspensão hídrica. As mudas foram rustificadas com as doses de 0, 50, 100 e 150 $\mu\text{mol L}^{-1}$ de metil jasmonato (MeJA). Posteriormente, foram transplantadas para vasos com solo e submetidas a suspensão hídrica por 12 dias. Durante esse período avaliou-se altura da parte aérea (H), diâmetro do coleto (DC), área foliar (AF), massa seca de folhas (MSF), de caule (MSC), de raiz (MSR) e total (MST), conteúdo relativo de água (CRA), índice de robustez (IR), índice de qualidade de Dickson (IQD), relação parte aérea/raiz (PA/R), condutância estomática (Cs) e evapotranspiração (ET). O experimento foi conduzido em delineamento de blocos casualizados, com cinco blocos. Os valores de IR, IQD, AF, CRA e MST apresentaram tendência quadrática, com resultados promissores proporcionados pelas doses de 50 e 100 $\mu\text{mol L}^{-1}$. Os valores de Cs e ET foram influenciados pelo déficit hídrico. Em condição de restrição hídrica, a aplicação de MeJA, em doses adequadas, promoveu modificações morfofisiológicas e indução de estratégias de defesa às plantas de *E. uniflora*. As doses de metil jasmonato entre 50 e 100 $\mu\text{mol L}^{-1}$ resultaram em mudas com maior tolerância às condições de déficit hídrico, por 12 dias.

Palavras-chave: pitangueira, regulador vegetal, metil jasmonato, estratégias de sobrevivência

Abstract

Using quality seedlings increases the chances of survival and development after planting. Hardening aims to modify the morphology of seedlings to acclimatize them to stressful field conditions. The objective of this study was to quantify the effects of chemical hardening with methyl jasmonate on the morphophysiology of *Eugenia uniflora* L. seedlings subjected to 12 days of water deficit. Seedlings were hardened with doses of 0, 50, 100, and 150 $\mu\text{mol L}^{-1}$ of methyl jasmonate (MeJA). Afterward, they were transplanted into pots with soil and subjected to water deficit for 12 days. During that period, we assessed shoot height (H), stem diameter (SD), leaf area (LA), leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM), total dry mass (TDM), relative water content (RWC), robustness index (RI), Dickson quality index (DQI), shoot/root ratio (S/R), stomatal conductance (Sc) and evapotranspiration (ET). The experiment was conducted in a randomized block design with five blocks. Variables RI, DQI, LA, RWC, and TDM values presented a quadratic trend, with promising results from the 50 and 100 $\mu\text{mol L}^{-1}$ doses. Sc and ET values were influenced by water deficit. Under conditions of water restriction, application of MeJA in adequate doses promoted morphophysiological changes and induced defense strategies in *E. uniflora* seedlings. Doses of methyl jasmonate between 50 and 100 $\mu\text{mol L}^{-1}$ resulted in seedlings with greater tolerance to water deficit for 12 days.

Keywords: Pitangueira, plant regulator, methyl jasmonate, survival strategies

INTRODUCTION

Eugenia uniflora L., popularly known as pitangueira, can be found as a dense shrub, measuring 2 to 4 m in height, or as a small, branched tree measuring 6 to 9 m in height with a crown measuring an average of 3-6 m in diameter. The species is native to Brazil and because of its adaptability can be found in all regions as well as in other countries such as Paraguay and Argentina (BEZERRA *et al.*, 2018).

The species has several uses, such as fresh fruit consumption and industrial use for manufacturing food and beverage. *E. uniflora* has also been used in the pharmaceutical and cosmetics industries. The tree is recommended for use as a hedge and for ornamental and urban planting. In addition, *E. uniflora* has a high survival capacity in disturbed areas, has good adaptability to different types of soils as well as different climatic/environmental conditions, and can be used for restoring degraded areas (BEZERRA *et al.*, 2018).

Thus, research aimed at producing seedlings of the species is of great importance, as it helps in establishing plantings and increasing survival and performance in the field since this is the first stage of production. Using seedlings with desirable attributes increases the chances of survival and development after planting

(GROSSNICKLE; MACDONALD, 2018). One way of promoting survival and growth after planting is hardening, a process carried out on seedlings in the nursery that mainly aims to modify their morphology to acclimatize them to field conditions (JACOBS; LANDS, 2009).

The application of plant regulators as a hardening technique for seedlings of woody species has been widely studied. The research areas are focused on ways of mitigating the effects of stress caused by lack of water and increasing tolerance. In this regard, some promising results have been observed. Mazzuchelli *et al.* (2014) studied the application of salicylic acid in hardening of *Eucalyptus urophylla* x *Eucalyptus grandis* hybrid seedlings. Gonçalves *et al.* (2015) studied the use of plant regulators and potassium phosphite in the hardening of *E. grandis* x *E. urophylla* hybrid seedlings.

Jasmonic acid and its derivatives, jasmonates, are endogenous growth regulators, originating from linolenic acid, which occurs in various plant species. Such regulators are involved in activating plant defense mechanisms by working as stress signals (DEUNER *et al.*, 2015). Using this substance to harden seedlings is regarded as a practical application and has been confirmed as a viable alternative by authors who verified its effects through morphophysiological responses of seedlings (HEBERLE *et al.*, 2018; CADORIN *et al.*, 2015; LIMA *et al.*, 2020).

In light of the above, this research hypothesis is that the application of adequate doses of methyl jasmonate stimulates changes in the morphology and physiology of *E. uniflora* seedlings, making them more vigorous and tolerant to field conditions. Due to the importance of the species and the need for information on woody species native to the Brazilian flora, this study aimed to quantify the effects of chemical hardening with methyl jasmonate on morphometry and physiology in seedlings of *E. uniflora* L. subjected to 12 days of water deficit.

MATERIAL AND METHODS

The *E. uniflora* seedlings were purchased from the forest nursery of the Instituto Água e Terra - IAT from Toledo-PR. The seeds were harvested from mature trees in forest remnants, located at least 100 meters apart. These seedlings were grown in polypropylene plugs with a capacity of 180 cm³ of commercial pine bark-based substrate and controlled-release macro- and micronutrient fertilizer (Basacote® Mini 6 M 16-8-12(+2)).

The research was conducted from July 2021 to January 2022, at the Protected Crop and Biological Control Station "Professor Dr. Mário César Lopes" of the Agricultural Sciences Center of the Western Paraná State University (UNIOESTE). The station is located in Marechal Cândido Rondon, in the Western Region of the Paraná state, with latitude of 24° 33' 24" S, longitude of 54° 05' 67" W and altitude of 420m. According to the Koppen climate classification, the regional climate is a Cfa type, mesothermal, humid subtropical with evenly distributed rainfall throughout the year (1600 to 1800 mm) and hot summers. The average annual air temperature varies between 22 and 23 °C and the relative humidity varies between 70% and 75% (NITSCHE *et al.*, 2019).

Before the treatments were applied, the seedlings were acclimatized for approximately 60 days in a protected shade house, with low-density, anti-UV polyethylene film 150 microns thick, equivalent to 80% transmissivity. During that period, seedlings were kept under sprinkler irrigation with an average of 4.0 mm of water (watering frequency three times a day, at 7:00 am, 3:00 pm, and 5:00 pm). Fertilization was carried out with a nutrient solution based on NPK fertilizer in the 10-15-15 formulation as required.

Prior to the methyl jasmonate (MeJA) applications, seedlings showed an average of 32.1 cm height and a stem diameter of 4.1 mm. The experimental design was a randomized blocks, consisting of four treatments and five blocks with five plants each. The treatments comprised increasing doses of methyl jasmonate and were diluted in water in the proportion of each dose. A non-ionic surfactant were added to each application to improve the distribution and foliar absorption of the product, as well as to reduce the surface tension of the droplets and their contact angle with the leaf surface. The product used was Agral-Syngenta®, according to the manufacturer's guidelines.

The solution was applied to the seedlings once a week for eight weeks at the following doses: 0 (control), 50, 100, and 150 µmol L⁻¹, using foliar sprays with a manual backpack sprayer. Throughout the treatment period, irrigation occurred three times a day and the applications were carried out in the evening, after the last irrigation.

At the end of the treatments, the seedlings remained in the trays for another two weeks for the effect of the final application. Afterward, they were transplanted into three-liter pots filled with local soil, classified as Eutroferic Red Latosol with a very clayey texture (SANTOS *et al.*, 2013) and placed on benches out of reach of the irrigation system. At the time of installation, the weight of the pot with dry soil+plant and the pot with wet soil+plant were determined. Moist soil was obtained by applying 1.5 L of water. The weight of the water was obtained by subtracting the weight of wet soil+plant from the weight of dry soil+plant. The seedlings remained acclimatized in the pots in a shade house for 16 days, a period in which they were irrigated with 0.5 L of water every two days.

After the acclimatization period, the suspension of irrigation started. On the first day, the seedlings were irrigated again with 1.5 L of water, after which they remained in the pots for 12 days without irrigation. During that period, the weight of the pots was assessed daily in the evening on a digital scale with a capacity of 30 kg (± 0.005 kg) to determine water loss. Evapotranspiration (ET) was determined by the difference between the weight of the pots on the day and their weight on the previous day, through the calculation of the amount of water lost in liters, according to the methodology adapted from Abreu *et al.* (2015).

During the water deficit period, the seedlings were assessed for shoot height (H) and stem diameter (SD). At the end of the experiment, leaf area (LA), leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM), total dry mass (TDM), and relative water content (RWC) were evaluated.

Shoot height was measured in centimeters (cm) using a graduated ruler, from the base of the shoot to the apical bud. Stem diameter was expressed in millimeters (mm) and measured at the limit between the shoot and the substrate using a digital caliper. Leaf area (cm^2) was assessed through the direct destructive method, using a portable meter model LI-3000A (Li-Cor area meter, USA). The seedlings were divided into leaves, stems and roots to determine their dry mass (g^{-1}) and dried in an air-circulation oven at 65°C until they reached a constant mass. From the data on height (H), stem diameter (SD), and dry mass, the robustness index (RI), shoot/root ratio (S/R) and the Dickson quality index (DQI) were determined.

The robustness index (RI) was obtained from the ratio between H and SD. The DQI was determined based on H, SD, TDM, shoot dry mass (SHDM=LDM+SDM), and RDM, according to the equation presented by Dickson *et al.* (1960). From the TDM and LA values, the dry biomass allocations (in g g^{-1}) were calculated for the leaves (LBA = LDM/TDM), the stem (SBA = SDM/TDM), and the roots (RBA = RDM/TDM), and the shoot/root ratio (S/R = SHDM/RDM).

To determine the RWC, 30 leaf disks of approximately 10 mm in diameter were removed from each plant and the fresh mass of the disks was determined on an analytical balance. Immediately after the procedure, the disks were transferred to a petri dish containing 35 mL of distilled water and kept at 25°C for six hours. After that period, the disks were removed from the petri dish and placed on paper towels to remove excess moisture. Then, the mass of the turgid disks was determined. Afterward, the disks were transferred to the oven at 65°C for 72 hours to determine the dry mass of the disks (DM). The RWC was calculated according to the equation described by Barrs and Weatherley (1962), whereas the data was expressed in percentage (%).

Stomatal conductance (Sc) was measured using a digital porometer (SC-1 Leaf Porometer, Decagon®). The measurements were conducted at the beginning of the experiment (0 days) and at 12 days of water deficit. Measurements were carried out on the abaxial side of expanded leaves in the morning, starting at 8:00 am, and the results were expressed in $\text{mmol m}^{-2} \text{s}^{-1}$.

During the acclimatization period and water deficit of the seedlings, the relative humidity values as well as air temperature were recorded daily. This procedure was carried out using a thermo-hygrometer datalogger with a temperature and relative humidity sensor (KlimaLogg Smart model), as presented in Figure 1.

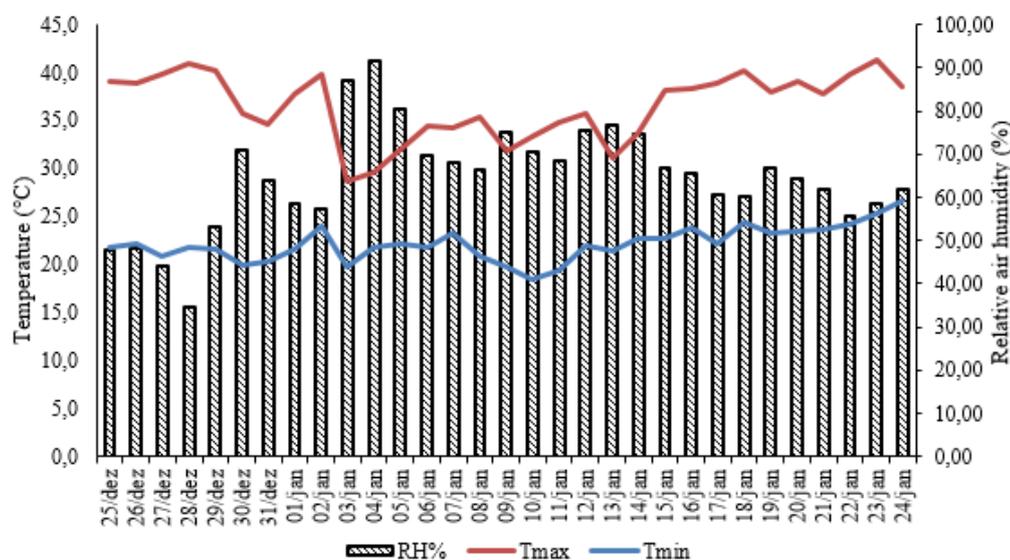


Figure 1. Maximum (Tmax) and minimum (Tmin) air temperature values and average relative humidity (RH%) were observed in a shade house during the water deficit experiment. Marechal Cândido Rondon-PR, 2021/22.

Figura 1. Valores de temperatura do ar máxima (Tmax) e mínima (Tmin) e umidade relativa do ar média (RH%) observados em ambiente de cultivo protegido durante a realização do experimento com suspensão hídrica. Marechal Cândido Rondon-PR, 2021/22.

The data were analyzed in a split-plot design to evaluate the parameters: H, SD, Sc, and ET. The plots correspond to the four doses of methyl jasmonate and the split-plots include the evaluation periods. ET was assessed at four times, H and SD were assessed at three times and Sc was measured at two times.

Data were analyzed for normality of residual distribution using the Lilliefors test and for normality of variance using the Bartlett test. Then, data were submitted for analysis of variance using SISVAR 5.6 statistical software (FERREIRA, 2014). Results were obtained by regression analysis.

RESULTS

Table 1 shows the averages of the height (H) and stem diameter (SD) of *E. uniflora* seedlings hardened with doses of methyl jasmonate (MeJA) over 12 days of suspension of irrigation. The doses of MeJA did not cause any significant changes in the growth (H and SD) of the seedlings compared to the control treatment.

Table 1. Mean values of shoot height (H) and stem diameter (SD) of *E. uniflora* seedlings exposed to doses of methyl jasmonate, after 0, 6, and 12 days of water deficit.

Tabela 1. Valores médios de altura de parte aérea (H) e diâmetro do coleto (DC) de mudas de *E. uniflora* submetidas a doses de metil jasmonato, após 0, 6 e 12 dias de suspensão hídrica.

Doses of methyl jasmonate ($\mu\text{mol L}^{-1}$)	Shoot height – H (cm) ^{ns}			Doses of methyl jasmonate ($\mu\text{mol L}^{-1}$)	Stem diameter - SD (mm) ^{ns}		
	0 days	6 days	12 days		0 days	6 days	12 days
0	37.9	37.9	37.8	0	4.33	4.54	3.95
50	36.9	37.6	37.9	50	4.62	4.46	4.17
100	39.5	39.7	39.7	100	4.85	4.96	4.63
150	38.0	37.9	38.1	150	4.32	4.35	3.93

^{ns} not significant in relation to doses of jasmonate and days of water deficit

In general, the measurements of height and stem diameter are considered adequate indicators of seedling quality. However, these parameters must be combined with other attributes to predict the growth pattern of the seedling, such as the seedling quality indices, robustness index, and Dickson quality index, all of which showed a quadratic trend, as well as the shoot/root ratio (Figure 2).

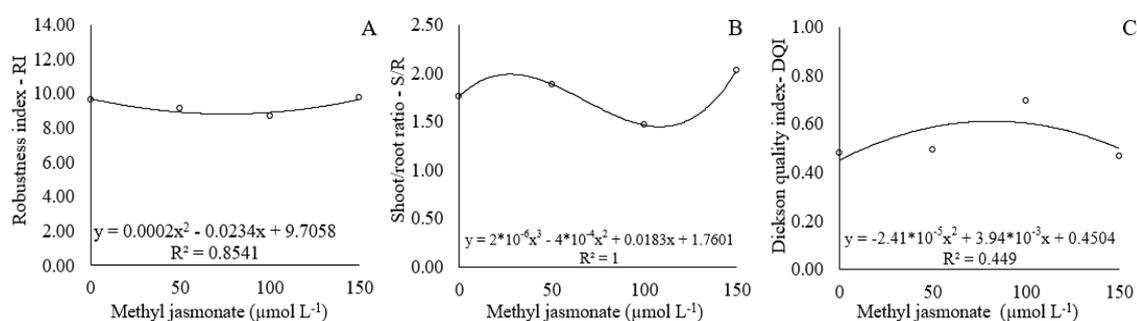


Figure 2. Quality indices of *E. uniflora* seedlings hardened with different doses of methyl jasmonate after 12 days of water deficit.

Figura 2. Índices de qualidade de mudas de *E. uniflora* rustificadas com diferentes doses de metil jasmonato, após 12 dias de suspensão hídrica.

The robustness index (RI), Figure 2A, showed a decrease of 5.2% and 9.8% for the doses of 50 and 100 $\mu\text{mol L}^{-1}$, respectively, compared to the control (0 $\mu\text{mol L}^{-1}$). Above 150 $\mu\text{mol L}^{-1}$, the average RI value tended to increase. Similarly, there was a decrease in the shoot/root ratio (S/R), Figure 2B, between the jasmonate doses of 50 and 100 $\mu\text{mol L}^{-1}$, and an increase from 150 $\mu\text{mol L}^{-1}$ onwards. The calculation of the Dickson quality index (DQI), Figure 2C, resulted in a 33% increase observed at the 100 $\mu\text{mol L}^{-1}$ dose compared to the 0 $\mu\text{mol L}^{-1}$ dose, with an average of 0.603.

The aforementioned results for the quality indices of *E. uniflora* seedlings showed that, up to a dose of 100 $\mu\text{mol L}^{-1}$ of MeJA, the seedlings developed better. From the 150 $\mu\text{mol L}^{-1}$ dose onwards, the effect of the chemical stimulant begins to hinder the seedling's quality due to the stress caused by the hormonal increase in the plant.

The leaf area (LA) results indicated a quadratic increase up to the dose of 100 $\mu\text{mol L}^{-1}$ (Figure 3A). The same trend was observed for the relative water content (RWC) values (Figure 3B), but to a lesser extent, since the average values were close to 17%, regardless of the dose applied.

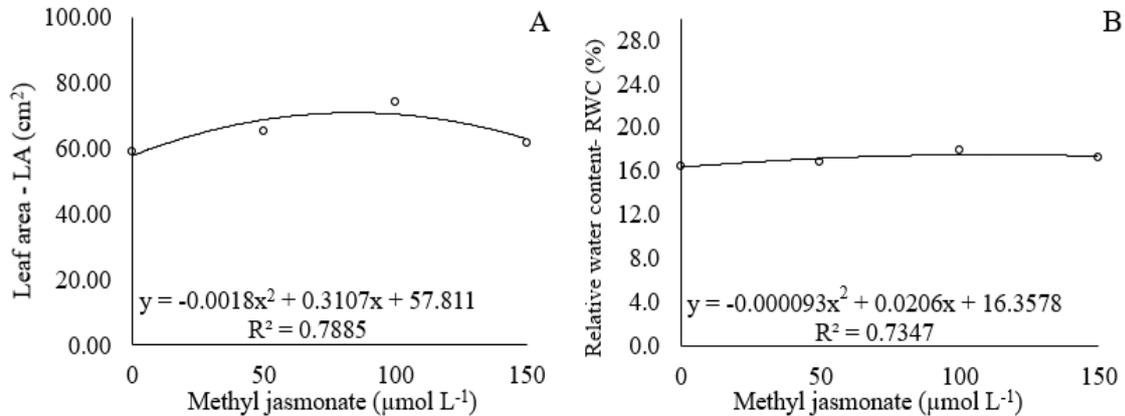


Figure 3. Leaf area - LA (A) and relative water content - RWC (B) of *E. uniflora* seedlings hardened with different doses of methyl jasmonate after 12 days of water deficit.

Figura 3. Área foliar – AF (A) e conteúdo relativo de água – CRA (B) de mudas de *E. uniflora* rustificadas com diferentes doses de metil jasmonato, após 12 dias de suspensão hídrica.

Figure 4A depicts a higher accumulation of total dry mass (TDM) in seedling hardened with doses of up to 100 $\mu\text{mol L}^{-1}$ of MeJA. Figure 4B displays dry mass allocation values for leaf (LBA), stem (SBA), and root (RBA).

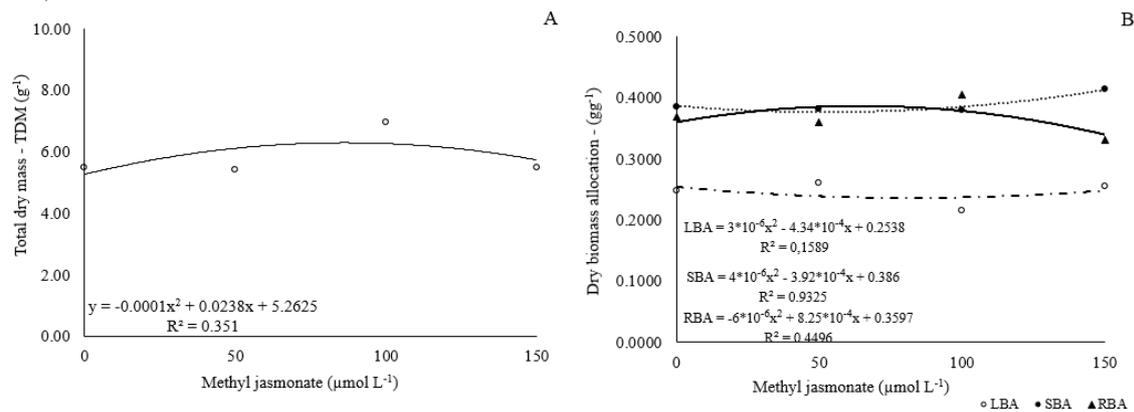


Figure 4. Total dry mass - TDM (A) and dry biomass allocation to leaves (LBA), stem (SBA), and root (RBA) (B) of *E. uniflora* seedlings hardened with different doses of methyl jasmonate after 12 days of water deficit.

Figura 4. Massa seca total – MST (A) e alocação de biomassa seca para folhas (ABF), caule (ABC) e raiz (ABR) (B) de mudas de *E. uniflora* rustificadas com diferentes doses de metil jasmonato, após 12 dias de suspensão hídrica.

The results observed for TDM (Figure 4A), with the highest dry mass accumulations caused by the doses of 50 and 100 $\mu\text{mol L}^{-1}$, corroborated the data observed for LA. The leaf area reflects the energy conversion resulting from photosynthesis to the production of photoassimilates for the plant. Therefore, it can be concluded that larger LA results in higher dry mass production. Concerning dry biomass allocation (Figure 4B), there was an increase in values between the doses of 50 and 100 $\mu\text{mol L}^{-1}$ for RBA and a decrease in LBA and SBA values in the same dose interval.

The values for stomatal conductance - Sc - (Figure 5A) and evapotranspiration - ET - (Figure 5B) were influenced by the water deficit imposed on the seedlings since there was a decrease of over 50% between the initial and final values in all the treatments evaluated.

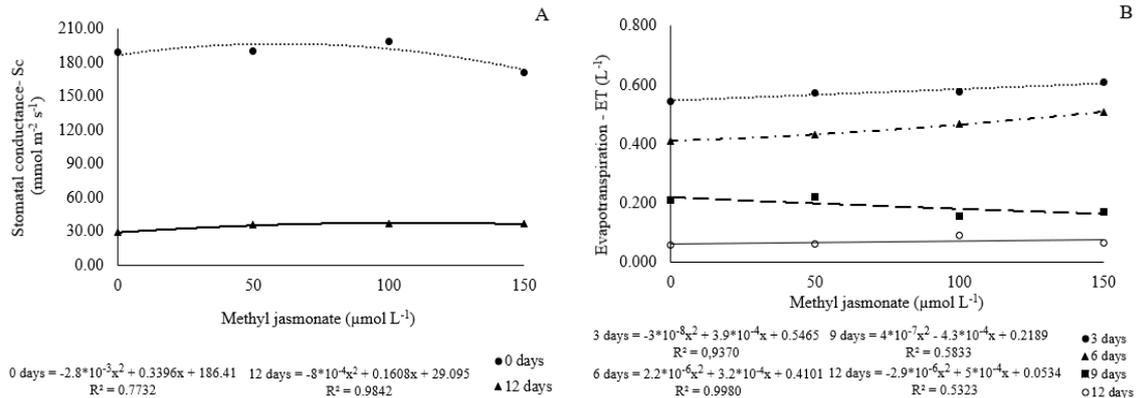


Figure 5. Stomatal conductance - Sc (A) and evapotranspiration - ET (B) of *E. uniflora* seedlings hardened with doses of methyl jasmonate after 12 days of water deficit.

Figura 5. Condutância estomática - Cs (A) e evapotranspiração - ET (B) de plantas de *E. uniflora* rustificadas com doses de metil jasmonato, após 12 dias de suspensão hídrica.

For the ET values (Figure 5B), there was a significant effect only among the days of water deficit, with no disparities found between the doses of MeJA applied. Thus, it can be inferred that the environmental conditions mainly influenced this parameter. The climatic data of the environment observed in Figure 1 support the results obtained for ET. When irrigation was suspended, the ambient temperatures were high (with a maximum temperature of 30 to 40 °C) and the relative humidity varied between 60% and 70%. As a result, there was a higher vapor pressure deficit and, consequently, higher evapotranspiration.

DISCUSSION

The quality of the seedlings can be assessed from a morphological and physiological perspective. Morphological quality is based on physical attributes such as height and diameter, while physiological quality is based on the seedling's internal functions. However, these attributes complement each other, as the morphological characteristics of the plant can be considered a physical manifestation of its physiological activities (HAASE, 2008).

Morphological attributes are considered a reliable measure of seedling quality since they retain their characteristics in the identity of the seedling for prolonged periods after planting and the start of growth in the field. However, such attributes only measure the overall size of the seedling, growth potential, and shoot/root balance, so they must be compared with the physiological quality. Therefore, seedlings must be produced to be physiologically ready for planting in the field environment (GROSSNICKLE; MACDONALD, 2018).

Rorato *et al.* (2018) also explained that for slow-growing forest species, such as *E. uniflora*, morphological attributes express a "past" situation since the seedlings take longer to manifest changes. On the other hand, physiological attributes show the current state of the seedling's metabolism.

The robustness index is an example of a physiological attribute that represents the relationship between the height and diameter of the seedling (plant), with a lower ratio indicating balanced growth (HAASE, 2008). Although there were no differences in the height and diameter measurements between the MeJA doses, the quadratic effect observed for RI (Figure 2A) demonstrated that the doses were capable of altering the quality of the seedlings. This index predicts the quality and survival rates of seedlings in the field when environmental conditions are considered (SILVA *et al.*, 2007; HEBERLE *et al.*, 2018). The doses of 50 and 100 μmol L⁻¹ presented the most promising results. On the other hand, higher doses do not promote this balance due to hormonal stress on the plant.

Heberle *et al.* (2018) found reductions in the shoot height/stem diameter ratio in seedlings of *Handroanthus impetiginosus* (Mart. ex DC.) Mattos and *Patagonula americana* L. when doses of jasmonic acid were applied. Cadarin *et al.* (2015) observed lower RI values in *Cordia trichotoma* (Vell.) seedlings hardened with methyl jasmonate and stem bending in comparison to unhardened ones.

The S/R ratio measures the balance between the plant's transpiration area and water absorption area (HAASE, 2008). When plants are subjected to a stress situation that affects such ratio, typically the roots grow at the detriment of the shoot, enabling the roots to penetrate deeper soil layers for greater water absorption

(MAZZUCHELLI *et al.*, 2014). The authors observed a quadratic increase in the root/shoot ratio in *Eucalyptus urophylla* x *Eucalyptus grandis* seedlings hardened with different concentrations of salicylic acid and subjected to water restriction. For the examined *E. uniflora* seedlings, the doses of 50 and 100 $\mu\text{mol L}^{-1}$ of jasmonate resulted in a decreased shoot/root ratio, indicating that doses of methyl jasmonate within this range are recommended.

The DQI is considered a good indicator of seedling quality because its calculation takes into account the balance between robustness and dry weight distribution. In this case, the higher the Dickson quality index, the higher the quality of the seedlings (HEBERLE *et al.*, 2018). The results observed for *E. uniflora* corroborated this finding, since the quadratic increase trend observed up to a dose of 100 $\mu\text{mol L}^{-1}$ was consistent with the results found for RI and S/R.

The results for leaf area (Figure 3A) suggest that the use of MeJA, at an appropriate dosage, can induce greater leaf production under favorable growth conditions and increase its activity time before senescence under water deficit conditions. Therefore, the *E. uniflora* seedlings hardened with doses of 50 and 100 $\mu\text{mol L}^{-1}$ of MeJA kept their leaves attached longer than the unhardened ones. The unhardened seedlings displayed higher senescence and leaf abscission, which explains their higher LA value compared to the control.

Leaf area is related to the plant's production of photoassimilates and depends on the size and quantity of leaves, as well as their activity time. Initially, this parameter is expected to increase, followed by a decrease due to senescence (TAIZ *et al.*, 2017). Therefore, the application of treatments that increase LA for an extended period is recommended even when the plants are subjected to stress conditions. Such treatments improve the chances of survival and development by providing more dry mass production.

The RWC estimates the current water content in the leaf tissue relative to its maximum water content at full turgor. This content varies from 98% in transpiring and fully turgid leaves to around 30-40% in leaves with severe water deficit, depending on the species' characteristics (BARRS; WEATHERLEY, 1962). The RWC of the *E. uniflora* plants seen in Figure 3B represents a low level of cell turgor. However, in general, RWC is a good indicator of water status, considering that 100% indicates the absence of water deficiency (PEIXOTO *et al.*, 2011).

The RWC values seen in Figure 3B resulted in low water content values in the plant (approximately 17%), regardless of the dose of MeJA applied. This occurred because during the period in which the seedlings were subjected to water restriction (January 10 to 22 - Figure 1) the maximum air temperature was above 30 °C, the minimum air temperature was over 20 °C and the relative humidity ranged between 55% and 75%. Such conditions indicate a higher atmospheric evaporative demand, resulting in higher water loss by plants and higher evapotranspiration values. However, as the water deficit intensifies, plants employ survival strategies. Among these is stomatal closure at the most critical times of the day, which leads to a reduction in leaf transpiration and stomatal conductance, but also decreases photosynthetic efficiency by limiting the entry of carbon dioxide into the leaf and reducing gas exchange (MEDLYN *et al.*, 2017).

When this atmospheric condition is combined with an excessive reduction in the soil water volume, the plant fails to meet the atmospheric demand, even though it has defense mechanisms against the stress. Consequently, there is a loss of cell turgor due to water loss and the plant enters a state of water deficit. Then, various metabolic processes are triggered, including cell death in extreme cases (TAIZ *et al.*, 2017).

According to Dranski *et al.* (2018), acknowledging the changes in carbon absorption and allocation among the plant's components (i.e. leaves, stem, and roots) allows the comprehension of its growth plasticity. Therefore, dry mass allocation can be considered an important parameter for evaluating plant responses. The dry mass allocation results for leaves, stem and root indicated that, under water deficit conditions, *E. uniflora* seedlings directed more energy toward root development as a survival strategy, since those organs are inclined to develop toward more humid soil layers, resulting in less shoot development. These results corroborated those depicted in Figure 2B for the S/R index, in which there was a lower shoot/root ratio after 12 days of water deficit. The findings also implied that the plant reallocated more resources to root development as a mechanism for tolerating water deficit.

Greater root growth in relation to the shoot is a common adaptive feature of plants subjected to water stress, which allows them to obtain water from other soil layers. This response may be associated with the mechanism of water stress tolerance since plants tend to allocate more dry mass to the root system under low soil water availability, enabling greater root growth. When the water potential is suddenly reduced in the roots, the osmotic adjustment occurs rapidly to allow partial recovery of turgor. Such adjustments enable the roots to resume growth even at low water potential. In contrast, under similar decreases in water potential, osmotic adjustment in the shoot occurs slowly, resulting in its growth inhibition (LIMA *et al.*, 2014).

The increase in dry mass for other plant organs is dependent on the production of assimilates in the leaves (BELO *et al.*, 2015). Consequently, treatments that lead to a greater increase in leaf area, as observed for the *E. uniflora* seedlings treated with 50 and 100 $\mu\text{mol L}^{-1}$ of MeJA, result in greater dry mass allocation to other regions of the plant, especially in water deficiency conditions. In this context, plants have survival mechanisms that can transform the leaves, considered sink under normal conditions, into an energy source to maintain vital activities.

The results for *E. uniflora* seedlings showed that stomatal conductance and, consequently, evapotranspiration were influenced by water deficit. Taiz *et al.* (2017) explained that stomatal regulation occurs according to atmospheric and water changes. Stomatal conductance indices are often used as indicators of water deficit. Stomatal conductance is related to the rate of water loss through the stomata and represents the plant's response to environmental factors, directly affecting the rate of photosynthesis and transpiration. Atmospheric weather conditions, especially air temperature and humidity, directly influence the pattern of stomatal conductance (CADORIN *et al.*, 2016). These authors observed a quadratic trend in the stomatal conductance of *Cordia trichotoma* seedlings over 12 days of water restriction, which they attributed to a decrease in temperature and variations in air humidity over the days following the imposition of water deficit.

Water deficit affects gas exchange and the production of photoassimilates (TAIZ *et al.*, 2017). However, plants have tolerance mechanisms, such as stomatal control, which can allow them to survive to some extent under unfavorable water availability conditions. Thus, it was observed that, under water deficit conditions, the application of methyl jasmonate to *E. uniflora* seedlings promoted morphophysiological changes and induced defense strategies to minimize the effects of stress, such as a reduction in stomatal conductance and greater partitioning of dry mass to the roots to acclimatize to the environment.

CONCLUSIONS

- Methyl jasmonate can be used to harden *Eugenia uniflora* seedlings. However, the recommended dose must be observed, as excessive amounts can adversely affect the plant's performance.
- Doses of methyl jasmonate between 50 and 100 $\mu\text{mol L}^{-1}$ resulted in the production of *E. uniflora* seedlings with higher tolerance to 12 days of water deficit conditions.

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