

## How does water stress affect *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson throughout the initial growth phase?

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### Abstract

*Corymbia citriodora* has the potential to be used in the expansion of the forestry sector in Brazil, as it presents greater tolerance to abiotic stresses than other commonly cultivated species. The plants are exposed to several stress events throughout their cycle that can compromise their growth and development. This study aimed to evaluate physiological, biochemical, and growth characteristics in *C. citriodora* plants grown under different irrigation regimes at different ages. Plants of *C. citriodora* grown in pots were submitted to five irrigation regimes (20, 35, 50, 65, and 80% of the maximum soil water retention capacity) for 105 days in a greenhouse. Plant growth, gas exchange, leaf water potential, chlorophyll, carotenoids, and proline contents, and lipid peroxidation by MDA content were evaluated at the end of this period. More restrictive irrigation regimes reduced seedling growth. Water availability affected physiological, biochemical, and morphological parameters. Water stress associated with plant growth increased stress levels.

**Keywords:** Gas exchange; eucalyptus; forest seedlings; morphological adaptations.

**Abbreviations:** ROS\_reactive oxygen species; A\_assimilation rate; *Ci*\_intracellular CO<sub>2</sub>; *E*\_Transpiration; *gs*\_stomatal conductance; IR\_irrigation regimes; MDA\_malondialdehyde; DSL\_diameter at soil level; LDM\_leaf dry mass; S+BDM\_stem + branch dry mass.

### Introduction

Forests planted with eucalyptus have significant importance in the economy of several countries, with the possibility of diversified uses, such as the production of energy, cellulose, wood for sawmills, and fiberboard. The rapid growth of eucalyptus stands out in comparison to other forest species. The management of these areas seeks to minimize environmental restrictions on plant growth, such as adverse climate factors that cannot be controlled, currently constituting a major challenge for eucalyptus production in the face of climate change (Reis et al., 2021).

Eucalyptus cultivation in Brazil has expanded to areas with limitations to forest growth, such as low annual rainfall, to meet the increased demand for wood-derived products (Ibá, 2021). The low soil water availability affects water and nutrient absorption, compromising forest productivity (Stape et al., 2004). In this sense, forest improvement programs must seek the development of genotypes adapted to the new areas of cultivation to obtain economically viable productivity (Hubbard et al., 2020).

*C. citriodora*, synonym *Eucalyptus citriodora* (Hook.), has aroused commercial interest, being cultivated by small-and medium-sized producers in Brazil (Souza et al., 2020). It features wood that is durable and crack resistant. Large extensions of areas cultivated with *C. citriodora* are not common, and the species has a longer cycle compared to *Eucalyptus* species, which may be one of the factors that

contribute to the small number of studies on the species (Cunha et al., 2019). In Brazil, the cultivation of the genus *Corymbia* is mainly intended for the production of woody biomass for energy and wood for fence posts (Brondani et al., 2018), but it can be used for the production of cellulose, essential oil, and carbon sequestration, in addition to being tolerant to water deficit, pests, and diseases (Healey et al., 2021).

Planted forests have a long cycle and are exposed to adverse factors. The constancy and extent of the water deficit are expected to increase shortly, affecting forest productivity (Singh et al., 2020). Thus, understanding the strategies that plants use to minimize the impacts of abiotic stresses at a physiological and biochemical level is important for understanding the behavior of the species in adverse environments, such as water scarcity and increased temperature. Plants under water deficit, for example, use mechanisms to alleviate the impact caused by stress, eliminating reactive oxygen species (ROS), and minimizing the degradative effect of oxidation (Pan et al., 2018).

Water stress affects plant species differently, reducing water potential and gas exchange and increasing ROS content (Osakabe et al., 2014). However, few studies that evaluate the effects of water stress on *C. citriodora* on a time scale can be found in the literature. We hypothesize that the time of exposure to water stress of newly planted seedlings of *C.*

*citriodora* is crucial for the plants to activate adaptation mechanisms. This study aimed to evaluate physiological, biochemical, and growth characteristics in *C. citriodora* plants grown under different irrigation regimes at different ages.

## Results

### Physiological characteristics

The gas exchange showed variations in response to irrigation regimes (Figure 2), whereas increased by more water availability. The net assimilation rate ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) had a quadratic adjustment, with higher values for seedlings at 35 days, followed by the evaluation at 105 days, with intermediate values for the irrigation regimes IR2, IR3, IR4, and IR5. The intracellular  $\text{CO}_2$  ( $C_i$ ,  $\mu\text{mol mol}^{-2}$ ) had a linear adjustment for water regimes throughout the evaluations, showing higher values as the water availability increased. Moreover,  $C_i$  values increased with the advancing age of seedlings, different from what was observed for  $A$ .

Transpiration ( $E$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) presented a linear adjustment in the three evaluation periods for the irrigation regimes, increasing with the higher water availability and decreasing with the seedling age. However, transpiration was similar in the evaluations at 70 and 105 days in more restrictive irrigation regimes (IR1 and IR2). The stomatal conductance ( $g_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) also showed a linear adjustment, increasing with higher water availability. The highest values were obtained at 35 days, with similar values in the other two evaluations and a slight superiority for the evaluation carried out at 105 days relative to 70 days.

Leaf water potential (Figure 2) in the predawn showed a quadratic adjustment as a function of irrigation regimes in the three evaluation periods. The values showed a trend to decrease with the age of evaluation and increase with higher water availability, but with stabilization in the three irrigation regimes with higher water availability (IR3, IR4, and IR5). Water potential in IR1 reached values close to  $-1.2$  and  $-1.4$  MPa, while the other irrigation regimes showed values higher than  $-0.4$  MPa, showing that IR1 provided water limitation to the seedlings. Leaf water potential in the evaluation at noon reached more negative values, but with a behavior similar to that observed in the predawn and higher differentiation between evaluation times. Leaf water potential reduced with advancing plant age, with a sharper decrease at 105 days than those observed at 35 and 70 days.

Biomass accumulation decreased leaf water potential.

Chlorophyll and carotenoid content decreased as water availability and seedling age increased (Figure 3). Variations in chlorophyll a, chlorophyll b, and chlorophyll a+b contents showed linear adjustments, depending on the water regimes at 35 and 70 days, and a quadratic adjustment at 105 days.

Carotenoids had a linear adjustment at 35 days, a quadratic adjustment at 70 days, and a linear adjustment at 105 days, with a trend to stabilize from IR3 (50% of the field capacity), mainly in the evaluation carried out at 70 days.

### Biochemical characteristics

Lipid peroxidation, expressed as malondialdehyde (MDA) concentration, showed linear adjustments as a function of irrigation regimes at 35 and 70 days. A trend towards stabilization was observed at 105 days for IR3, IR4, and IR5. IR1 (the most restrictive treatment) provided a 33.14% increase in MDA content at 35 days. MDA concentration increased with the advancing age of the plants and reduced

as water availability increased, with a trend to stabilize from IR3 (50% of field capacity) (Figure 4).

Proline content increased under irrigation regimes with lower water availability, regardless of the evaluated period. Proline content showed a linear adjustment at 35 and 70 days and quadratic adjustment at 105 days after the establishment of water regimes. IR1 provided a 45.64% increase in the proline content at 35 days.

### Growth characteristics

Growth variables increased with seedling age, as expected, and water availability, with quadratic adjustments for height, diameter at soil level (DSL), leaf dry mass (LDM), stem + branch dry mass (S+BDM), number of leaves, and leaf area, while root dry mass presented a linear adjustment (Figures 5 and 6). Height, DSL, and the number of leaves showed a trend to stabilize between the irrigation regimes IR3 and IR4, with a reduction in IR5, suggesting a water excess in this regime with 80% of the field capacity.

This is more evident at the lowest age (35 days) when the plants were smaller and with less water demand. Also, the characteristics showed a higher increase with an increase in plant age as a function of the irrigation regimes. Leaf area at the three ages, LDM at 35 days, and S+BDM at 35 and 70 days showed a stabilization between the irrigation regimes IR4 and IR5. Root dry mass had a linear adjustment, with a small increase as a function of the increase in water availability at 35 days and higher responses at 75 days and, mainly, 105 days.

## Discussion

### Physiological characteristics

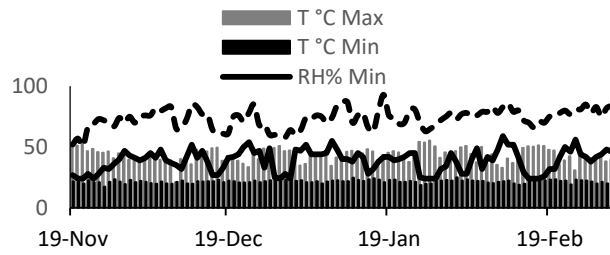
Water stress alters physiological and biochemical mechanisms in plants (Souden et al., 2020), and these changes vary with age. Thus, the same water regime can provide different changes depending on the stage of development and water demand of the plants. The rates of net assimilation ( $A$ ,  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ), transpiration ( $E$ ,  $\text{mmol m}^{-2} \text{ s}^{-1}$ ), and stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{ s}^{-1}$ ) were higher at 35 days, decreasing at the ages of 70 and 105 days, as seedling growth led to a higher demand for water so that the plants could maintain their physiological and biochemical mechanisms. N3ia Junior et al. (2019) observed that plants can reduce leaf expansion to mitigate the impact on photosynthesis as a strategy under water deficit.

Stomatal closure affects gas exchange, reducing transpiration and biomass accumulation. These effects are dependent on water availability (Utkhao and Yingjajaval, 2015). The assimilation rate ( $A$ ) and stomatal conductance ( $g_s$ ) in *Eucalyptus citriodora* seedlings (= *C. citriodora*) were negatively affected by water stress (Brilli et al., 2013), corroborating with the results obtained here under irrigation regimes with less water availability, mainly in IR1. Stomatal closure under water deficit is a strategy adopted by plants to reduce water losses through transpiration, as reported by Urban et al. (2017). The variation of leaf water potential between irrigation regimes was more drastic in the regime of lower water availability (IR1), which provided more negative potentials in the predawn period, with small differences in the other irrigation regimes. It also occurred in the evaluations performed at noon, but changes were higher between irrigation regimes, accentuating with the advancing age of seedlings. Furthermore, water demand increases with

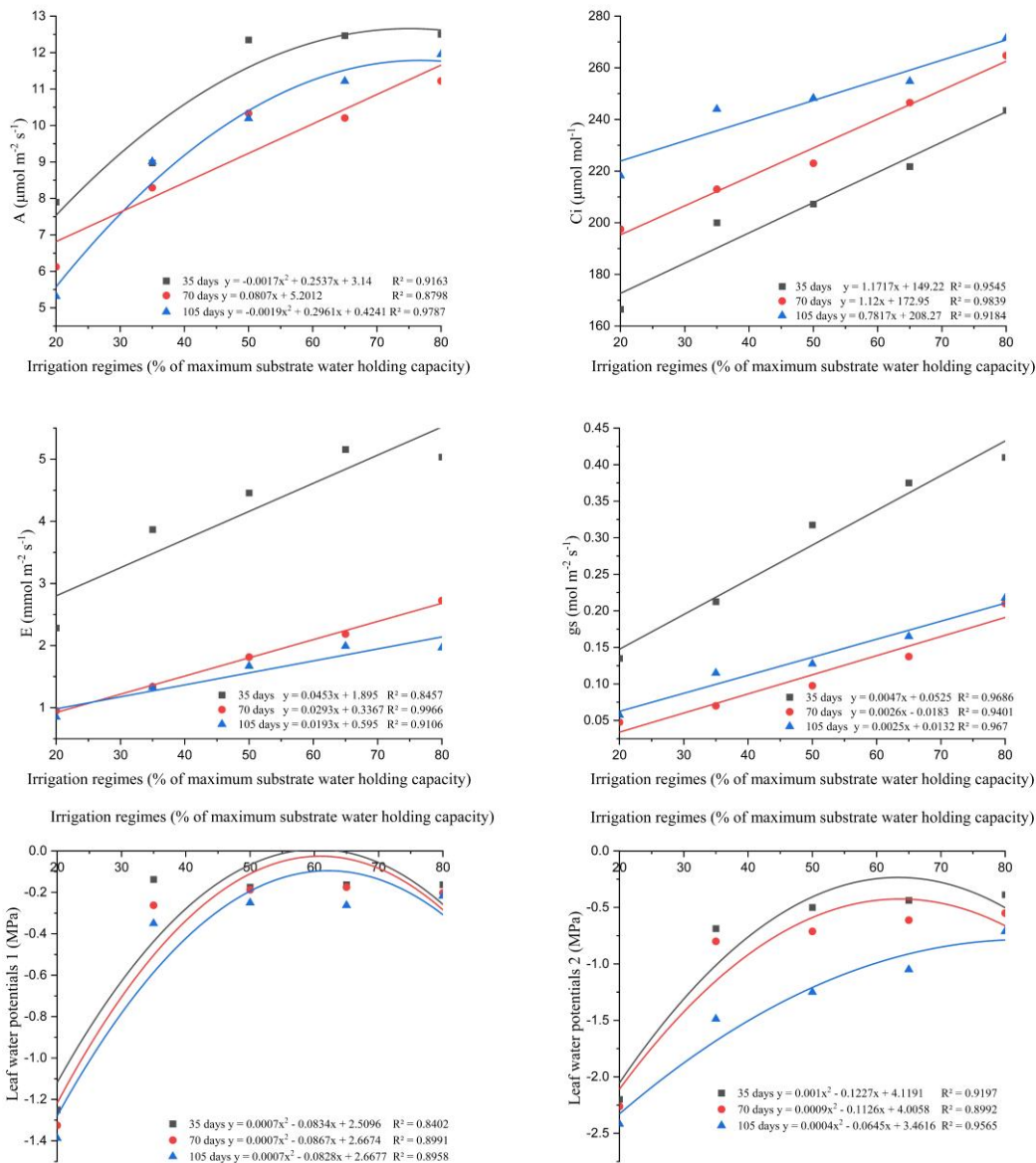
**Table 1.** Chemical characterization of the soil used in the experiment.

| pH  | OM | P resin | K   | Ca | Mg | H+Al | SB   | T    | V  |
|-----|----|---------|-----|----|----|------|------|------|----|
| 5.6 | 4  | 4       | 1.8 | 9  | 3  | 12   | 13.8 | 25.8 | 53 |

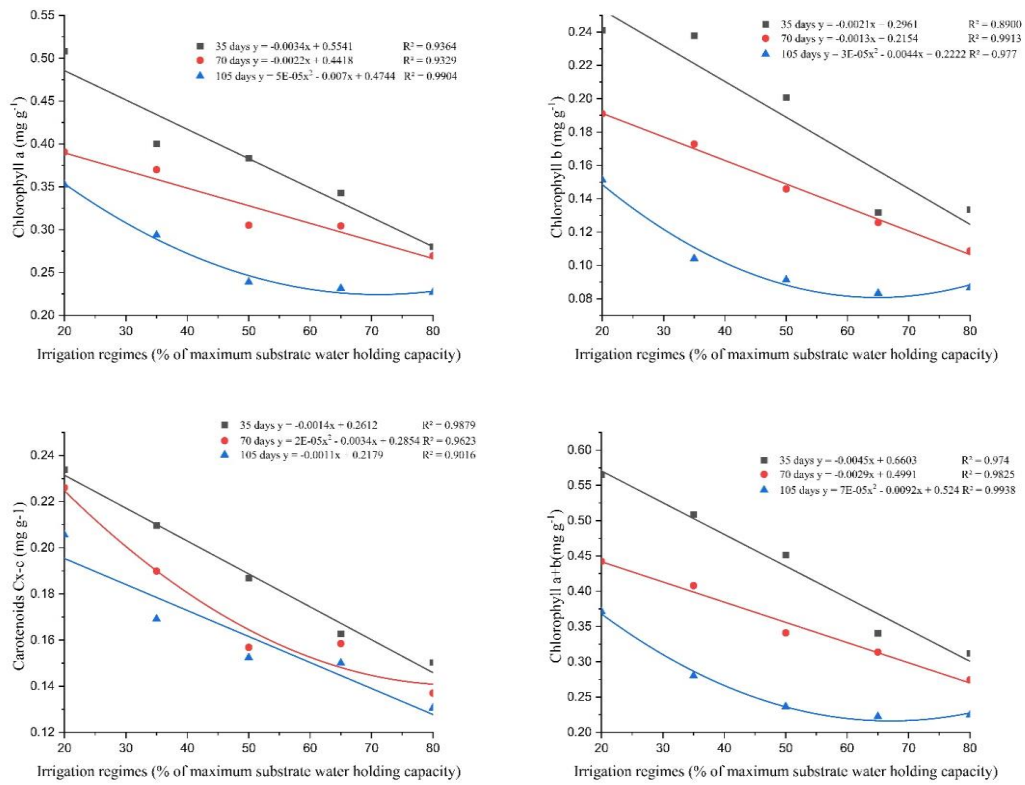
OM – organic matter; SB – sum of bases; T – cation exchange capacity, V – base saturation



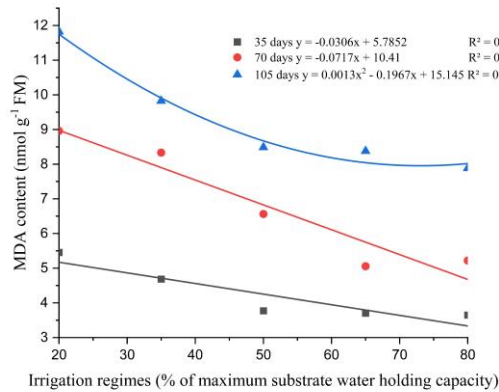
**Figure 1.** Daily maximum ( $T\text{ }^{\circ}\text{C Max}$ ) and minimum ( $T\text{ }^{\circ}\text{C Min}$ ) temperatures and maximum ( $\text{RH\% Max}$ ) and minimum ( $\text{RH\% Min}$ ) relative humidity obtained inside the greenhouse during the experimental period (November 2019 to February 2020).



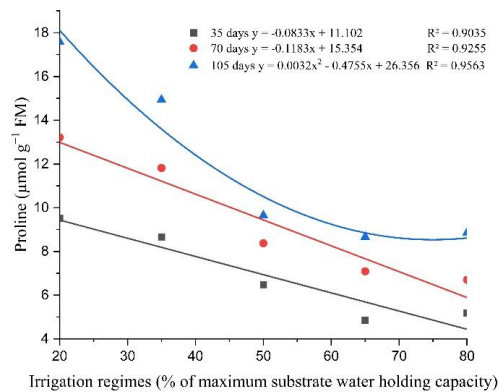
**Figure 2.** Net assimilation rate ( $A$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), transpiration ( $E$ ,  $\text{mmol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mol m}^{-2} \text{s}^{-1}$ ), intracellular  $\text{CO}_2$  ( $C_i$ ,  $\mu\text{mol mol}^{-1}$ ), leaf water potential 1 (in the predawn period,  $-\text{MPa}$ ), and leaf water potential 2 (at noon,  $-\text{MPa}$ ) in *C. citriodora* plants submitted to five irrigation regimes (IR1, IR2, IR3, IR4, and IR5, that is, 20, 35, 50, 65, and 80% of the maximum substrate water retention capacity, respectively), evaluated at 35, 70, and 105 days after the establishment of water regimes.



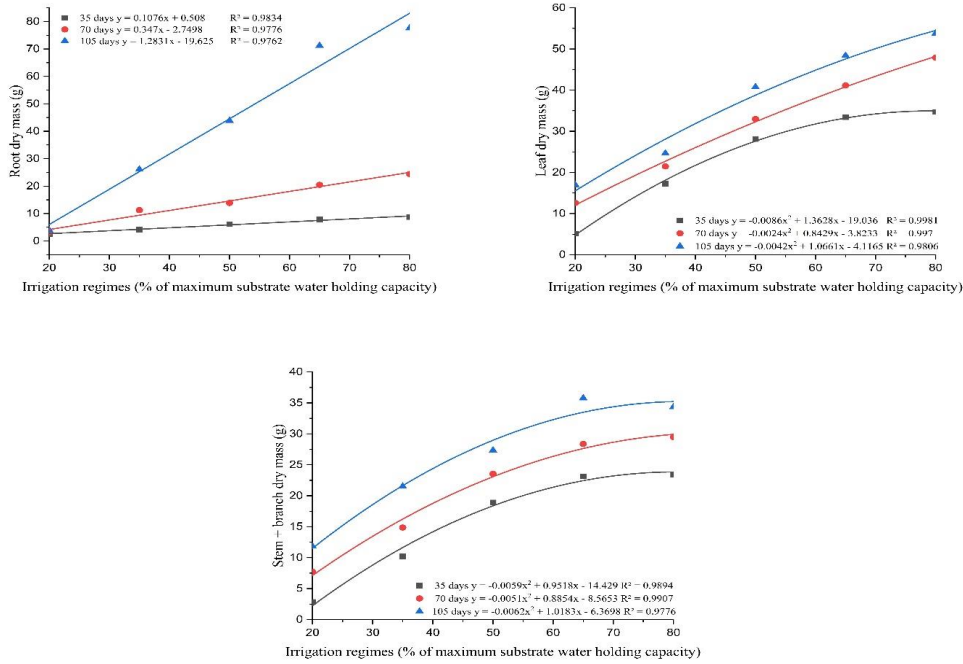
**Figure 3.** Contents (mg g<sup>-1</sup>) of chlorophyll a (Chl-a), chlorophyll b (Chl-b), chlorophyll a+b (Chl a+b), and carotenoids (Cx+c) in *C. citriodora* plants submitted to five irrigation regimes (IR1, IR2, IR3, IR4, and IR5, that is, 20, 35, 50, 65, and 80% of the maximum substrate water retention capacity, respectively), evaluated at 35, 70, and 105 days after the establishment of water regimes.



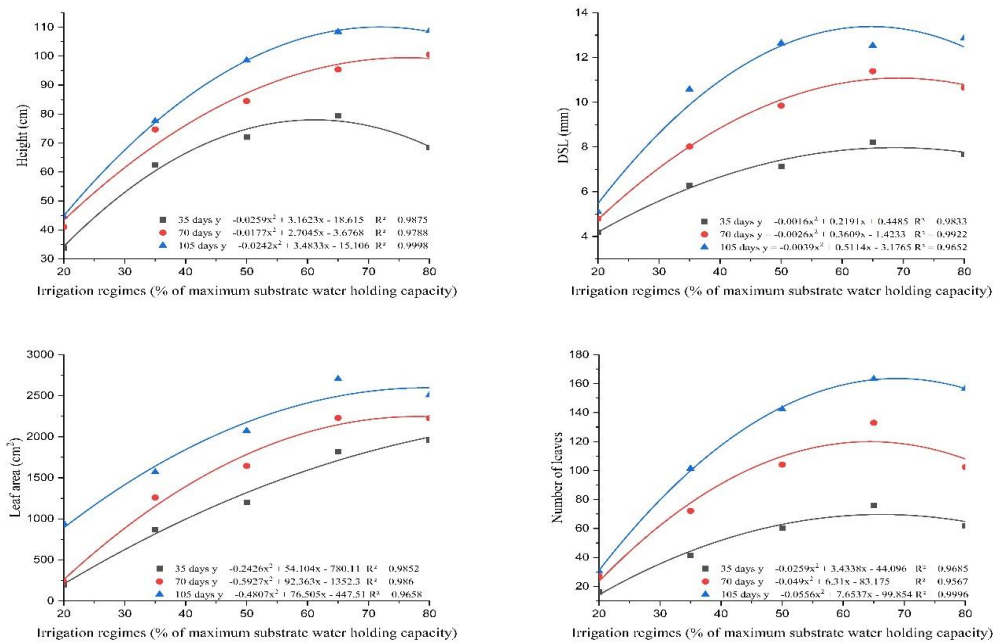
**Figure 4.** Malondialdehyde content (MDA, nmol g<sup>-1</sup> FM) in *C. citriodora* plants submitted to five irrigation regimes (IR1, IR2, IR3, IR4, and IR5, that is, 20, 35, 50, 65, and 80% of the maximum substrate water retention capacity, respectively), evaluated at 35, 70, and 105 days after the establishment of water regimes.



**Figure 5.** Proline content (μmol g<sup>-1</sup> FM) in *C. citriodora* plants submitted to five irrigation regimes (IR1, IR2, IR3, IR4, and IR5, that is, 20, 35, 50, 65, and 80% of the maximum substrate water retention capacity, respectively), evaluated at 35, 70, and 105 days after the establishment of water regimes.



**Figure 6.** Behavior of height (cm), diameter at soil level (mm), number of leaves, leaf area (cm<sup>2</sup>), root dry mass (g), stem + branch dry mass, and leaf dry mass of *C. citriodora* plants submitted to five irrigation regimes (IR1, IR2, IR3, IR4, and IR5, that is, 20, 35, 50, 65, and 80% of the maximum substrate water retention capacity, respectively), evaluated at 35, 70, and 105 days after the establishment of water regimes.



**Figure 7.** Behavior of root dry mass (g), stem + branch dry mass, and leaf dry mass of *C. citriodora* plants submitted to five irrigation regimes (IR1, IR2, IR3, IR4, and IR5, that is, 20, 35, 50, 65, and 80% of the maximum substrate water retention capacity, respectively), evaluated at 35, 70, and 105 days after the establishment of water regimes.

increasing plant age, as observed for gas exchange, which causes the water potential to decrease. The evaluations at noon at ages of 35 and 70 days showed little difference between the irrigation regimes IR3, IR4, and IR5, but a continuous reduction in the water potential was observed at 105 days with a decrease in water availability for the plants. The reduction in leaf water potential was accentuated as plants grew, which is due to the higher water demand of these plants with biomass accumulation and leaf area expansion. It leads to a reduction in available water,

reducing water potential by altering turgor and water uptake by plants (Dbira et al., 2018), leading to a reduction in the growth rate in the most restrictive water regimes. Chlorophylls fulfill essential functions in photosynthesis and are basic systems of the pigment-protein complex (Chen et al., 2018). Chlorophyll and carotenoid contents were reduced with the advancing age and seedling growth, which were lower with an increase in water availability. *E. camaldulensis* Dehnh. seedlings subjected to water stress mediated by sodium chloride and mannitol showed a

reduction in chlorophyll and carotenoid contents (Cha-um and Kirdmanee, 2010), unlike what was observed in the present study, in which chlorophyll increased in the lowest water regimes.

Water deficit causes damage to chloroplasts, which can reduce chlorophyll contents and produce reactive oxygen species (ROS) (Flexas et al., 2006). However, higher chlorophyll contents were observed under lower water availability, which suggests that gas exchange may have been reduced under these conditions by stomatal closure. Chlorophyll and carotenoid contents may favor chloroplast membranes and minimize oxidative degradation (Barros Junior et al., 2021), which may have partially favored seedlings under more restrictive irrigation regimes.

#### **Biochemical characteristics**

The increase in MDA content in the most restrictive irrigation regimes and more intensely with the increasing age of seedlings indicates membrane degradation by increased lipid peroxidation (Singh et al., 2020). Barros Junior et al. (2021) studied water stress in *E. urophylla* and found similar results. Lipid peroxidation causes irreversible damage to membrane integrity, increasing MDA content, constituting an indicator of oxidative stress (Ullah et al., 2017). The highest MDA contents indicate plant growth restriction caused by lower water availability, which increased with age and seedling growth. MDA and proline contents at 35 days in IR1 indicate a level of stress compared to the other water regimes, which was accentuated with seedling development.

As in MDA, the proline content was higher in the more restrictive water regimes (IR and IR2) and with the advancing age of seedlings. The higher proline accumulation helps to maintain pressure on leaf turgor (Tariq et al., 2019) and constitutes an osmotic adjustment mechanism to minimize the harmful effect of water stress. Some studies report proline accumulation under water stress in *E. grandis* (Tariq et al., 2019) and *E. tereticornis* (Singh et al., 2020). These authors stated that regulating the osmotic potential by increasing the proline content in plants under water deficit is a type of response of species and/or genotypes tolerant to water deficit (Cha-um et al., 2013). This adjustment mechanism can reduce H<sub>2</sub>O<sub>2</sub> and MDA contents, which are produced on a larger scale under stress (Cui et al., 2021) to ensure plant survival (Rezaei-Chiyaneh et al., 2021).

#### **Growth characteristics**

Growth in height and diameter were affected by irrigation regimes, but in different intensities as a function of the age of evaluation. Therefore, differences between irrigation regimes were smaller in younger plants due to their lower water demand, increasing with advancing plant age. In general, growth and biomass accumulation did not respond to the irrigation regime of 80% of field capacity (IR5), except for root dry mass, which suggests water excess under this condition, especially at the lowest age of evaluation (35 days). On the other hand, the more restrictive irrigation regimes (IR1 and IR2, that is, 20 and 35% of the field capacity, respectively) provided a significant reduction in growth traits, mainly with advancing plant age.

Reductions in growth and biomass accumulation are related to changes in physiological and biochemical characteristics, observed in irrigation regimes that are more restrictive to plants because plants regulate turgor under water deficit to maintain functional activities that are declining (Farrell et al,

2017). For instance, it can occur, through osmotic adjustment (Chen et al., 2020), production of reactive oxygen species or oxidative metabolism (Ulloa et al., 2021), and other mechanisms. These characteristics are affected before growth, regulating plant tolerance and survival.

The sensitivity of eucalyptus species to water stress varies between characteristics (Hakamada et al., 2017; Chaín et al., 2020; Reis et al., 2021). It could be observed in the response curves to irrigation regimes (Figure 5). For instance, root dry mass was little affected by water availability at 35 and 70 days probably because, until that moment, the water demand was small and totally supplied by a root system, which is still underdeveloped. However, the regime of lower water availability (IR1) at 105 days significantly limited root development, and root biomass accumulation had an expressive response to the increase in water availability. Roots are effective for successful seedling establishment, and a well-developed root system provides better access to water and nutrients, ensuring good plant development (Grossnickle et al., 2005). On the other hand, water deficit causes a low soil water potential, which makes it difficult for the roots to elongate and limits water and nutrient absorption (Chaín et al., 2020), consequently compromising the growth.

## **Materials and methods**

#### **Plant materials**

Seedlings were obtained from *C. citriodora* seeds collected from remaining trees from a 40-year-old open pollination progeny test installed in the municipality of Jaboticabal, State of São Paulo, Brazil (21°15' S, 48°19' W, 605 m asl). The regional climate is Cwa according to the Köppen classification (Alvares et al., 2013). The progeny test underwent an individual phenotypic selection, leaving the best 150 trees. Fruits collected from 15 of these trees were dried in a semi-shaded environment for later seed extraction and manual processing, which were later homogenized in a single seed lot for the experiment.

#### **Treatments**

Conducted in a greenhouse between November 2019 and March 2020 by submitting *C. citriodora* seedlings to five water regimes for 105 days. The greenhouse was covered with a 150- $\mu$ m thick transparent plastic film with sides covered with screens capable of intercepting 30% of the light. The maximum and minimum temperatures and relative humidity were monitored inside the greenhouse during the experimental period (Figure 1). The seedlings were produced from seeds germinated in 50-cm<sup>3</sup> tubes filled with Plantmax<sup>®</sup> substrate and sand. Only the most central seedling with the best development was maintained. The seedlings remained in the tubes for 90 days, when they reached adequate size for planting.

#### **Conduction of study**

The seedlings were planted in plastic pots filled with 7 kg of soil sample from the Jaboticabal region, classified as a very clayey-textured typic eutrophic Red Latosol, according to Santos et al. (2018), whose fertility analysis is shown in Table 1. The dried and sieved soil was properly corrected to raise the base saturation to 60% using a 3:1 Ca to Mg ratio. Phosphate fertilization was carried out in the same procedure, applying 100 mg P dm<sup>-3</sup>, using single superphosphate based on the crop requirements proposed

by Gonçalves et al. (1997).

### **Experimental design**

The seedlings were acclimatized for seven days after planting and before the establishment of water regimes. Water treatments consisted of daily maintenance of soil moisture at 20% (IR1), 35% (IR2), 50% (IR3), 65% (IR4), and 80% (IR5) of the maximum substrate water retention capacity, also called field capacity (Silva et al., 2014). The treatments corresponding to soil moisture levels were maintained for 105 days (when the experiment ended). The evapotranspired water was replaced daily by the gravimetric method, aiming to replace the amount of water sufficient to maintain the desired moisture of the treatments. The design was randomized in blocks, with four replications of two plants per pot, totaling 20 pots and 40 plants per evaluation.

### **Traits measured**

#### **Physiological analyses**

**Gas exchange and leaf water potential** – The net assimilation ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), transpiration ( $E$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), and stomatal conductance rates ( $g_s$ ,  $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) and intracellular  $\text{CO}_2$  ( $C_i$ ,  $\mu\text{mol mol}^{-2}$ ) were analyzed at 35, 70, and 105 days after the establishment of water regimes in the period between 8:00 and 10:00 h, using an LCPro+ infrared gas analyzer (IRGA) (ADC BioScientific Ltd.) coupled to an artificial light source set to provide a photosynthetically active photon flux of  $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$  and under ambient  $\text{CO}_2$  concentration ( $400 \pm 10 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ). These evaluations were performed on fully expanded leaves in the upper third of the canopy.

Leaf water potential was determined in the predawn and at noon, using the method established by Scholander et al. (1965) on leaves detached from the plant and placed in the pressure chamber.

#### **Photosynthetic pigments**

Carotenoid and chlorophyll contents were determined following the methodology described by Lichtenthaler (1987). For this purpose, leaf discs with 0.025 to 0.030 g of fresh mass were collected from one plant of each replication per treatment, placed in Eppendorf with 2 mL of 80% acetone, maintained for 48 hours under cooling at temperatures from 6 to 10 °C, with subsequent reading in a spectrophotometer at 470, 647, and 663 nm. The results were interpolated in a standard curve to determine the amount of these compounds ( $\text{mg g}^{-1}$  of leaf fresh mass).

#### **Biochemical analysis**

Malondialdehyde (MDA) and proline contents were determined in plant samples collected during the experiment (35, 70, and 105 days) from one plant per pot in the four replications.

#### **MDA content**

Estimated by the content of substances reactive to thiobarbituric acid. MDA concentration was determined using 0.2 g of leaves macerated in trichloroacetic acid, followed by centrifugation at 10,000 rpm for 15 minutes at  $-4$  °C. TBA (thiobarbituric acid reactive) was added to the supernatant. The mixture was placed in a dry bath at 95 °C for 30 minutes and then the solution was clarified. The concentration of MDA equivalents was calculated using an

extinction coefficient of  $1.55 \times 10^{-5} \text{ mol}^{-1} \text{ cm}^{-1}$ , with readings at 535 and 600 nm and results expressed in  $\mu\text{mol mg}^{-1}$  of fresh mass (Gratão et al., 2012).

#### **Proline content**

Determined according to the methodology of Bates et al. (1973), that is, the manual homogenization in a porcelain mortar of 0.5 g of fresh plant material with 10 mL of 3% sulfosalicylic acid and filtering. Proline contents were calculated for the standard curve and based on fresh mass, expressed in  $\mu\text{moles of proline g}^{-1}$  of fresh mass.

#### **Growth analysis**

The following variables were analyzed: plant height (cm), measured with a ruler graduated in mm; collar diameter (mm), determined with a digital caliper; number of leaves, determined by manually counting the number of leaves per plant; leaf area ( $\text{cm}^2$ ), determined with the leaf area analyzer Li-Cor 3100; and dry mass of plant components (g), determined on an analytical balance (0.001 g) after drying leaves, stem + branches, and roots in an air circulation oven at  $65 \pm 3$  °C until constant mass. The parameters related to plant biomass were analyzed with four replications per treatment, using 20 experimental units (pots) with 40 seedlings (mean per experimental unit).

#### **Statistical analysis**

The data were subjected to analysis of variance, followed by polynomial regression to express the evaluated characteristics as a function of water regimes. These analyses were performed using the AgroEstat® software (Barbosa and Maldonado Júnior 2015), with plots constructed using the Origin® software.

### **Conclusions**

Physiological, biochemical, and growth parameters in *Corymbia citriodora* plants are affected by irrigation regimes, regardless of the age of evaluation, but low water availability becomes more harmful with age and biomass accumulation. The alterations manifested by *C. citriodora* plants to the low water availability aim to guarantee plant survival to the detriment of higher growth and biomass accumulation.

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