








## ARTICLE

# Hydrogel and arduino system in the transplantation of *Schinus terebinthifolia* for urban afforestation

Hidrogel e sistema arduino no transplante de *Schinus terebinthifolia* para arborização urbana

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**Abstract:** Urban forest provide medium and large vegetation cover in urban areas. Planting native trees on sidewalks is a viable approach to reduce damages caused by extensive urbanization. The use of hydrogels seems to increase the success of seedling transplantation in urban environments. Thus, this study aims to evaluate the efficacy of *Schinus terebinthifolia* in urban afforestation, focusing on its adaptation and post-transplant survival using hydrogel and being monitored by an arduino system. The concentrations of the commercial hydrogel used were 0.75, 1.5, 3.0, and 6.0 g L<sup>-1</sup>, and two controls were also established: a control treatment without irrigation and without hydrogel, and a control with daily water irrigation. The evaluation was continuous for 14 days, and the parameters analyzed were substrate temperature and moisture, relative water content (RWC), electrolyte extravasation (EEE), chlorophyll content, and biochemical compound. A completely randomized design was adopted, consisting of 6 treatments with 9 replicates each. The results indicate that *S. terebinthifolia* is a highly resilient species suitable for urban afforestation, showing remarkable tolerance to transplantation and water restriction. It was observed that the use of hydrogels significantly contributes to maintaining substrate moisture, resulting in greater stability of the transplanted seedlings. The Arduino system allowed for continuous and precise evaluation of substrate conditions, optimizing the management of urban afforestation and validating the efficiency of the applied treatments. Positive responses were observed when using hydrogels in terms of relative water content, membrane stability, and antioxidant activity, even under water restriction. The viability of *Schinus terebinthifolia* for urban afforestation stands out, through the application of hydrogels and the use of the Arduino system to monitor parameters such as temperature and humidity.

**Keywords:** antioxidant system, native species, stress, urban afforestation.

**Resumo:** As florestas urbanas fornecem cobertura vegetal de médio e grande porte em áreas urbanas. O plantio de árvores nativas nas calçadas é uma abordagem viável para reduzir os danos causados pela extensa urbanização. O uso de hidrogéis parece aumentar o sucesso do transplante de mudas em ambientes urbanos. Assim, este estudo tem como objetivo avaliar a eficácia de *Schinus terebinthifolia* na arborização urbana, focando na sua adaptação e sobrevivência pós-transplante utilizando hidrogel e sendo monitorado por sistema arduino. As concentrações do hidrogel utilizadas foram 0,75; 1,5; 3,0 e 6,0 g L<sup>-1</sup>, sendo também estabelecidos dois controles: um tratamento controle sem irrigação e sem hidrogel, e um controle com irrigação diária com água. A avaliação foi contínua durante 14 dias, e os parâmetros analisados foram temperatura e umidade do substrato, teor relativo de água (CR), extravasamento de eletrólitos (EEE), teor de clorofila e compostos bioquímicos. Adotou-se delineamento inteiramente casualizado, composto por 6 tratamentos com 9 repetições cada. Os resultados indicam que *S. terebinthifolia* é uma espécie altamente resiliente e adequada para arborização urbana, apresentando notável tolerância ao transplante e à restrição hídrica. Observou-se que o uso de hidrogéis contribuiu significativamente para a manutenção da umidade do substrato, resultando em maior estabilidade das mudas transplantadas. O sistema Arduino permitiu avaliação contínua e precisa das condições do substrato, otimizando o manejo da arborização urbana e validando a eficiência dos tratamentos aplicados. Respostas positivas foram observadas ao utilizar hidrogéis em termos de conteúdo relativo de água, estabilidade da membrana e atividade antioxidante, mesmo sob restrição hídrica. Destaca-se a viabilidade de *Schinus terebinthifolia* para arborização urbana, através da aplicação de hidrogéis e utilização do sistema Arduino para monitoramento de parâmetros como temperatura e umidade.

**Palavras-chave:** arborização urbana, espécies nativas, estresse, sistema antioxidante.

## Introduction

Currently, over 50% of the global population resides in urban areas, with this percentage rising to 86% in Brazil, where a significant majority of the population is concentrated in urban regions. This trend of population concentration in urban cities gives rise to numerous adverse effects, including the creation of heat islands, a lack of green spaces, and alterations in water resources (Silva et al., 2020; Nadal et al., 2022; Souza et al., 2022).

Incorporating natural elements through urban tree planting is crucial for mitigating environmental issues arising from urbanization. Urban forests enhance the quality of life for city dwellers, offering not only leisure and comfort but also essential ecological services. These green spaces play a pivotal role in mitigating the adverse effects of urban sprawl, contributing to the transformation of urban centers into more sustainable and environmentally adaptive habitats (Souza et al., 2022; Luz et al., 2023).

Among native species suitable for urban street afforestation, *Schinus terebinthifolia* is distinguished. It is a widely recognized species that is readily accessible to the population, though it has been underutilized for sidewalk planting. The use of native trees in urban afforestation is fundamental, as these species are adapted to the local climatic and edaphic conditions, contributing to a higher survival rate and reduced maintenance needs. Furthermore, native trees play a crucial role in the conservation of urban biodiversity, providing habitat and food for local fauna. They also aid in maintaining hydrological cycles and air purification, capturing carbon and other pollutants more effectively, which is essential for combating heat islands and improving the quality of life in cities (Martin et al., 2020; Storch-Böhm et al., 2022).

Urban afforestation comprises the trees that are inserted into urban environments, including trees present along streets and avenues and on sidewalks. However, several elements influence the cultivation of tree

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species, with soil and water being the most limiting factors. Soil occupies the second most crucial role in this context, as it is common for the urban environment to become highly impermeable due to compaction and the formation of dense layers. Water accounts for 90% of the chemical constitution of plants and is extremely important for the maintenance of their physiological and metabolic activities (Silva et al., 2020).

Decreased water availability is one of the factors that causes stress in plants; it affects their performance and may change their biological efficiency and productive potential (Pereira et al., 2020). The use of water-retaining polymers, popularly known as hydrogels, has emerged as a viable technology to mitigate water stress. These materials are composed of polymer chains formed by one or more hydrophilic components joined by covalent and/or electrostatic bonds organized in three-dimensional networks surrounded by water molecules (Neves et al., 2022).

These compounds have great relevance in agricultural and forestry activities, being able to absorb and retain 100 to 400 times more water and/or biological material than their own weight. Thus, they store water and nutrients for plant consumption while promoting greater porosity in the soil, as they gradually release the absorbed water (Nomura et al., 2019). To improve the effectiveness of water-retaining polymers, new technologies that encompass the concept of the Internet of Things (IoT) are employed, allowing the connection and control of programs with which environmental factors such as water and soil temperature can be monitored (Bhakta et al., 2019).

Arduino, a platform for the IoT, consists of a simple and low-cost microcontroller that is used to develop control, automation and interactivity applications. Through this platform, it is possible to connect devices and program the exchange of information between the Arduino

and the components that are connected to it. The Arduino is compatible with several operating systems, such as WiFi networks, sensors, and GPS (Bhakta et al., 2019).

This study aims to evaluate the efficacy of *Schinus terebinthifolia* in urban afforestation, focusing on its adaptation and post-transplant survival using hydrogel and being monitored by an arduino system. Specifically, the study seeks to understand the mechanisms that confer high plasticity and tolerance to water stress on *Schinus terebinthifolia*, as well as to investigate the role of hydrogel in maintaining constant substrate moisture, thereby facilitating plant stability after transplanting. Additionally, it intends to validate the efficiency of monitoring substrate moisture and temperature using the arduino system, aiming to offer an innovative technological approach to the management of urban afforestation.

## Materials and methods

### Experimentation site

The experiment was conducted in a greenhouse in the region of Lavras, Minas Gerais State, with an average minimum temperature of 14.80 °C, a maximum temperature of 26.50 °C, and an annual rainfall of 1,408 mm.

### Substrate analysis

The commercial substrate Basaplant® was used, and its physicochemical characteristics (Schafer & Lerner, 2022) were determined in the Substrate Laboratory of the Horticulture Department of the Federal University of Rio Grande do Sul, according to the 17th Normative Instruction of the Ministry of Agriculture, Livestock and Supply-MAPA (Brasil, 2009), based on the average of three replicates per sample (Table 1).

**Table 1.** Analysis of horticultural substrate, sample no. 3239, performed in the Substrate Laboratory, Department of Horticulture, Federal University of Rio Grande do Sul.

Parameter	Unit	Value
pH	M <sup>+</sup>	4.08
Electrical conductivity	mS cm <sup>-1</sup>	1.45
Wet density	kg m <sup>-3</sup>	468.39
Dry density	kg m <sup>-3</sup>	462.21
Current humidity	%	22.67
Total porosity	%	74.44
Air space	%	25.93
Readily available water	%	18.57
Buffering water	%	4.20
Remaining water	%	25.73
CRA (10)	%	48.51
CRA (50)	%	29.93
CRA (100)	%	25.73

According to the information provided by the manufacturer on the product label, the commercial substrate consists of peat, charcoal, vermiculite, NPK and micronutrients. The amounts of nutrients are expressed based on the results of the analysis performed by the Water

Resources Laboratory of the Campus of Agricultural Sciences of the Federal University of Espírito Santo according to the methodology described in the Manual of Chemical Analysis of Soils, Plants and Fertilizers (Table 2).

**Table 2.** Values of the chemical attributes of the Basaplant® substrate.

Parameter	Unit	Value
No.	g kg <sup>-1</sup> mg	5.03
Q	g kg <sup>-1</sup> mg	1.42
K	g kg <sup>-1</sup> mg	1.04
Ca	g kg <sup>-1</sup> mg	1.46
Mg	g kg <sup>-1</sup> mg	4.01
Mn	mg kg <sup>-1</sup>	31.81
Zn	mg kg <sup>-1</sup>	30.40

### Seedling production

Seeds of *S. terebinthifolia* were collected in the third quarter of 2018 and processed according to Brasil (2009) with adaptations for this species. For sowing, 120 cm<sup>3</sup> tubes were filled with commercial substrate and slow-release fertilizer (Osmocote®; formulation: 15-10-10 NPK, plus 3.5% Ca, 1.5% Mg, 3% S, 0.02% B, 0.05% Cu, 0.5% Fe, 0.1% Mn, 0.004% Mo and 0.05% Zn). After the establishment of the seedlings, those with an average height of 13 cm were transplanted into polypropylene bags measuring 20 cm x 26 cm x 0.16 cm. Substrate was added in a 3:2:1 proportion of sieved substrate, manure and commercial substrate, until a volume of 100 cm<sup>3</sup> was reached. Next, the seedlings were transplanted into tubes with a volume of 3,800 cm<sup>3</sup> and cultivated until they reached the standard used for urban afforestation, with a height greater than 180 cm, single trunk and diameter at breast height (DBH) of 3 cm. Importantly, throughout the development of the seedlings, they were irrigated daily until the beginning of the experiment.

### Sensor calibration

The data collection system consisted of an Arduino (MEGA 2560), two 400-hole breadboards, 24 analog capacitive humidity sensors and 24 digital DS18B20 temperature sensors attached using the one-wire method. Each board transmitted data to the Arduino microcontroller. The sensors were placed in 24 pots containing 12 liters of commercial substrate, which were irrigated until reaching field capacity. Substrate moisture and temperature were monitored daily for 10 days by sensors connected to the Arduino.

The evaluations were divided into three intervals: morning (4:00 am to 12:00 pm), afternoon (12:00 pm to 8:00 pm) and evening (8:00 pm to 4:00 am). After receiving the substrate moisture and temperature data, different concentrations of commercial FORTH® hydrogel were added to the pots, and the substrate was irrigated up to field capacity. To improve data accuracy, a 5TE capacitance sensor was used to verify substrate moisture and temperature; the sensor was inserted at a depth of 2 cm below the end of the sensor rod and with a minimum radius of 10 cm of substrate surrounding the sensor (Ariswati and Titisari, 2020). The measurements were collected on days 1, 7 and 14 after the experiment was set up.

### Experimental conditions

Twenty-five-liter pots containing 12 liters of commercial substrate were used, and six different concentrations of the commercial hydrogel FORTH® were incorporated into the substrate. The treatments were defined as follows: no irrigation and no hydrogel; daily irrigation without the addition of hydrogel; without irrigation and with the addition of 0.75, 1.50, 3.00, and 6.00 g.L<sup>-1</sup> hydrogel. All treatments were irrigated with water until reaching field capacity at the time of establishment. Each treatment consisted of 9 replicates, each with one *S. terebinthifolia* seedling that met the urban afforestation criteria.

### Physiological analyses

To calculate the relative water content (RWC) of the plants, 10 leaflets were collected from each replicate on the day of installation and on the 14th day of the experiment. These leaflets were immediately weighed to obtain the fresh mass using a precision scale. Then, they were placed in Petri dishes containing distilled water for 24 hours. After this period, the excess water was removed, and the leaflets were weighed again to obtain the turgid mass. The samples were then placed in an oven with forced air circulation at 60 °C for 24 hours and weighed again to obtain the dry mass. The RWC was estimated using the following equation, expressed as a percentage:

$$RWC = ((FM - DM)/(TM - FM)) \times 100$$

where FM = fresh mass, DM = dry mass and TM = turgid mass.

To analyze the electrolyte extravasation, a circular cutter was used to obtain five leaf discs with an area of 1.13 cm<sup>2</sup> each from each repetition. These discs were washed and placed in Petri dishes containing 20 mL of deionized water. The plates were kept at 25 °C for 90 minutes, and then the initial conductivity of the medium (Xi) was measured using a conductivity meter (Digimed DM31). Subsequently, the Petri dishes were placed in a drying oven (Dubesser SSD 11 L) at 80 °C for 90 minutes. After cooling the contents of the plates, the final conductivity (Xf) was measured. Electrolyte extravasation is expressed as the percentage of conductivity relative to total conductivity and is calculated using the following equation (Campos and Thi, 1997):

$$\% \text{ Conductivity} = (Xi/Xf) \times 100$$

### Determination of the contents of chloroplast pigments

Samples of 1 g of leaflets were collected from the median region of the leaves. The material was immediately macerated in 80% acetone. The resulting extracts were filtered using quick filter paper and transferred to 10 mL volumetric flasks, and 80% acetone was added until the final volume was reached. The readings were performed in a spectrophotometer at wavelengths of 470, 645 and 663 nm (Campos and Thi, 1997).

### Biochemical analyses (leaf and root)

Samples were collected for biochemical analysis on days 1, 7, and 14 after transplantation. Three replicates of each treatment were selected, and leaflets of three leaves located in the middle third of the plant were collected. The roots were cleaned and collected. For the quantification of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and lipid peroxidation, 0.2 g of fresh matter (leaves and roots) was collected, macerated in liquid nitrogen containing 2% polyvinylpyrrolidone (PVPP) and homogenized in 1.5 mL of 0.1% (w/v) trichloroacetic acid (TCA). The samples were centrifuged at 12,000 × g for 15 minutes at 4 °C. The supernatant was collected for quantification of H<sub>2</sub>O<sub>2</sub> and lipid peroxidation.

The determination of H<sub>2</sub>O<sub>2</sub> was performed by measuring the absorbance at 390 nm in a reaction medium containing 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide (Velikova et al., 2000). The quantification of lipid peroxidation was performed using thiobarbituric acid (TBA) and TCA, and the readings were obtained in a spectrophotometer with wavelengths of 535 nm and 600 nm (Buege and Aust, 1978). The method used for the quantification of proline was adapted from Bates et al. (1973). A volume of 2 mL of the sample extract was used, to which 2 mL of acetic acid and 2 mL of acid ninhydrin solution were added. The mixture was heated in a water bath at 100 °C for 60 minutes, and after cooling in an ice bath, the samples were read at 520 nm.

### Antioxidant metabolism

The enzymatic extract was obtained by macerating 0.2 g of the fresh material in liquid nitrogen containing 5% PVPP. Next, 1.5 mL of extraction buffer containing 400 mM potassium phosphate with pH 7.8, 10 mM EDTA and 200 mM ascorbic acid was added. Superoxide dismutase activity was determined by the ability of the enzyme to inhibit the photochemical reduction of nitroblue tetrazolium, as proposed by Giannopolitis and Ries (1977), at a reading of 560 nm. Catalase activity was determined by the reduction in absorbance at 240 nm, measured every 15 seconds for 3 minutes, and monitored by H<sub>2</sub>O<sub>2</sub> consumption (Havir and Mchale, 1987). Ascorbate peroxidase activity was determined by the decrease in ascorbate absorbance ( $\epsilon = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ ) at 290 nm, measured every 15 seconds for 3 minutes, according to Nakano and Asada (1981).

### Statistical design

A completely randomized design was adopted, consisting of 6 treatments with 9 replicates each. Two control groups were established: one without the use of hydrogel and without irrigation and the other without the use of hydrogel but with daily irrigation using water. In addition to the controls, treatments with hydrogel concentrations of 0.75 g L<sup>-1</sup>, 1.5 g L<sup>-1</sup>, 3.0 g L<sup>-1</sup>, and 6.0 g L<sup>-1</sup>, all without irrigation, were tested. The means were assessed by analysis of variance, and comparison between means was performed using the Scott-Knott test, with a significance level of 5%, in Sisvar® software (Ferreira, 2019).

### Results and Discussion

The permanent wilting point of *S. terebinthifolia* was determined; this point was reached after 14 days of water restriction, causing the plants to begin the senescence process. The temperature remained stable with a maximum value of 24.3 °C and a minimum value of 10.8 °C.

The relative humidity of the air was monitored throughout the experiment, with an average of 62.91%. The variation in environmental humidity was moderate, with a maximum recorded value of 67.20% and a minimum of 52.40%.

The calibration of the Arduino system generated a standard for the observed temperature value, as shown in Fig. 1A. During the morning, the temperature remained milder, below 21 °C. In the afternoon and evening, the temperature of the vessels rose, ranging from 22 °C to 24 °C on the days on which readings were taken.

In the same experiment, substrate moisture (Fig. 1B) was monitored continuously, and it was observed that in the control treatment without hydrogel and without irrigation, there were more oscillations that decreased over time. This can be explained by the porosity of the substrate, which was not filled by the hydrogel or water.

The substrate exhibits a porosity of 74%. In contrast to the results of the control treatment, the pots that received the hydrogel showed consistent data throughout the day, and the higher the concentration of hydrogel was, the smaller the variations observed.

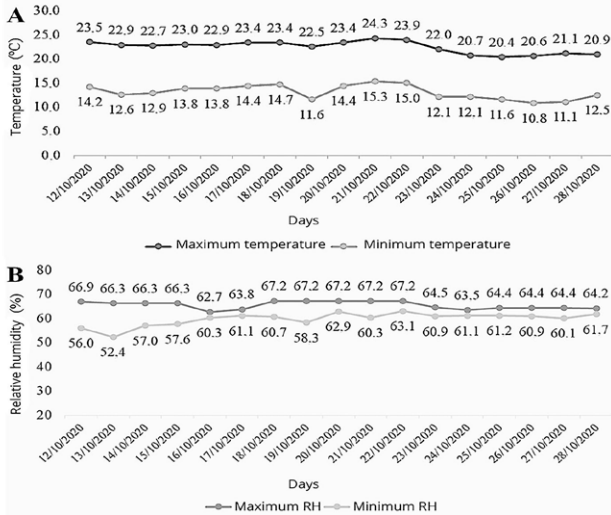


Fig. 1. (A) Temperature and (B) substrate moisture in vessels without seedlings under the different treatments over the course of 10 days of monitoring.

The substrate temperature in the pots containing the *S. terebinthifolia* seedlings (Fig. 2A) was measured over the 14 days of the experiment, with milder morning temperatures and higher average temperatures in the afternoon and evening. On the 8<sup>th</sup> day of the experiment, there was a drop in temperature due to factors external to the substrate. However, the maximum temperature recorded throughout the experiment did not exceed 24 °C, and the minimum recorded temperature was 18.5 °C.

The substrate moisture content (Fig. 2B) varied in the control treatments, which may be related to the interference with the sensor created by the substrate porosity, since the sensor uses the difference in voltage resistance as a reading standard. However, in the treatments that received the highest concentrations of hydrogel, the moisture content remained constant throughout the experiment.

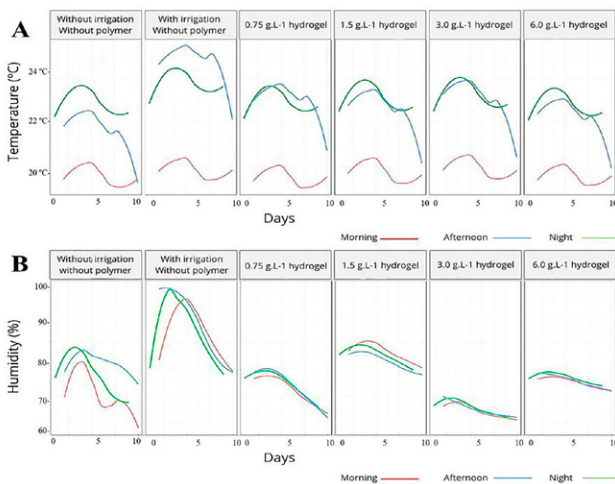


Fig. 2. (A) Temperature and (B) substrate moisture after transplanting *Schinus terebinthifolia* seedlings to the different treatments over 14 days of readings taken by the Arduino system.

Substrate temperature and moisture were evaluated using the 5TE Pro-Chek sensor. These measurements revealed that the substrate temperature on days 0 and 14 was higher in all treatments than on the other days. This finding may be related to the temperature of the external environment, since the data obtained with the sensors of the Arduino system showed a drop in temperature between days 7 and 8, corroborating the data obtained by this sensor.

On the first evaluation day, shortly after the experiment was set up, humidity exceeding 80% was observed in all the treatments. In the subsequent evaluations, in the pots without hydrogel and without irrigation, the substrate water content decreased to approximately 60%, representing the treatment with the greatest decrease in substrate water content. The other treatments showed no significant differences (Fig. 3).

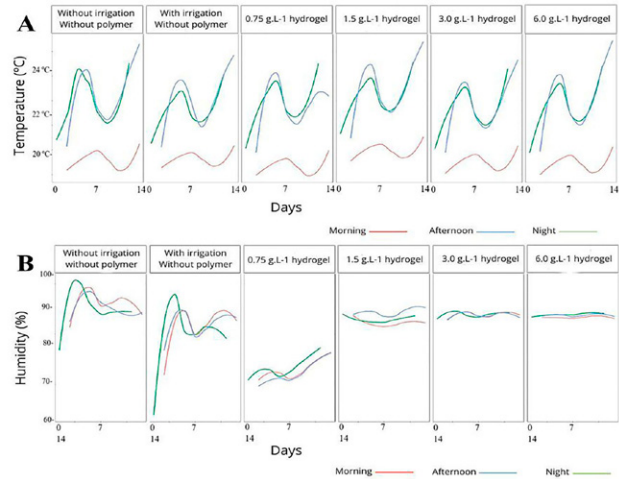


Fig. 3. (A) Temperature and (B) substrate moisture at field capacity in vessels with *Schinus terebinthifolia* seedlings under the different treatments over 14 days of readings taken with 5TE Pro-Check equipment. The same capital letters (treatments) and lowercase letters (days) indicate values that do not differ from each other according to the Scott-Knott test at 5% significance.

The RWC in the leaves of *S. terebinthifolia* did not show significant differences between treatments, exceeding 80% 14 days after transplanting. The seedlings were tolerant to water restriction, as this type of restriction can result in a decline in leaf water potential, followed by loss of cell turgor, reduction in cell expansion and division, protein synthesis, stomatal conductance and photosynthesis (Nascimento et al., 2015). This observed tolerance can also be explained by the fact that some species have faster stomatal closure mechanisms, a strategy that prevents water loss to the environment and increases drought resistance (Fatima et al., 2023).

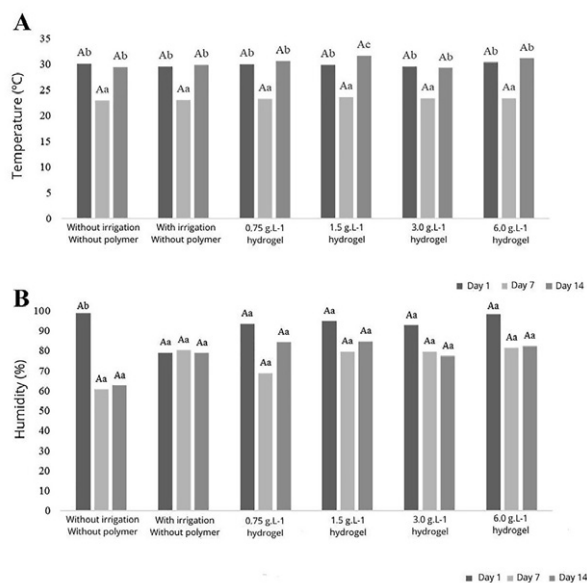
It is possible that the RWC of seedlings of *S. terebinthifolia* did not differ significantly after transplanting under water restriction due to the direct relationship between osmotic adjustment and the accumulation of low molecular weight organic solutes in the plants under lower water availability (Fatima et al., 2023). This result was also observed in a study with *Hymenaea courbaril*, in which the plants maintained a high RWC and adjusted their leaf concentrations under water limitation (Nascimento et al., 2015).

The treatment with 0.75 g L<sup>-1</sup> had the highest chlorophyll *a* concentration (Fig. 4A), followed by the treatment without the addition of hydrogel and with constant irrigation. The other treatments showed no significant differences. The chlorophyll *b* content (Fig. 4B) in the seedlings grown in the treatment with 6.0 g L<sup>-1</sup> hydrogel was higher than that in the other treatments. The treatments without hydrogel and with constant irrigation, as well as the treatment with 1.5 g L<sup>-1</sup> of hydrogel, had the lowest chlorophyll *b* contents; these treatments provided less stressful conditions than the others. The results obtained for carotenoids showed no difference between treatments, with a general mean of 1.32 μg g<sup>-1</sup> MF.

Plants produce chlorophyll *b* and carotenoids under stressful conditions because these pigments act as photo protectors, preventing chlorophyll

oxidation during photosynthesis and reducing the damage caused by light to the photosynthetic apparatus and membranes (Cunha Neto et al., 2020). Plants under water stress conditions undergo changes in physiological, biochemical, molecular and morphological processes, resulting in changes in the pathways related to photosynthesis and chloroplast pigments. In addition, the effects of these variations in water availability compromise the establishment capacity of most plants (Rocha et al., 2018).

In this sense, the content of chloroplast pigments is directly related to the stress level of the plant and may be higher or lower according to the degradation suffered (Cintra et al., 2020). For the parameter of electrolyte extravasation (REE) (Fig. 4C), the treatments with 0.75 g L<sup>-1</sup>, 3.0 g L<sup>-1</sup> and 6.0 g L<sup>-1</sup> hydrogel showed no differences, exhibiting the highest REE values. The other treatments showed lower levels of REE and did not differ from each other.



**Fig. 4.** (A) Chlorophyll *a*, (B) chlorophyll *b* and (C) electrolyte extravasation in *Schinus terebinthifolia* seedlings in different treatments during 14 days of evaluation. Bars with the same letters do not differ from each other according to the Scott–Knott test at 5% significance.

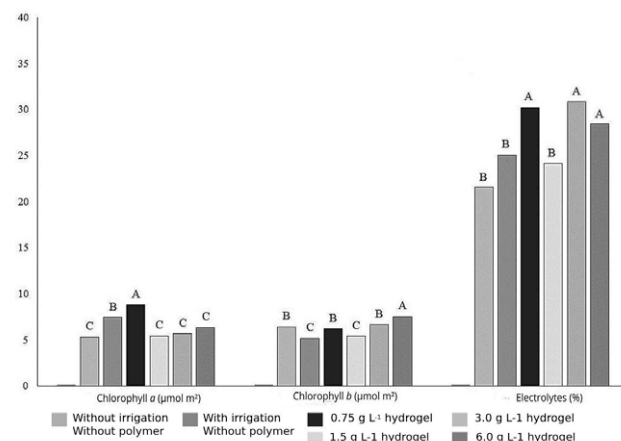
The low concentrations in the treatments without hydrogel and in the treatment with 1.5 g L<sup>-1</sup> of hydrogel may be related to the stomatal closure that occurred during the suspension of irrigation. Stomatal closure may lead to a reduction in the intracellular concentration of CO<sub>2</sub> and a decrease in the incorporation of this gas, consequently resulting in a reduction in the flow of noncyclic electrons (Cintra et al., 2020). Thus, it is possible to determine whether there is a lower availability of carbon dioxide in the environment, which is involved in the biochemical limitation of photosynthesis (Jacinto et al., 2019).

The increase in REE in the treatments with 0.75, 3.0 and 6.0 g L<sup>-1</sup> of hydrogel can be attributed to the consequences of the limitation of photosynthesis imposed by the lower water availability, which results in a disruption of the membrane integrity triggered by the overproduction of reactive oxygen species (ROS) (Dranski et al., 2017).

However, the species studied is considered a pioneer and has characteristics, such as taproots, that allow greater adaptation to adverse conditions and tolerance to environmental changes (Silva et al., 2020). Young plants subjected to different soil and climate conditions may exhibit phenotypic plasticity in response to abiotic stressors, such as reduced water content, which may limit plant survival and development (Saracho et al., 2020).

The quantitative analysis of proline in the leaves of transplanted seedlings revealed significant differences in the levels of this amino acid among the applied treatments at three evaluation points. On the first day without water, seedlings grown in substrate without hydrogel and with irrigation showed the highest levels of proline. There were no significant

differences between the other treatments during this period. However, from the seventh day, it was observed that treatments with higher concentrations of hydrogel recorded elevated proline levels, a trend that continued on the 14th day after water restriction. Initially, the control treatments (with and without irrigation) exhibited the highest proline levels, but on days 7 and 14, the treatments with higher doses of hydrogel stood out, indicating a differentiated response over the evaluated period (Fig. 5A).



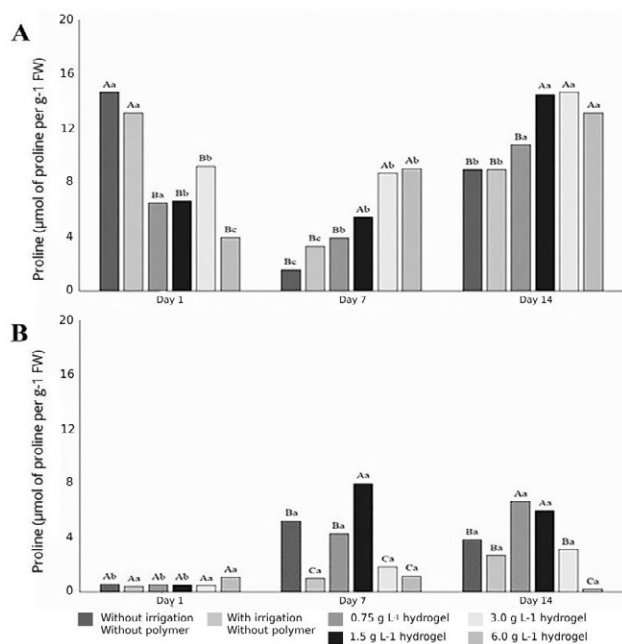
**Fig. 5.** Quantification of proline in the leaves (A) and roots (B) of *Schinus terebinthifolia* under the different treatments over the 14 days of evaluation. The same capital letters (treatments) and lowercase letters (days) indicate that the values do not differ from each other according to the Scott–Knott test at 5% significance.

Initially, the proline content in the roots showed no significant differences. However, after seven days, the treatment with 1.5 g L<sup>-1</sup> hydrogel exhibited the highest proline concentration, followed by the treatment with 0.75 g L<sup>-1</sup> and the control without irrigation. After 14 days of water restriction, treatments with hydrogel concentrations of 0.75 g L<sup>-1</sup> and 1.5 g L<sup>-1</sup> recorded higher proline levels, while in the control treatments (with and without irrigation) and in the treatment with 3.0 g L<sup>-1</sup>, the proline levels did not show significant differences. In the treatment with 6.0 g L<sup>-1</sup>, the proline level was lower than in the other treatments. When comparing the root results across the evaluation days, it was observed that the control treatment with irrigation and the treatments with the two highest concentrations of hydrogel remained consistent, whereas the control treatments without irrigation and with 0.75 g L<sup>-1</sup> and 1.5 g L<sup>-1</sup> hydrogel exhibited variations on the first day, with lower proline levels than in subsequent evaluations. Under water limited conditions, plants are able to perform osmotic adjustments to maintain cell turgor. This allows plants to protect themselves during short periods of stress (Dranski et al., 2017).

These osmotic adjustments occur due to the accumulation of solutes, such as proline, in the vacuole or cytosol, which results in the maintenance of the cellular water balance and in the preservation of enzymatic and protein activities and the integrity of the cell membrane (Dranski et al., 2017). In addition, some of these solutes play a role in protecting metabolism against the accumulation of harmful byproducts arising from water stress. Proline is one of the most studied amino acids in plants due to its rapid response under stress conditions (Govêa et al., 2018).

Plants under stress conditions tend to have an increase in proline content compared to non-stressed plants, and this factor may be an important factor in the search for more resistant plants (Govêa et al., 2018). However, although in the literature the proline content is often associated with stress levels in plants, it is still unclear whether an increase in this amino acid will always occur in all species under stress conditions.

The amount of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in the leaves (Fig. 6A) did not differ significantly between the first day of evaluation and the 14<sup>th</sup> day under water restriction. However, 7 days after transplanting, the highest H<sub>2</sub>O<sub>2</sub> values were found in the control treatment with irrigation, followed by treatment with 3 g L<sup>-1</sup> hydrogels.



**Fig. 6.** Quantification of hydrogen peroxide in (A) leaves and (B) roots of *Schinus terebinthifolia* in the different treatments during the 14-day evaluation period. The same capital letters (treatments) and lowercase letters (days) indicate that the values do not differ from each other according to the Scott–Knott test at a 5% significance level.

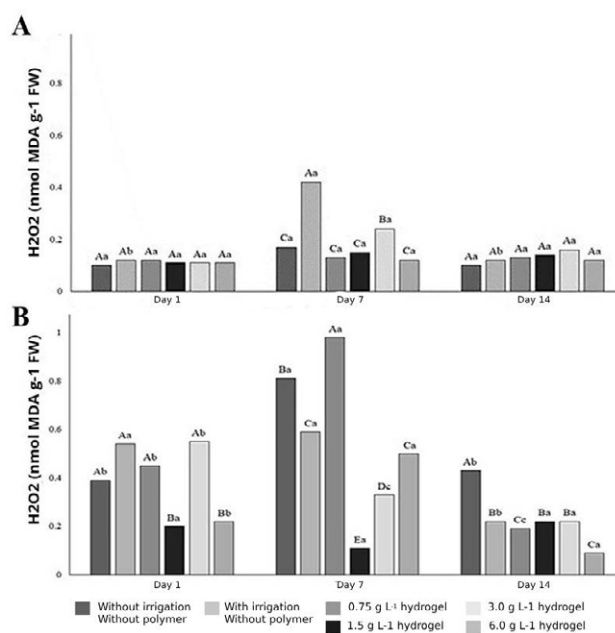
For the seedlings cultivated with constant irrigation, there were differences in the concentrations of  $H_2O_2$  in the leaves 7 days after transplanting. However, the other treatments did not show significant variations over the evaluation days. On the first day of evaluation, the control treatments (with and without irrigation) and treatment with hydrogel concentrations of  $0.75\text{ g L}^{-1}$  and  $3.0\text{ g L}^{-1}$  had higher levels of  $H_2O_2$  in the roots, while treatments with concentrations of  $1.5\text{ g L}^{-1}$  and  $6.0\text{ g L}^{-1}$  had lower concentrations.

After 7 days of water restriction, the treatment with the smallest amount of hydrogel had the largest amount of  $H_2O_2$ , followed by the control without irrigation, the control with constant irrigation and the treatment with  $6.0\text{ g L}^{-1}$  hydrogel. The smallest amounts of  $H_2O_2$  were observed at hydrogel concentrations of  $3.0\text{ g L}^{-1}$  and  $1.5\text{ g L}^{-1}$ . At 14 days of water restriction, the control treatment without irrigation had the highest  $H_2O_2$  levels, while the other treatments had lower levels. There were differences in the quantification of  $H_2O_2$  over the three days evaluated, and the treatment without irrigation and without hydrogel and the treatment with  $0.75\text{ g L}^{-1}$  hydrogel had the highest  $H_2O_2$  concentrations after 7 days of water restriction.

The seedlings grown without hydrogel and with constant irrigation showed high levels of  $H_2O_2$  in the first evaluations, and this level decreased by the third evaluation. The treatment with  $1.5\text{ g L}^{-1}$  hydrogel did not show variations in the level of  $H_2O_2$  throughout the evaluations, while the values in the treatments with  $3.0\text{ g L}^{-1}$  and  $6.0\text{ g L}^{-1}$  oscillated. Low levels of  $H_2O_2$  act as signaling molecules for the expression of defense-related genes, while high concentrations can damage membranes and lead to cell death (Sharma et al., 2022).

The production, metabolism and removal of ROS are essential for plant growth and development. The regulation and elimination of these compounds are part of the defense mechanism of plants and may increase tolerance to various types of environmental stress (Dranski et al., 2017), as can be observed when comparing the contents of ROS at 7 and 14 days after transplanting.

Lipid peroxidation in leaves (Fig. 7A) did not differ between treatments, but there was variation among the days without irrigation. In the control treatment without irrigation, lipid peroxidation decreased beginning at 7 days after the start of the experiment, while the treatment with  $1.5\text{ g L}^{-1}$  of hydrogel had a higher content at 7 days of water restriction, differing from the other treatments.



**Fig. 7.** Quantification of lipid peroxidation in the (A) leaves and (B) roots of *Schinus terebinthifolia* grown in the different treatments over 14 days of evaluation. The same capital letters (treatments) and lowercase letters (days) indicate that the values do not differ from each other according to the Scott–Knott test at a 5% significance level.

The intensity of oxidative stress can be assessed based on the level of lipid peroxidation, which plays a central role in membrane deterioration. Lipid peroxidation is caused by high concentrations of ROS, damaging the membrane system and resulting in cell death. However, because the different treatments presented WRCs greater than 80% in the leaves, it was possible to conclude that there was no irreversible damage due to the level of peroxidation of the membranes, suggesting that the antioxidant defense system was activated during the water restriction period (Dranski et al., 2017).

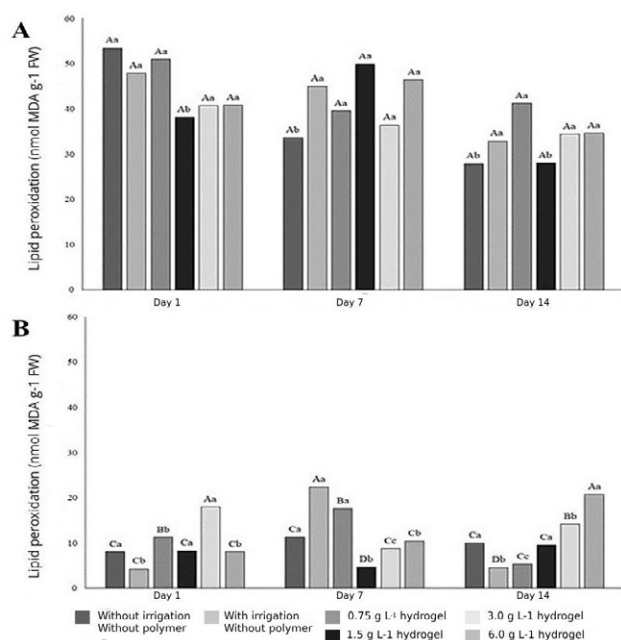
WRC values of up to 60% in the leaves do not cause irreversible damage. After the rehydration of plants, there was a rapid recovery of photosynthetic activity and the level of membrane peroxidation, suggesting that the antioxidant defense system was activated after water suspension. Under normal conditions, plants are generally well adapted to minimize damage due to the inevitable formation of ROS during photosynthesis (Fujita and Hasanuzzaman, 2022).

The level of lipid peroxidation in the roots differed between the treatments on the different evaluation days. The treatment with the highest concentration of hydrogel showed the highest lipid peroxidation level on the first day, while the control treatment with constant irrigation had the lowest lipid peroxidation level. At 7 days, the control treatment with constant irrigation had the highest lipid peroxidation value, while the treatment with  $1.5\text{ g L}^{-1}$  hydrogel had the lowest value. At 14 days, the treatment with the highest concentration of hydrogel had the highest lipid peroxidation content. In general, the control treatment with constant irrigation had the lowest levels of lipid peroxidation.

Differences were observed throughout the evaluations, and the control treatment without irrigation did not show significant variations over the evaluation period. The control treatment with irrigation showed higher levels of lipid peroxidation at 7 days of water restriction, as well as the treatment with  $0.75\text{ g L}^{-1}$  hydrogel. On the other hand, the treatment with  $1.5\text{ g L}^{-1}$  hydrogel had the lowest level of lipid peroxidation in the second evaluation, unlike the other treatments. The treatments with higher concentrations of hydrogel, i.e.,  $3.0\text{ g L}^{-1}$  and  $6.0\text{ g L}^{-1}$ , had lower values of lipid peroxidation at 7 days and higher concentrations at the first and third evaluations, respectively.

The superoxide dismutase (SOD) activity in the leaves of the seedlings in the different treatments differed on the first and third days of evaluation. On the first day, the control treatment with irrigation and the treatment with the highest concentration of hydrogel had higher levels of

SOD activity than the other treatments, which did not differ from each other. At 14 days of water restriction, the control treatments and those with the lowest concentrations of hydrogel had higher SOD levels than the other treatments.



**Fig. 8.** Quantification of superoxide dismutase in (A) leaves and (B) roots of *Schinus terebinthifolia* in the different treatments over 14 days of evaluation. The same capital letters (treatments) and lowercase letters (days) indicate that values do not differ from each other according to the Scott–Knott test at a 5% significance level.

Over the duration of the experiment, the treatments without irrigation and without the addition of hydrogel, together with the treatment with 0.75 g L<sup>-1</sup> hydrogel, had increased SOD levels over time. In the other treatments, there were no significant differences in SOD levels. In the roots, the treatments with higher hydrogel concentrations had higher SOD contents in the first and third evaluations. In the second evaluation, the control treatment with constant irrigation and the treatment with 6.0 g L<sup>-1</sup> hydrogel had the highest SOD contents. The control treatment without irrigation and the treatments with 1.5 g L<sup>-1</sup> and 3.0 g L<sup>-1</sup> of hydrogel showed no significant differences in SOD contents over the days evaluated.

The control treatment with constant irrigation and 6.0 g L<sup>-1</sup> hydrogel had the highest SOD levels at 7 days, while the treatment with 0.75 g L<sup>-1</sup> hydrogel had the lowest SOD level on the third day of evaluation. Some treatments showed an increase in activity in the leaves over the evaluated days due to water deficit, and SOD was the first enzyme to manifest itself under this condition.

The enzymes catalase (CAT) and ascorbate peroxidase (APX) in the leaves and roots of the seedlings did not differ significantly based on the tests performed. Regardless of the type of metabolism, water scarcity results in a reduction in photosynthetic activity and an increase in plant respiration, which leads to an excessive production of ROS. To minimize the cytotoxic effects of ROS, plants activate a complex antioxidant system in which specific enzymes act to neutralize the action of these radicals, starting with SOD.

To avoid the accumulation of ROS, plants have enzymatic and nonenzymatic defense systems that allow the elimination of these compounds and thus protect against oxidative stress. The variation in water availability can trigger the activation of this antioxidant system (Rocha et al., 2018). Importantly, SOD is the first enzyme involved in the plant defense system when the cell detects a stress signal and is responsible for the direct formation of different ROS. In response to SOD activity, the H<sub>2</sub>O<sub>2</sub> level increases, initiating the action of secondary neutralization enzymes, such as CAT and peroxidases, which are responsible for the conversion of H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O + ½ O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O + R(O)<sub>2</sub>, respectively (Pereira et al., 2020).

## Conclusions

Based on the results obtained, we can conclude that *Schinus terebinthifolia* is an excellent option for urban afforestation. This plant has mechanisms that allow its stability after transplanting, demonstrating plasticity and high tolerance to water restriction. In addition, the results indicated that the use of hydrogel may be a viable approach after transplanting because it maintains constant substrate moisture. Monitoring through the Arduino system was efficient, and the results were validated by the other sensors used.

## Competing Interests

The authors declare no competing interests.

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## Author Contribution

All authors contributed to the study conception and design.

## Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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