WATER-RETAINING POLYMER MITIGATES THE WATER DEFICIT IN *Schinus terebinthifolia*: PHOTOSYNTHETIC METABOLISM AND INITIAL GROWTH

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ABSTRACT

The use of water-retaining polymer (hydrogel) can mitigate the damage to the photosynthetic apparatus and increase the robustness of young plants under water deficit conditions. Thus, the aim of this study was to evaluate the effect of water-retaining polymer on the morphophysiological aspects of *Schinus terebinthifolia* Raddi submitted to water deficit. The studied water regimes were characterized by three plant irrigation management: 1) control: irrigated daily, 2) stress: water deficit (irrigation suspension) and 3) stress + hydrogel: water deficit + water-retaining polymer. The plants were submitted to irrigation suspension until photosynthesis approached zero [1st P0], followed by resumption of irrigation (REI) for 15 days. After REI, until recovery of photosynthetic rate of plants, the maintenance of irrigation of all plants for 90 days, characterizing the final recovery (125th day of experiment). The photosynthetic rate reached values close to zero in plants under stress on the 20th day of water restriction, whereas the plants in conditions with hydrogel did not show a decrease in leaf metabolism. Excluding intercellular CO_2 concentration, physiological parameters were lower on 1st P0 in plants under water deficit grown without hydrogel than those in plants grown with hydrogel, but all plants showed physiological plasticity. The use hydrogel contributed to the maintenance of the photosynthetic capacity of *S. terebinthifolia* under water deficit.

INTRODUCTION

Tropical regions, particularly the Cerrado ecoregion, are characterized by different vegetation types and climatic seasonality that are caused by water fluctuations, leading to water deficit in certain periods of the year as a consequence of precipitation fluctuations (Fonseca et al., 2017). The low water availability in the soils of the Cerrado region can cause functional and metabolic damage to plants, such as stomatal closure, reduced Rubisco carboxylation efficiency, and increased intercellular CO_2 concentration and production of reactive oxygen species (ROS), thereby compromising plant photosynthetic capacity and growth (França et al., 2017; Resende et al., 2019).

Adverse environmental conditions may hinder the survival success and initial establishment of plants in priority reforestation areas, during the recovery of degraded areas, and in integrated sustainable production systems. In this sense, it is necessary to establish the technologies that can mitigate the harmful effects of water deficit in young plants, because they need to maintain their physiological processes and production of photoassimilates, especially in the implementation phase.

It has been shown that the use of water-retaining polymers (hydrogels) contributes to the retention of water in the soil, making it available to plants in dry periods (Felippe et al., 2016; Silva et al., 2019), as well as improving aeration and the cation exchange capacity, and reducing nutrient leaching (Bartieres et al., 2016; Konzen et al., 2017). However, information on the ecophysiology of fruit tree species in the seedling phase associated with the use of hydrogels is scarce.

Among the species with bioecological potential for being cultivated in the Cerrado phytophysiognomy, *Schinus terebinthifolia* Raddi (Anacardiaceae), a pioneer plant species popularly known as the Brazilian pink pepper or red

pepper, is widely distributed in South America, including Brazil with its diverse biomes. In adult phase, this species can reach a height of 5 to 10 m, and it bears small and reddish fruits, which are attractive to wild fauna (Oliveira Júnior et al., 2013; Viégas et al., 2018). The species has potential for use in integrated production systems and/or degraded areas with the goal of ecosystem service restoration.

The aim of this study was to evaluate the effect of water-retaining polymer on the morphophysiological aspects of *S. terebinthifolia* subjected to water deficit conditions. We intended to test the hypothesis that although water restriction reduces the efficiency of leaf metabolism, the addition of hydrogel to soil can mitigate damage to the photosynthetic apparatus and increase the tolerance of this plant species to water deficit in the initial growth phase for the recovery and enrichment of Cerrado areas.

MATERIAL AND METHODS

Ripe fruits of *S. terebinthifolia* (Access Registration No. A9CDAAE - CGEN-MMA, from 10/15/2018) were collected from matrices located in the Horto Medicinal Plants (22°11'43.7"S and 54°56'08.5"W, 452 m a.s.l.), Federal University of Grande Dourados - UFGD, Dourados - MS, Brazil. After manual pulping, the seeds were immersed in sodium hypochlorite (2%) for five minutes, and sowing was carried out in 72-cell expanded polystyrene trays filled with Tropstrato®.

Sixty days after emergence, the seedlings were transplanted to 290 cm^3 polyethylene tubes and placed in a nursery with 50% shade and daily irrigations until they reached an average height of 10 cm, which occurred 30 days after transplanting. Then, they were transplanted to plastic pots filled with 10 kg of dry substrate consisting of Dystrophic Red Latosol + sand (3:1, v/v) with daily irrigations maintaining 70% of the water retention capacity (WRC) in the substrate for 30 days. This was the acclimatization period before the submission of plants to different water regimes.

The experiment was carried in a nursery with 30% shading and additional protection on the top and on the sides of the plastic cover with thickness of 150 μm, in order to provide protection from precipitation. The water regimes included three irrigation practices, i.e., three groups: 1) Control: plants irrigated daily, 70% of the WRC was maintained in the substrate according to Souza et al. (2002), 2) Stress: water deficit, i.e., suspension of irrigation, and the photosynthetic rate was monitored every two days until the values approached zero [1st P0], after which irrigation was resumed, and 3) Stress + hydrogel: water deficit + water-retaining polymer, the procedures were similar to those in the previous water regime.

The water-retaining polymer (Forth Gel® hydrogel), consisting of polyacrylic potassium polyacrylamide (soil conditioner - class E; cationic exchange capacity= 53.22 cmol$_c$ dm^3), was added to a container at a dose of 4 g L^{-1} of water and it remained in the container for 30 minutes, until the product had a gel appearance. Following this, 100 mL of the product was added at the time of seedling transplantation to each stress + hydrogel pot near the plant roots.

During the experimental period, the evaluations of morphophysiological characteristics were carried out in four periods: (1) T0 (beginning of the experiment: day zero, one day before suspending the irrigation of the corresponding treatments), (2) 1st P0 (first time photosynthesis was close to zero: 20th day), (3) REI (daily resumption of irrigation on substrate during 15 days after the 1st P0: 20th to 35th day), this time when the plants under stress presented photosynthetic rate close to those of the control plants. After this period, all plants were irrigated daily for another 90 days, corresponding to the final recovery period (4) (REC), and the final evaluation was performed on the 125th day of the experiment.

Irrigation was carried out according to the established water regimes, being those under daily irrigations, according to Souza et al. (2002), REI and REC were maintained under 70% of the WRC in the substrate; WRC of 100% in the substrate was determined using the water content retained after draining, and the WRC of 70% was obtained using a simple three rule as a function of weight. The pots were weighed on an analytical balance (0.001 g precision) and individualized irrigation was performed daily, with enough water to reach the pre-established of substrate weight.

The treatments were arranged in a subplot scheme, with water regimes allocated in the plots, and the evaluation periods in the subplots were conducted in a randomized block design, with four replications. Each experimental unit consisted of four pots with two plants in each pot. In each evaluation period, four plants were evaluated by repetition for non-destructive characteristics and one plant for destructive characteristics, and the following evaluations were carried out:

(a) Gas exchange: measured using fully expanded sheets, the rate of CO_2 assimilation (photosynthesis): A (μmol CO_2 m^2 s^{-1}), intracellular CO_2 concentration – C_i (μmol CO_2 m^2 s^{-1}), stomatal conductance – g_s (mol H_2O m^{-2} s^{-1}), and transpiration – E (mmol^{-1} H_2O m^2 s^{-1}) were quantified using a portable LCIPro-SD photosynthesis meter (IRGA - *Infra Red Gas Analyzer*) (Model ADC BioScientific Ltd.). Measurements were made between 8 and 10 a.m. with photosynthetically active radiation of 865 μmol m^2 s^{-1}. Subsequently, water use efficiency – WUE (μmol CO_2/mmol H_2O), Rubisco carboxylation – A/C_i (μmol m^{-2} s^{-1}/μmol mol), and intrinsic water use – $_iWUE$ (mmol^{-1} H_2O) were calculated as A/E, A/C_i and A/g_s ratios, respectively.

(b) Soil Plant Analyzer Development (SPAD) index: measured using a portable chlorophyll meter (Konica Minolta 502) in the morning (8 to 11 a.m.).

(c) Growth: measured by counting the number of fully expanded leaves, after which the plants were collected, separated into different plant parts, and the leaf area was determined using an area integrator (LI-COR, 3100 C – Area Meter, Nebraska, USA). The leaf limbs and roots were placed in an oven with forced air circulation at 60 ± 5 °C until a constant dry mass was obtained and weighed on a precision scale (0.0001 g precision).

(d) Physiological indices: calculated from the leaf area and dry biomass production data; leaf area to specific leaf area ratio was calculated as well (Benincasa, 2003).

The data were analyzed using analysis of variance (ANOVA), and the results that were significant by F test ($p < 0.05$), were used in comparing the means using Tukey test for water regimes and evaluation periods ($p \leq 0.05$). Statistical analysis were performed using the SISVAR software (Ferreira, 2019).

RESULTS AND DISCUSSION

The rates of CO_2 assimilation (A), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were influenced by the interaction between water regimes and evaluation periods. The photosynthetic rate (A) reached values close to zero (1st P0) in plants under stress (1.01 μmol CO_2 m^2 s^{-1}) at 20 days of water restriction (Figure 1a). During this period, the plants subjected to daily irrigation (control) and stress + hydrogel maintained their A values, indicating that the addition of hydrogel effectively contributed to the maintenance of water conditions in the plant, thereby preventing dehydration.

When performing the resumption of irrigation (REI), we observed an increase in A in all plants, including those that were only subjected to stress, which maintained lower REI values when compared to the other plants in this period, obtaining values similar to the other plants only at the end of the experiment, thus extending the period required for recovery (REC). However, plants with added water-retaining polymer did not show a decrease in photosynthetic rate during irrigation suspension, but maintained values close to those of plants irrigated daily throughout the experimental period. The use of hydrogel favors the ability to maintain water in the soil as it can store high water volume, especially the excess water, and make it available to plants in dry periods (Silva et al., 2019).
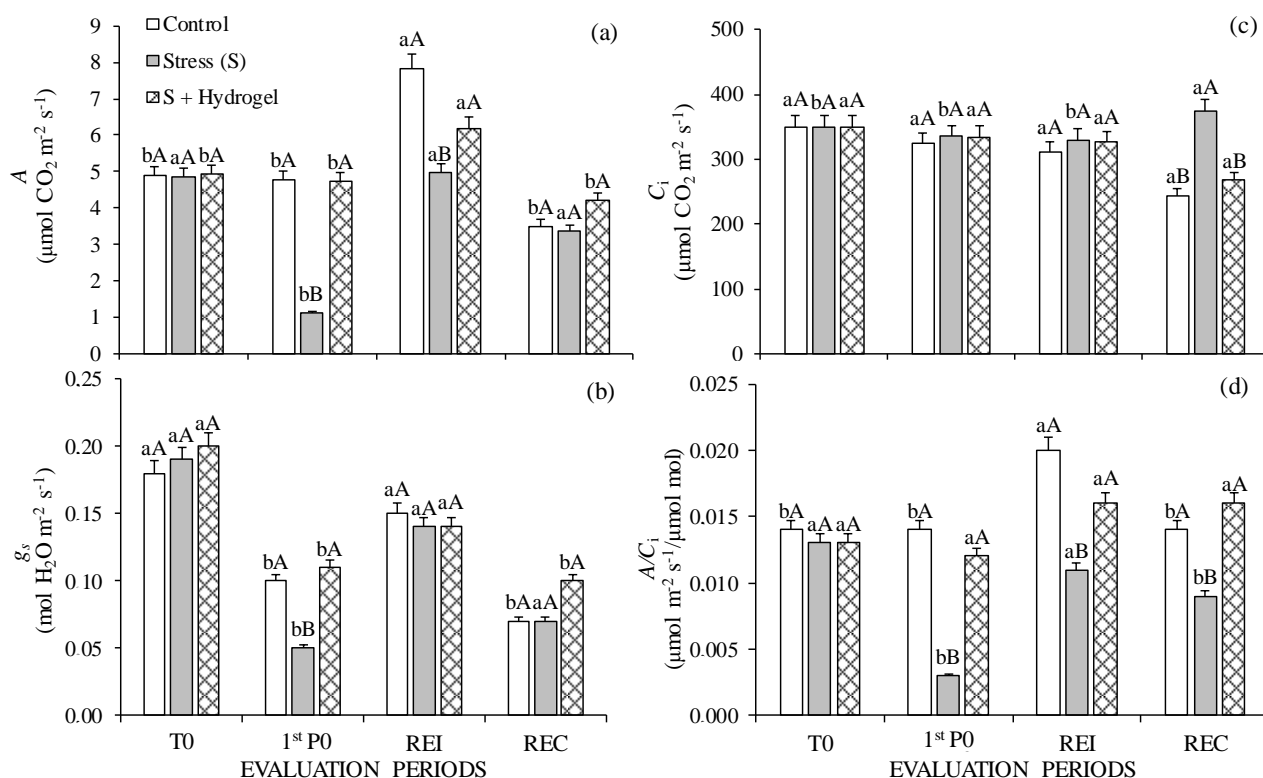

FIGURE 1. Variations in gas exchange characteristics in young plants of *S. terebinthifolia* under water regimes and evaluation periods. Photosynthesis – A (a), stomatal conductance – g_s (b), intracellular CO_2 concentration – C_i (c) and intrinsic efficiency of carboxylation – A/C_i (d). Lowercase letters compare the same water regime in the different evaluation periods, and uppercase the different water regimes on the same period (Tukey; $p < 0.05$).

The g_s value was higher in T0 than in others evaluation periods, independent of the water regimes, as these plants were not yet subjected to stress conditions (Figure 1b). In the 1st P0, plants grown under stress conditions had lower g_s (0.05 mol H_2O m^{-2} s^{-1}) than under control, with a tendency to reestablish their values after re-irrigation. Lower g_s directly influence the decrease in CO_2 entry into the substomatic chamber, limiting its assimilation capacity. In the REI period, g_s values were higher than those in 1st P0, with no difference between the water regimes, whereas in REC, these values showed the same trend as A, that is, from the moment daily irrigation was started, the recovery capacity of the plants increased. This indicated physiological plasticity of the investigated plants.

Until the start of the REI period, the intercellular CO_2 concentration remained similar under all water regimes. On the other hand, in REC, we recorded a higher C_i in plants under stress when compared to that in control plants, indicating lower efficiency of carboxylation in plants

under these conditions, with the increase in CO_2 in the tissues. However, when we observed the patterns of C_i (Figure 1c) and A (Figure 1a), we found that the plants under stress had a photosynthetic rate similar to that of control plants, suggesting that other factors besides stomatal factors interfered with the photosynthetic efficiency of the investigated plants.

The decrease in water availability, influenced by the increase in abscisic acid, induces stomatal closure (França et al., 2017), reducing the assimilation and diffusion of CO_2 (Ashraf & Harris, 2013), as we observed in *S. terebinthifolia*. Conditions of low water availability result in a decrease in stomatal conductance (Wong et al., 1979). However, lower g_s availability favors the optimization of water use by reducing transpiration rate (Kröber et al., 2015), as observed in the present study, in the plants in the 1st P0.

We observed lower values of A/C_i in plants under stress when compared to that in control plants in all periods of evaluation except at T0 (Figure 1d). The decrease in

Rubisco carboxylation under low water availability (1st P0) is, among other factors, a consequence of lower g_s, reflecting a higher C_i. The increase in C_i is a reflection of lower Rubisco carboxylation, and this response can result in the damage to thylakoid membranes (Rejeb et al., 2014; Choudhury et al., 2017). When irrigation was resumed, the plants showed an increase in A/C_i similar to that in the other water regimes, including under stress, as this also increased the gas exchange in the same period, favoring the entry of CO_2 and maximizing photosynthesis. In the REC, the plants showed a recovery capacity, but under stress, their recovery capacity was lower than that of the plants irrigated daily and lower than that of the plants under stress + hydrogel.

Leaf transpiration (E) was influenced only by the evaluation periods, with the highest E observed in plants at T0 (5.35 mmol H_2O m^{-2} s^{-1}), and the lowest in plants at 1st P0 (1.14 mmol H_2O m^{-2} s^{-1}), which was not statistically different from these values in REI (Figure 2a). The reason for the lower E value in *S. terebinthifolia* in the 1st P0 was the stomatal closure that was formed as a consequence of irrigation suspension, or what favors the reduction of water loss by tissue (Osakabe et al., 2014). After the 1st P0, there was a gradual increase in E, mainly in the REC (4.01 mmol H_2O m^{-2} s^{-1}), demonstrating that this condition was overcome with irrigation recovery, as observed in the REI and REC.
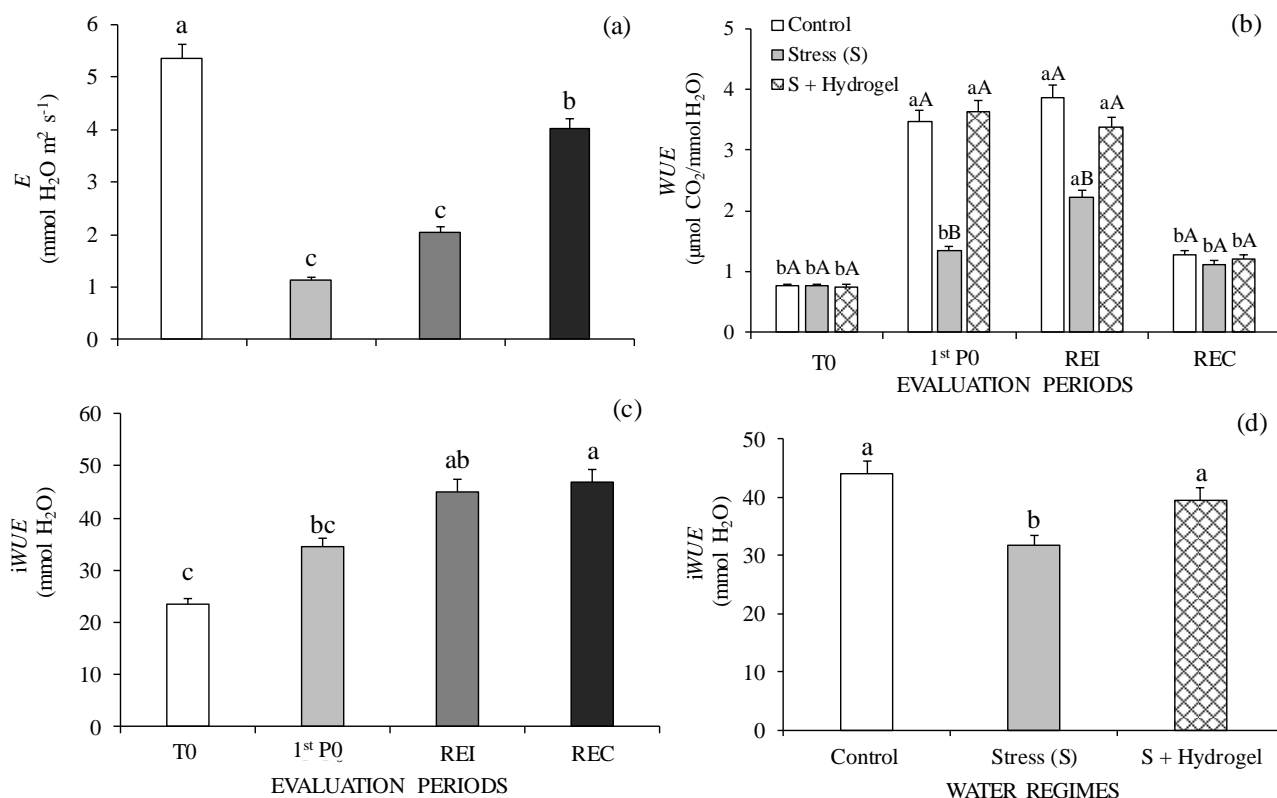

FIGURE 2. Variations in water use in young plants of *S. terebinthifolia* under differents water regimes and evaluation periods. Leaf transpiration – E (a), water use efficiency – WUE (b) and intrinsic water use efficiency – $_iWUE$ (c, d). (a–c–d) Equal letters in columns do not differ from each other (Tukey; $p < 0.05$). (b) Lowercase letters compare the same water regime in the different evaluation periods, and uppercase the different water regimes on the same period (Tukey; $p < 0.05$).

Water use efficiency (WUE) was influenced by the interaction between the factors under study (Figure 2b), and the irrigated plants kept under stress conditions with hydrogel showed higher values of WUE in the 1st P0 when compared to those submitted to the deficit in function reduction of E and A. Compared to control plants, we observed an increase in WUE in plants under stress, and their WUE was lower when compared to those in plants in the other water regimes in the 1st P0 and REI. In the REC, we observed a decrease in relation to the other periods of evaluation, and therefore, the values were similar.

We found that $_iWUE$ was influenced by the factors in isolation and increased during the evaluation periods (Figure 2c), with a lower value in plants under stress when compared to that in control plants (Figure 2d), which is a result of lower g_s reflecting lower WUE and A. Therefore, the addition of hydrogels can contribute in maintaining the capacity of physiological processes under stress conditions

such as intermittent water deficit. With regard to water regimes, results similar to ours were observed by Nunes et al. (2017) in *S. terebinthifolia* seedlings under intermittent water restriction, suggesting that the reduction of these characteristics ($_iWUE$) indicate water saving.

The SPAD index, number of leaves, and leaf area were influenced by the interaction of the investigated factors. The SPAD index decreased in the 1st P0 in plants under water deficit (Figure 3a). The lowest SPAD index was observed in plants submitted to water deficit as a result of lower water availability in the substrate, and although they recovered after the resumption of irrigation, their SPAD index did not reach the values of the plants under continuous irrigation or those cultivated with hydrogel, supporting the hypothesis that water deficit is stressful for *S. terebinthifolia*.

Under conditions of low water status in the soil, cell turgor decreases and photosynthetic pigments degrade due

to collapses in chloroplasts as a consequence of increase in carbohydrates and ROS, which causes oxidative damage (Zhang et al., 2014). Similarly, other species found in Cerrado phytophysiognomies, such as *Hymenaea courbaril* L. (Freitas et al., 2018) and *Dipteryx alata* Vogel. (Matos et al., 2018), also showed a reduction in chlorophyll content as a consequence of lower water availability in the soil.
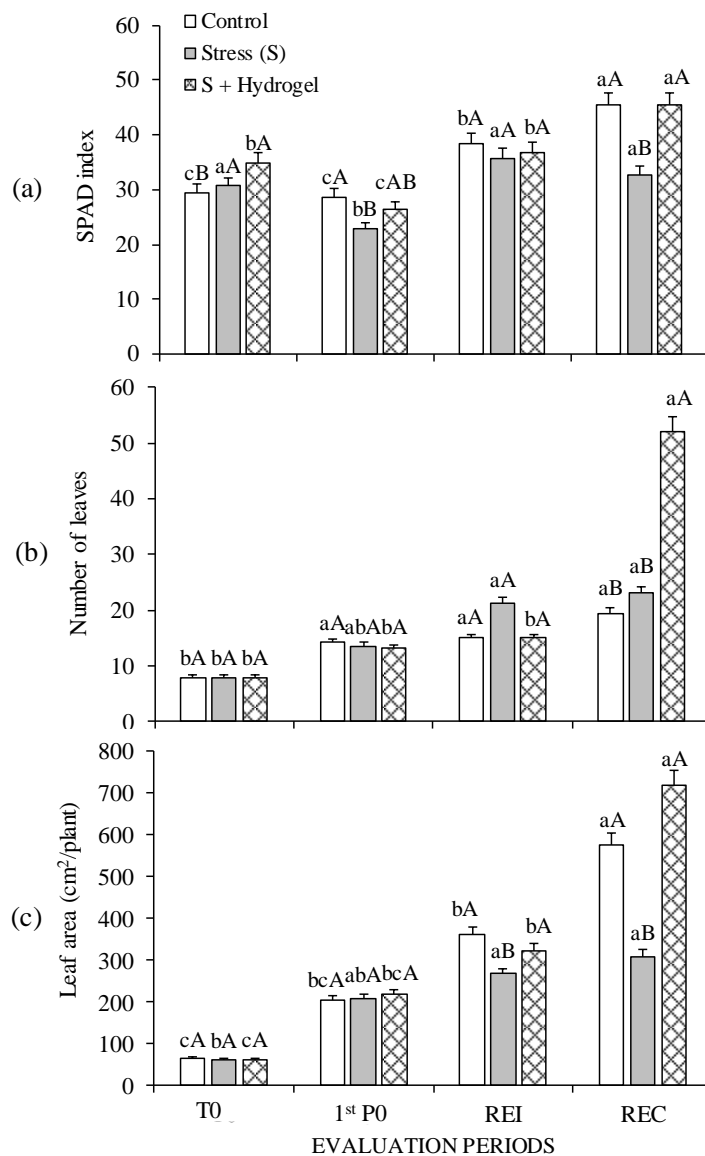

FIGURE 3. Variations in growth characteristics in young plants of *S. terebinthifolia* under water regimes and evaluation periods. Chlorophyll index – SPAD (a), number of leaves (b) and leaf area (c). Lowercase letters compare the same water regime in the different evaluation periods, and uppercase the different water regimes on the same period (Tukey; $p < 0.05$).

The number of leaves did not vary in T0 and 1st P0 periods regardless of the water regime (Figure 3b). This pattern in T0 was a consequence of the standardization of seedlings (eight leaves) at the time of transplanting; however, under irrigation suspension, the investigated species showed a tolerance to this adverse condition in the 1st P0, possibly because of the fact that under reduced photosynthetic activity, it initially maintained the translocation of photoassimilates to the vegetative organs.

However, compared to the other water regimes, we observed an increase in the number of leaves in plants under water deficit in REI, demonstrating that the plants quickly responded to physiological stress (Freitas & Silva, 2014). Nevertheless, in the REC, the plants showed a higher number of leaves in the stress + hydrogel treatment than in the other water regimes in the same period, which was possibly because of the prolonged effect of hydrogels on the soil, i.e., the polyacrylamide present on the hydrogel increased water availability for a longer period of time.

The largest leaf areas (LA) were observed in plants irrigated daily and under stress + hydrogel in the REI and REC (Figure 3c) because of the greater water availability in these conditions. The lower LA in plants subjected to stress may suggest they used strategies for reducing transpiration per unit area with the aim to maintain the integrity of photosynthetic machinery. These strategies have been reported in the literature as a protective response in several species, for example in the seedlings of *Callophyllum brasiliense* Cambess. (Reis et al., 2020); these plants also had a smaller leaf area when subjected to water deficit, similar to those observed in the present study. We found that *S. terebinthifolia* plants were tolerant to water deficit for several days (1st P0), maintaining a leaf area close to that of plants in the other treatments. However, the

responses of plants to the stress conditions in the 1st P0 caused subsequent negative responses, that is, smaller LA even after the resumption of irrigation in plants grown without hydrogel.

The leaf dry mass was influenced only by the evaluation periods, in which the highest leaf dry mass values occurred over time (Figure 4a). These results were a consequence of the fact that even under conditions of water suspension and subsequent re-irrigation, the investigated plants optimized the available resources at the end of the experimental period, demonstrating the recovery capacity and increase in photoassimilates in the REC. Regarding the dry root mass, in the 1st P0, the plants under stress +

hydrogel showed higher dry root mass values when compared to those in the other water regimes (Figure 4b), demonstrating the beneficial effect of hydrogel, mainly because it was in close contact with the roots, thereby increasing the potential of the plant for nutrient and water absorption. During the REI, we observed an increase in root biomass in all water regimes. However, in the REC, the largest supply of root biomass occurred in plants irrigated daily and under stress + hydrogel, whereas those without the addition of hydrogel had lower root biomass values, indicating that the water deficit impaired the translocation of photoassimilates in roots even when the stress conditions were not present anymore.
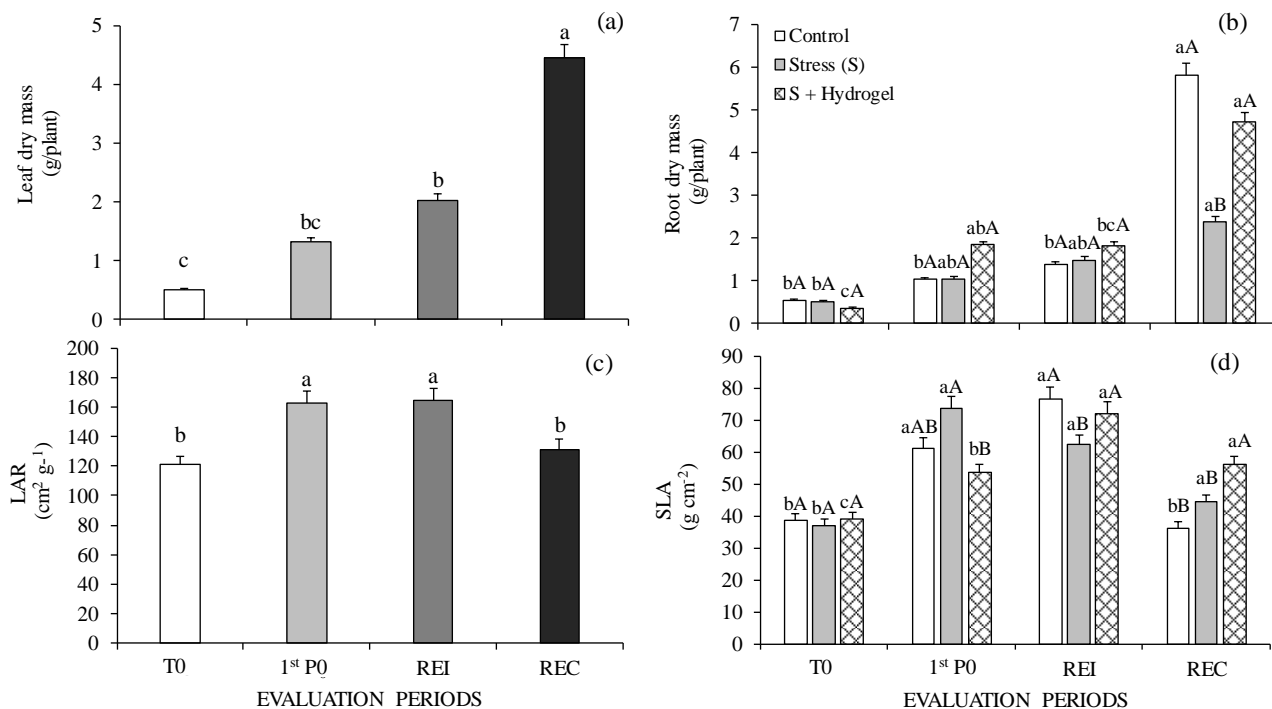

FIGURE 4. Variations in dry mass and physiological indexes characteristics in young plants *S. terebinthifolia* under different water regimes and evaluation periods. Leaf dry mass (a), root dry mass (b), leaf area ratio – LAR (c) and specific leaf area – SLA (d). (a–c) Equal letters in columns do not differ from each other (Tukey; $p < 0.05$). (b–d) Lowercase letters compare the same water regime in the different evaluation periods, and uppercase the different water regimes on the same period (Tukey; $p < 0.05$).

The leaf area ratios were higher in plants in the 1st P0 and REI (Figure 4c), indicating that although the *S. terebinthifolia* seedlings had considerable LA (Figure 3c), they showed lower biomass accumulation in these two periods, because in the 1st P0, the plants were in stress conditions, and in the REI, the plants did not have sufficient time to fully recover the *A* (Figure 1a) and biomass production in these cultivation conditions. The specific leaf area (SLA) was influenced by the interaction between water regimes and evaluation periods, presenting similar values in T0 for all treatments. In the 1st P0, the SLA of all treatments increased with increasing stress (Figure 4d). Compared to stress, plants under stress + hydrogel had higher SLA in the REI and REC.

The responses of leaf parameters to different water availabilities vary between different species. According to Campelo et al. (2015), the seedlings of *Swietenia macrophylla* (King), *Handroanthus serratifolius* (Vahl), and *Handroanthus impetiginosa* (Mart.) Matos showed lower SLA values when submitted to water deficit when

compared to those in control plants, which was a different response from that observed in the present study for *S. terebinthifolia*. The variation in SLA can be explained by the increase in leaf thickness in response to water restriction, which was reflected in higher leaf biomass.

In general, it is worth noting that in addition to maintaining the water storage capacity in the soil, water-retaining polymers contribute to the mitigation of nutrient leaching (Navroski et al., 2015), especially when they are applied close to the roots. In this way, they increase the contact surface between roots and the rhizosphere, favoring nutrient intake and stabilizing plant metabolic processes, including *A*, g_s, A/C_i, and leaf morphological characteristics of *S. terebinthifolia*.

The use of water-retaining polymers has proved to be a promising practice for silvicultural activities in areas with irregular rainfall or inefficient irrigation system size, as it contributes to reduced costs of seedling replacement due to their greater survivability.

CONCLUSIONS

Our results confirmed our hypothesis that the efficiency of leaf metabolism in *S. terebinthifolia* plants would be reduced under water restriction and that the addition of hydrogel to the soil would contribute to the maintenance of photosynthetic capacity and growth of this species, even under water deficit conditions, in addition to enhancing plant responses and biomass allocation in these conditions. In addition, we emphasized that *S. terebinthifolia* has the potential to recover gas exchange after stress periods, demonstrating its environmental resilience owing to its physiological plasticity.

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