

Contrasting responses of three tropical hardwood saplings species to a short-term drought experiment in Bahia, Brazil

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Research Article

Keywords: water stress, photosynthesis, stomatal conductance, isoprene, abscisic acid

Posted Date: October 21st, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-5153163/v1>

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Additional Declarations: No competing interests reported.

Abstract

The global demand for tropical hardwood continues to rise. However, exacerbated by a warming climate, high temperatures, and drought conditions during the dry season in many tropical regions is likely a contributing factor in the low survival rates of some planted hardwood tree seedlings grown under natural field conditions without watering. Here, we present a leaf-gas exchange and chlorophyll fluorescence experiment with tree seedlings of three species (*Astronium fraxinifolium* - AF, *Cariniana legalis* - CL, and *Handroanthus serratifolius* - HS) under well-watered and water stress conditions. Following the cessation of watering, leaf temperatures increased as soil water content and transpiration rates decreased. A gradual reduction of soil water content over 4-days negatively impacted assimilation net CO₂ rates (A_{net}), stomatal conductance (g_s) and transpiration (E) with CL showing the greatest reduction in A_{net} (94%), HS (90%), and AF the smallest reduction (77%). Moreover, the decline in A_{net} was not solely attributed to partial stomatal closure, as F_v/F_m photosynthetic parameters derived from chlorophyll fluorescence also declining throughout the drought. While HS did not show detectable emissions of volatile isoprenoids, AF and CL maintained leaf isoprene emissions in the light throughout the drought. Drought induced the leaf accumulation of abscisic acid in HS, although an unknown interference following ABA leaf extraction prevented its quantification in AF and CL. *The results indicate that common tropical hardwood species in Brazil are highly sensitive to water stress, with partial stomatal closure and isoprenoid synthesis playing an important role in the thermotolerance of photosynthesis during moisture stress.*

1. INTRODUCTION

Planted forests play a central role in meeting the growing global demand for tropical wood. However, sustaining the productivity of these planted forests represents a major challenge in the future scenarios from climate change (Payn et al., 2015, Nambiar, 2019). Although efforts to increase the productivity of plantations with tropical species are still incipient, it is essential to develop forests with effective tree species that can meet this demand (Nepal et al., 2019, FAO, 2022).

With ongoing climate change, rising temperatures and more frequent and intense drought events threaten the role of planted forests. Environmental stressors affect productivity, leading to differential mortality and requiring adaptive responses from tree species (Williams & Jackson, 2007, Marengo et al. 2011, IPCC, 2019). In this scenario, it is crucial to address how tropical tree species respond to stresses such as drought. Many studies have been driven by concerns about forest growth, productivity and carbon storage, affected by climate change (Marchin et al., 2020).

However, most studies have focused on survival and growth traits and do not provide an understanding of the mechanisms behind species performance (Campoe et al., 2014; Santos Junior et al., 2006; Werden et al., 2018). The physiological functional characteristics of plants can indirectly affect an individual's performance (Violle et al, 2007). Such characteristics can help to understand the mechanisms used by different species to favor their establishment (Campoe et al., 2014; Martínez-Garza

et al., 2013). Characteristics related to the ability of species to tolerate drought, such as photosynthesis (Anet) and stomatal conductance (gs), can help in species selection and increase initial performance for planting (Martínez-Garza et al., 2013; Ostertag et al., 2015; Werden et al., 2018).

In the setting of water and heat stress conditions, plants respond by closing their stomata to reduce water loss through transpiration (E), a mechanism that prevents damage (Nobel, 1999) but negatively affects Anet and gs (Cornic 2000, Evans et al. 2009). In this sense, the initial establishment of seedlings of tree species is a critical step for the success of forest plantations (Ostertag et al., 2015; Werden et al., 2018, Muller et al., 2019). Also, different abiotic factors, including high irradiance and temperature and soil moisture conditions, can impair their survival and growth (Campoe et al., 2014).

Strategies to ameliorate the negative effects of climatic stressors vary greatly between species, which can lead to a strong differential mortality between species in the initial establishment of seedlings in the plantation. Kobe and Vrisendorp (2011) analyzed 53 species of tropical trees and reported that mortality strongly depended on physiologically based life history traits. This reinforces the idea that the initial seedling establishment of young individuals comprises one of the most vulnerable life history phases for species (Kitajima & Fenner, 2000), under stressful environmental conditions. This may result in different initial mechanisms of biochemical responses to water stress and may be related to stability (homeostasis), preventing the shortening of plant life (Jones et al., 2012).

As plants exceed the physiological limits of humidity and temperature, for example, protective biochemical pathways are induced that can alleviate the negative effects of oxidative stress, with the production of volatile organic compounds VOC's, in the prevention of irreversible damage to tissues that can cause death (Jardine et al. 2014). Furthermore, studies indicate that VOCs act as effective plant antioxidants (Vickers et al. 2009, Loreto and Schnitzler 2010), but may vary between species under extreme environmental conditions (Rodrigues et al., 2020, Jardine et al. 2020). Furthermore, under conditions of high temperatures and drought, plants can produce the phytohormone abscisic acid (ABA), which stimulates stomatal closure, leading to a reduction in E, by regulating gs (Laloi et al., 2004, Brodribb & Feild, 2010, McAdam & Brodribb, 2018).

To the best of our knowledge, a scientific study in which the physiological and biochemical responses of *A. fraxinifolium*, *C. legalis* and *H. serratifolius* under contrasting conditions of water availability has not been performed before. These species were selected due to their ecological and economic relevance, as well as their distribution in tropical areas of Brazil. In this context, the main objective of this study was to investigate the leaf physiological strategies of seedlings in response to an experimental short-term drought. In addition, we aim to discuss aspects of underlying responses to defense mechanisms against water stress. Here, we aim to provide a better understanding of how the vital processes and responses of tree seedlings relate to their tolerance and susceptibility to water deficit, which could impact their successful use in the planted forest projects.

2. MATERIAL AND METHODS

2.1 Study area

The experiment was carried out in an experimental nursery in the district of Trancoso, located in the municipality of Porto Seguro, BA. It is situated close to the Pau Brazil National Park (PNPB) which represents one of the largest continuous of dense rainforest in the Coastal Tablelands in Brazil (Fig. 1). Previous studies (Silva & Nascimento 2001) highlighted the high richness of plant species in these areas and current surveys indicate that this area is one of the most diverse tropical forests in the world (Amorim, 2011). Currently, the region where the PNPB is inserted is called “Central Corridor of the Atlantic Forest” and includes the south of Bahia, the state of Espírito Santo in almost all its territorial extension and small areas of the east of Minas Gerais. This Corridor covers about 12 million hectares, with native forest covering approximately 12% of its area (Mori et al., 1983). The climate is type Af, hot and humid, without a dry season, according to the Köppen classification (Alvares et al. 2013), with altitude ranging between 10 and 80 meters above sea level. The average temperature is 22.6°C, ranging from 18.9°C to 27.9°C. Figure 2 shows the mean air temperature and solar radiation during the days the experiment was conducted.

2.2 Species selection

We selected three hardwood species: (1) *Astronium fraxinifolium* Schott, from the Anacardiaceae family, popularly known as gibatão and Gonçalves-alves. It is mainly found in rocky and dry lands (Lorenzi, 2002), with wide occurrence in the forest savannas of Central Brazil. It also occurs in transition areas between other biomes such as the Amazon Forest and the Atlantic Forest (Silva-Luz, 2017). *A. fraxinifolium* (AF) has excellent quality wood, is widely exploited for commercial use, and is often used in reforestation (Colmanetti and Barbosa, 2013), and recovery of degraded areas of the Brazilian Cerrado (Miranda et al., 2011). (2) *Cariniana legalis* Mart. O. Kuntze belongs to the Lecythidaceae family, popularly known as Jequitibá (Smith et al., 2016). It is an endemic tree of Brazil, it is found in the Amazon, followed by the Atlantic Forest (Prance and Mori, 2004). *C. legalis* (CL) is suitable for use in silvicultural management and restoration programs (Miranda Neto et al., 2012). (3) *Handroanthus serratifolius* (Vahl) SO Grose is a tree species of the Bignoniaceae family, popularly known as Ipê-amarelo. In Brazil, this tree can be found in the Amazon in the North region, in Ceará in the Northeast region, extending to the state of São Paulo in the Southeast region. It occurs in Evergreen Seasonal Forest, Ombrophylous Forest and Dense Ombrophylous Forest (Zappi et al., 2015). *H. serratifolius* (HS) wood is widely used for various applications, used in urban afforestation, and recommended for reforestation and forest restoration of degraded areas, especially in saline soils (Zappi et al., 2015).

2.3 Experimental design

The seedlings were grown in plastic bags (diameter 15 cm, height 25 cm) containing a mixture of humus soil and vermiculite in a 9:2 ratio. These plants were grown in a nursery, where they were given an unlimited supply of water and nutrients and light conditions similar to those found in their natural habitats (Fig. 3. a). All environmental factors, except irrigation water used in place of rain, were

maintained to mimic natural conditions. In early January, when the average plant height for AF, CL and HS reached 35, 45 and 60cm, respectively, water stress was imposed. All plants were irrigated daily until the beginning of the experiment. The seedlings of the three species were grown in a two-factor design (Species $\times$ water irrigation regime) with three replications (Fig. 3. b). For the first group (Well-watered, Control), the soil was watered slowly by hand twice a day, and for the second group (Drought, Dry), irrigation was drastically suspended during the experiment period short-term drought which lasted 5 days and then rehydrated on the fourth day.

2.4 Measurements of gas exchange (Anet), stomatal conductance (gs), leaf temperature (Tleaf) and Soil Moisture (VWC)

Gas exchange parameters were measured on three leaves per plant of each species, which were fully mature and exposed to direct light. Data collections were performed between 7:00 and 17:00 with a 2:00-hour interval during the experimental days. A portable infrared gas analyzer in an open system configuration (LI-6400, Li-Cor, Lincoln, NE, USA) was used to measure the following parameters: CO₂ assimilation (Anet, $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$), stomatal conductance (gs, $\text{mol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$), transpiration rate (E, $\text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$), Fv/Fm and ETR. Water use efficiency (WUE) and intrinsic water use efficiency (WUEi) ($\text{mmol CO}_2 \text{ mmol H}_2\text{O}^{-1}$) were determined as the ratio between Anet/E and Anet/gs, respectively (Fischer & Turner, 1978; Gago et al. 2014). The leaf temperature regulation capacity during the experiment was obtained by calculating the difference between air and leaf temperatures ($\text{DT} = T_{\text{air}} - T_{\text{leaf}}$) (Souza et al. 2004).

To verify the progression of stomatal behavior under natural conditions, during the days of the experiment, the stomatal conductance was measured with the SD-1 Porometer equipment (Decagon Devices®, WA, USA) with an interval of 10 minutes, every hour, between 8:00 AM and 3:00 PM. Leaf instantaneous temperature was measured using Thermal Imager (Model E5, Flir, USA) at a distance of 0.5 m from each leaf. The Hydrosense II portable sensor (Model CS659, Campbell, USA) was used to measure soil moisture content. Measurements were performed hourly between 8:00 AM and 6:00 PM local time, for both treatments Control and drought.

2.5 ABA concentration measurements

Leaves were collected on the fourth day between 9:00 AM and 3:00 PM local time, respectively. The leaves were bagged, identified, and stored in a portable freezer at -20 (°C). After the field stage, the samples were taken to the Laboratory of Molecular Genetics at the State University of Santa Cruz-UESC in Ilhéus-BA. The method proposed by (Kim et al., 2012) was used to extract and quantify leaf abscisic acid. Leaf tissue was ground in liquid nitrogen and extracted in the dark in ABA extraction buffer (80% methanol with hydroxytoluene 100 mg. L⁻¹ butide and 500 mg. L⁻¹ citric acid) for 16 hours with constant stirring at 4°C. The supernatant was collected after centrifugation (10,000 xg) and diluted 10 times with TBS buffer (50 mM TRIS, 1 mM MgCl₂, 150 mM NaCl, pH 7.8). Subsequently, the ABA

concentration was quantified by ELISA with the Phytodetek ABA test kit (Agdia, Elkhart, IN, USA). After completing the procedure established in the described protocol, the optical density was read by a plate spectrophotometer at a wavelength of 405 nm and the standard curve was calculated to determine the concentration of leaf ABA.

2.6 Emissions of volatile isoprenoids and net photosynthesis under controlled environmental conditions

Gas exchanges and isoprene emission rates were quantified from leaves during changes in photosynthetically active radiation (PAR) using the (LI-6400XT, LI-COR Inc., USA) controlled parameters temperature, irradiance, and CO₂ concentration, under standard conditions (30°C leaf temperature, 1000 $\mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ PAR level and 400 ppm CO₂ concentration). A modification in the LI6400XT was made so that a fraction of the air leaving the leaf chamber was diverted to the Less -P for the collection of VOC's. Samples were automatically collected in thermal desorption (TD) tubes using a portable autosampler (Less -P, Signature Science LLC., Austin, TX, USA), lasting 10 min, passing 750 ml of air sample from the compartment of the leaf through each tube in 10 minutes (75 ml min⁻¹). Once a leaf was placed in the chamber with the initial conditions (30°C leaf temperature, 400 ppm reference CO₂, 0 $\mu\text{mol m}^{-2} \cdot \text{s}^{-1}$ PAR), the sample and infrared reference gas analyzes were performed. combined and the autoprograms on both LI -6400XT and Less-P have been started. For the LI-6400XT, a light curve program consisted of recording data every 30 seconds while controlling PAR for 10 minutes. After collecting the samples in the field, the thermal desorption tubes were transported to the forest management laboratory of the National Institute for Research in the Amazon (INPA) in Manaus, Brazil. For the analysis of adsorbed isoprene, an automatic thermal desorption system (TD-100, Thermal Desorber, Markes International, UK) coupled to a gas chromatography system (7890A series, Agilent Technologies, USA) and mass spectrometer (Agilent ChemStation, Agilent Technologies, USA) (TD-GC-MS) system was calibrated for isoprene using m/z 67 as described by Jardine et al., (2014, 2016,2020).

2.7 Statistical analysis

The data were submitted to ANOVA's having as main effects species and treatment; species was analyzed as a random effect and treatment as a fixed effect. When there was a significant interaction between species $\times$ treatments ($p \leq 0.05$), individual species responses were analyzed by Tukey 's t test ($p < 0.05$). All statistical analyzes were performed using the free software R version 3.6.0 (R DEVELOPMENT CORE TEAM, 2019). All data were tested for normality with the Shapiro Wilk test.

3 RESULTS

3.1 Physiological responses to water stress

The experimental treatment gradually reduced the volumetric soil water content of potted plants from field capacity ($\sim 30\%$, control VWC) to severe water deficit for AF, $3.06 \pm 1.19\%$, CL, $2.6 \pm 0.26\%$ and HS

$2.16 \pm 1.34\%$ (95% CI) during one period of the experiment (**Supplementary Fig. 1. a**). There was a significant difference in the soil VWC between the control and dry treatments ($p < 0.001$, $n = 24$) in the maximum drought intensity on the fourth day of the experiment. The average soil VWC was higher for the control treatment in relation to the drought treatment, for all studied species (20–30% vs. 2–3%, respectively). Furthermore, the effect of the drought treatment on the soil VWC VPD leaf and Relative Humidity leaf variables during the experimental days are presented (**Supplementary Figure SFigure 1. b and c**).

3.2 Physiological responses to water deficit

During the experiment with young seedlings of *A. fraxinifolium* (AF), *C. legalis* (CL) and *H. serratifolius* (HS) in a full sunlight, subjected to two conditions of water regime, one in ideal conditions (Control) and another with water deficit conditions (Drought) the gas exchange results were observed, which demonstrate the means and confidence intervals at 95% and the minimum and maximum of the CO_2 assimilation rate (A_{net}), stomatal conductance (gs), transpiration (E), as well as WUE and WUEi. The results of the ANOVA for the treatments with the dependent physiological variables (**Supplementary Table – Stable 1**) are presented.

The mean A_{net} values for AF seedlings ranged from $3.52 \pm 0.20 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $2.3 \pm 0.19 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (95% CI) between control and drought treatments. For CL, the mean A_{net} values ranged from $3.83 \pm 0.09 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $1.98 \pm 0.12 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (95% CI). While for HS the mean A_{net} values ranged from $6.56 \pm 0.35 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $1.03 \pm 0.02 \mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (95% CI). The mean gs values for AF seedlings ranged from $0.0405 \pm 0.0019 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $0.0314 \pm 0.0014 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (95% CI) between the control and drought treatments. For CL, the mean gs values ranged from $0.0419 \pm 0.0007 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $0.0215 \pm 0.0020 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (95% CI). While for HS the average gs values ranged from $0.0637 \pm 0.0042 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $0.0117 \pm 0.0002 \text{ mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (95% CI). A statistically significant difference was observed for gs values observed between treatments ($p < 0.001$). The mean E values for AF seedlings ranged from $1.27 \pm 0.06 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $1.09 \pm 0.04 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (95% CI) between control and drought treatments. For CL the mean E values ranged from $1.31 \pm 0.07 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $0.784 \pm 0.069 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (95% CI). While for HS the mean E values ranged from $2.11 \pm 0.01 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ to $0.457 \pm 0.009 \text{ mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (95% CI). Thus, a statistically significant difference was observed for the E values observed between treatments ($p < 0.001$). The mean WUE values for AF seedlings between the control and drought treatments ranged from $88.5 \pm 3.11 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ to $70.8 \pm 2.85 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ (95% CI). For CL, the average WUE values ranged from $91.9 \pm 2.51 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ to $94.4 \pm 2.75 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ (95% CI). While for HS the average WUE values ranged from $108 \pm 3.24 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ to $88.5 \pm 1.94 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ (95% CI). Thus, a statistically significant difference was observed for the WUE values observed between

treatments ($p < 0.001$). The mean WUEi values for AF seedlings between the control and drought treatments ranged from $3.14 \pm 0.22 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ to $2.04 \pm 0.09 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ (95% CI). For CL, the mean WUEi values ranged from $3.13 \pm 0.22 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ to $2.57 \pm 0.06 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ (95% CI). While for HS the average WUEi values ranged from $3.17 \pm 0.08 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ to $2.28 \pm 0.08 \mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ (95% CI). Thus, a statistically significant difference was observed for the WUEi values observed between treatments ($p < 0.001$). The species showed losses in the Fv/Fm ratio values (**Supplementary Figure – SFigure 2**). The mean values for AF ranged from 0.65 ± 0.002 to 0.45 ± 0.004 and there was a reduction of 31.35% compared to the beginning of the experiment. The CL species presented mean values that ranged from 0.63 ± 0.005 to 0.59 ± 0.005 and was the one that showed the smallest reduction of 6.73%. The HS species presented mean values that ranged from 0.61 ± 0.004 to 0.48 ± 0.006 and a reduction of 22.01%.

It was observed that there was a statistically significant difference in physiological variables between species and treatments ($p < 0.001$). This interaction suggests that the different studied species respond differently to the treatments applied, resulting in varied strategies. Similar results were observed in the daily progression of physiological variables during 4 uninterrupted days in the Drought treatment (Fig. 4. **a-e**). Considering the beginning and the end of experiment, the CL species presented the highest loss rates (Fig. 4. **f-j**) in A_{net} (94.33%), g_s (91.66%), E (86.57%), WUE (59.01%) and WUE_i (29.46%), followed by HS which presented losses in A_{net} (90.27%), g_s (88.05%), E (82.44%), WUE (39.23%) and WUE_i (11.40%). In contrast, AF presented the lowest losses in A_{net} (77.23%), g_s (61.28%), E (41.24%), WUE (66.71%) and WUE_i (43.69%). Although CL presented the greatest losses, it was the species that had the highest recovery rate with rehydration on the 5th day of the experiment (Fig. 4. **f-j**) representing recovery in A_{net} (45.88%), g_s (83.75%), E (77.50%), WUE (50.20%) and WUE_i (31.37%), followed by AF which showed recovery in A_{net} (43.16%), g_s (50.73%), E (32.75%), WUE (46.61%) and WUE_i (26.63%). In contrast to the other HS, it presented the worst recovery rates in A_{net} (1.50%), g_s (20.50%), E (1.52%), WUE (1.64%) and WUE_i (-17.38%).

3.3 Effect of soil moisture on leaf temperature

For the variable leaf temperature (T_{leaf}), the averages between species were compared. The Fig. 5. **a**) shows thermophotographs with the maximum temperatures observed for each species. There was no significant difference in leaf temperature between AF and CL ($t = -0.41^\circ\text{C}$, $p > 0.05$). On the other hand, there was a significant difference in leaf temperature between AF and HS ($t = -1.72^\circ\text{C}$, $p = 0.012$). Finally, there was no significant difference in leaf temperature between CL and HS ($t = -1.31^\circ\text{C}$, $p \text{ value} > 0.05$) (Fig. 5. **b**). HS presented, on average, the highest values of leaf temperature, reaching a maximum of 48.6°C . The results presented in (Fig. 5. **c**) demonstrate a significantly ($p < 0.01$) greater dissipative capacity in HS e AF during the afternoon except at 9:00 h (low temperature). However, HS, obtained a reduction in the dissipative efficiency between 13 and 14:00h. While CL from 10:00 h presented greater dissipative efficiency of AF for maintenance of leaf temperature and cooling. In general, throughout the day there was a stronger effect of induced drought on CL as AF and HS showed dissipative capacity in

hours of higher air temperature. The ability to maintain and cool leaf temperature in relation to changes in air temperature was evaluated as an estimate of global energy dissipation through latent heat. Thus, a negative result of the ratio $DT = T_{\text{leaf}} - T_{\text{air}}$ ($DT < 0$) indicates loss of dissipative capacity by showing heating of the leaf surface in relation to air temperature, while positive values ($DT > 0$) result in plants with adequate dissipative efficiency.

3.4 Biochemical responses to water stress

Leaf ABA was present in *H. serratifolius*, with very low values in *C. legalis* and absent in *A. fraxinifolium*. The ABA concentration was higher at 9:00 am local time when compared to the values at 11:00 am and 3:00 pm. It was observed that isoprene emission was uncoupled from A_{net} and g_s in AF, mainly in the morning where A_{net} rates were higher as well as leaf temperature, being higher than $16 \text{ nmol m}^{-2} \cdot \text{s}^{-1}$ at 13:00 local time, while photosynthesis for that same horary decays. The CL species did not show this behavior, maintaining its A_{net} rates during the day while there was no considerable isoprene emission. At the end of the experiment, the HS species presented the lowest values of A_{net} and g_s at the same time, a higher concentration of ABA was observed in the early morning and a decrease in the late afternoon, opposing the behavior of A_{net} coupled to g_s .

During the five days of experiment with young seedlings in a full sunlight, submitted in two conditions of water regime, it was observed that the concentration of VOC's, especially isoprene was present in AF and CL and varied during the days of experiment, as well as contrasting its emission between species and keeping it partially constant in the control (Fig. 6. a). While for HS, it was not detected. Although the presence of 3-Carene compounds in AF and Sabinene in HS was also observed (Fig. 6. b). It was observed that the emission of VOC's was accentuated for AF in the morning, with a peak at local time and decreased throughout the late afternoon. In contrast CL did not show this behavior keeping the emission constant but much lower. Leaf ABA collections occurred at the end of the experiment and higher values were identified in the Drought treatment.

4 DISCUSSION

4.1 Effect of water stress on gas exchange

Stress definitions are usually associated with environmental factors that reduce the productivity or survival capacity of plants (Grime, 1989; Jones & Corlett, 1992). Water stress, for example, is one of the main limitations for plant growth and development, since water is a crucial factor for photosynthesis and other metabolic functions. We adopted the definition of stress proposed by Jones et al., (2013) according to which stress is a singular phenomenon caused by external disturbances, interrupting the hierarchical organization of a system, affecting its stability (homeostasis), decreasing life span and/or decreasing the reproductive capacity of plants. In the setting of water stress conditions, plants generally respond by closing stomata to reduce water loss through transpiration, which negatively affects photosynthesis, regulated by lower CO_2 concentration in chloroplasts (Cornic 2000, Evans et al. 2009,

Brodrigg & Feild, 2010, Deans et al., 2019). The importance of diffusive and metabolic limitations to the photosynthetic process during stages of water stress is still under debate (McAdam and Brodrigg, 2018, Deans et al., 2019, Marchin et al., 2020). As the drought progresses, stomatal closure occurs for longer and longer periods of the day in field-grown plants, starting in mid-morning (Tenhunen et al., 1987, Nobel, 1999, Manzoni et al., 2011). This depression in gas exchange simultaneously reduces daily carbon assimilation and water loss at the moment of greatest evaporative demand from the atmosphere (Cowan, 1982., Jones, 1992).

Based on the main results presented, we can infer that severe water stress in the present study had a significant impact on gas exchange and efficient water use (WUE) in seedlings of three plant species AF, CL and HS. The gradual reduction of the volumetric soil water content of the potted plants from field capacity to a severe water deficit resulted in a drastic and synchronized decrease in the rate of CO₂ assimilation (A_{net}), stomatal conductance (g_s) and transpiration (E) in all three species as found by Souza et al., (2004). These results associated with the physiological assessments indicated a reduction in the homeostatic capacity of the species, suggesting that they were seriously affected by the drought, characterizing a typical situation of severe stress (Marchin et al., 2020). Notably, each plant species reacted differently to water stress treatments, with some showing greater reductions in A_{net} and g_s than others. The species *H. serratifolius*, for example, was the most affected by water stress, showing the greatest reductions in gas exchange and efficient water use (WUE and WUEi), the opposite has already been reported for moderate drought induction (Yang et al., 2020). In the opposite, AF was the least affected species, showing the smallest reductions in gas exchange and efficient water use. These results suggest that different plant species have different mechanisms of adaptation to water stress, with some species being more tolerant than others. In addition, the results highlight the importance of monitoring soil water availability to adopt appropriate management strategies to minimize water stress when plants are in field condition. Other studies with tropical species have demonstrated a loss in F_v/F_m , as an indicator of photoinhibition, and that these species, regardless of ontogenetics, show a significant loss in efficiency when subjected to high irradiance environments, associated with a reduction in growth rate (Krause, et al. 2012). The apparent electron transport rate (ETR) can cause extensive damage to membranes evidenced by increases in lipid peroxidation. The response to photoinhibition of the studied species becomes evident when we evaluate the F_v/F_m ratio, so that all species, throughout the duration of the experiment with drastic reduction in irrigation, showed significant losses. This behavioural result has already been observed in other studies associated with a reduction in growth rate (Sobrado, 2008, Krause, et al. 2012). The F_v/F_m and ETR ratio values (**Supplementary Figure – SFigure 2**) indicate that the AF, and HS species have undergone chronic stress, a situation that generates irreversible damage to the photosystem (Osmond, 1994; Osmond and Grace, 1995). In contrast to CL which presented a non-chronic value of 0.5. This is evident when observing the lowest F_v/F_m values on the last day of the experiment, so that the damage suffered over the days in the photosystems is not recovered. It is necessary to study more carefully whether these species tolerate high irradiance and low water availability as a strategy for dissipating excessive energy and how they recover after a longer period of rehydration.

4.2 Leaf temperature and physiological responses

Leaf temperature is a key factor in assessing water stress, as it influences various physiological and biochemical processes in plants. Specifically, it can alter the rates of photosynthesis, transpiration, respiration, and the synthesis of secondary metabolites, among other critical functions. When temperatures rise over a short period, the impact on CO₂ assimilation (Anet) depends on whether the temperature exceeds the optimal range. While some plants acclimate to elevated temperatures without damage to their photosynthetic systems, leading to a higher thermal optimum (Berry and Björkman, 1980), drought conditions often intensify the negative effects when combined with heat. The degree to which photosynthesis responds to rising temperatures varies by species, and this may be linked to changes in CO₂ transport within the chloroplasts (Fares et al., 2011; Deans et al., 2019). For instance, isoprene production, which occurs in the chloroplast, is closely associated with photosynthesis, with a large portion of the carbon used in isoprene synthesis derived from primary substrates (Sharkey and Yeh, 2001). Nevertheless, how isoprene emission adapts to the combined effects of temperature increases and water stress is not fully understood. Some evidence suggests that high temperatures boost isoprene emissions in water-stressed plants, even as photosynthetic rates (Anet) decrease (Fortunati et al., 2008; Jardine et al., 2013, 2014). Our findings showed that the water deficit treatment triggered physiological responses consistent with drought stress. All species displayed partial stomatal closure under simulated drought, resulting in higher leaf temperatures in the Dry treatment group. Stomatal conductance and transpiration contribute to leaf cooling through evaporation (Nobel, 1974; Farquhar and Sharkey, 1982), so partial stomatal closure under drought conditions can lead to increased leaf temperature (Medina and Gilbert, 2015; Buckley, 2016; Buckley, 2017), which may be linked to xylem cavitation thresholds (Bartlett et al., 2016; Martin-StPaul et al., 2017), thereby helping limit tissue damage during water stress. The severity of the experimental drought resulted in wilting and leaf shedding in some species, particularly in HS, indicating that the water stress was intense. Moreover, Anet and isoprene emissions became decoupled, especially during the hottest parts of the day. These findings are consistent with previous research showing that isoprene production tends to increase with rising leaf temperatures (Jardine et al., 2016). Isoprene production is linked to the plant's defense mechanisms against oxidative and thermal stress. However, the same behavior was not observed for CL and HS, which may explain that when leaf temperature increases, plants need to invest more energy in maintaining their thermal homeostasis, which may reduce the availability of resources to produce isoprene. This was most evident with the isoprene flux behavior in AF during daylight hours, which may be an adaptation to avoid oxidative damage caused by sunlight and high temperatures. In summary, leaf temperature plays an important role in regulating VOC production, and these results may have important implications for understanding the effects of water stress on species emission when subjected to natural or induced water stress.

4.3 The effect of water stress and ABA

When the water supply is inadequate or the transpiration rate exceeds the water supply, the plants close the excessive stomatal opening as a first line of defense against the decrease of the internal water

potential. This is primarily induced by increased abscisic acid (ABA), which reduces sweating (McAdam and Brodribb, 2016). Stomatal closure causes a drop in CO₂ mesophilic, thereby increasing the concentration gradient for CO₂ diffusion and increasing WUEi (McAdam and Brodribb, 2016). ABA accumulation is considered a key factor that can optimize WUEi and shape plant adaptation to water stress (Campitelli et al., 2016), and concomitantly by increasing leaf temperature and DPV (Sampaio-Filho et al., 2018). Under stress conditions such as drought, plants produce ABA in large amounts to regulate their metabolism and protect themselves from dehydration (Yao et al., 2021a). Therefore, under water stress conditions, plants need to carefully balance stomatal closure to avoid excessive dehydration, but still allow sufficient CO₂ entry for photosynthesis (Yao et al., 2021b). Another important function of ABA is the regulation of gene expression. These regulatory factors may explain the results found in this study, where significant values of ABA concentration were observed only in HS in the drought treatment. Two hypotheses are presented here, (i) the experiment with ABA at the end failed to capture the interaction of ABA between species with treatments because water stress exceeded the ability of plants to respond and (ii) species differ in protective tactics to stress, corroborating the results found with isoprene emission in AF and (iv) HS was the only one to present significant concentrations of ABA because there was a low percentage of rehydration on the fifth day, as it presented a prolonged effect of ABA in the leaves. In summary, ABA plays a key role in regulating plant responses to environmental stress, helping to coordinate plant response to drought conditions and other forms of stress.

4.4 Induced drought and the preparation of seedlings before planting

It is evident, with the data presented, the importance of hardening young seedlings before planting in the field with the application of accentuated stresses, aiming at increasing resistance to lack of water (Marchin et al., 2020). Similar results were found in the study by Franco et al., (2005), with better seedling performance after severe conditions of lack of water. Other studies point in the same direction, with treatments for moderate water deficiency and severe water deficiency where the seedlings that suffered some degree of deficiency during hardening, showed greater tolerance to water stress (Villar-Salvador et al., 2013). This behavior of improvement in the growth performance of plants that have been under deficit irrigation, after a prolonged recovery period (irrigation in the first week maintained at field capacity) is a compensation for the loss in the primary productivity rate in response to the previous low water availability. It has already been observed in *Pinus resinosa* (Miller and Timme, 1994) and in *Quercus petraea* (oak) (Mijnsbrugge et al., 2016). Christersson (1972), demonstrated that non-rustified seedlings of tree species of the genus *Picea*, when planted in the field, were not physiologically prepared to tolerate stress events such as drought. Therefore, AF, CL and HS species suffered significant degrees of water deficit in pots, in simulation of field conditions, under water stress that characterized a drought event during the experiment. The present study highlights the importance of water deficit in the hardening process so that seedlings can be better prepared and acclimatized to the adverse conditions encountered in the first months of planting in the field. Without the stage of severe drought simulation

with Dry treatment, it would not be possible to observe the change in the behavior of the studied species. These results help to corroborate the importance of applying water stress events due to deficiency close to the point of permanent wilting, in the hardening phase, in order to condition the plant to obtain an increase in its performance under adverse conditions, when planted in the field.

5 CONCLUSION

This study provides important insights into the physiological and biochemical responses of three plant species subjected to water stress conditions. Gas exchange measurements, such as CO₂ assimilation rate and stomatal conductance, were negatively affected by water deficit, indicating a reduction in photosynthetic capacity and in the regulation of water loss by plants. In addition, water use efficiency and intrinsic water use efficiency were also influenced by water stress, showing the importance of plant adaptation to maximize the use of available resources. Leaf temperature and emissions of volatile organic compounds showed significant variations between species, indicating different response strategies to water stress. Absciscic acid concentration also showed variations, being higher in the morning, which suggests a specific temporal response of plants to stress. These findings underscore the complexity of plant responses to water stress and highlight the need to consider species diversity when developing management and conservation strategies in water-scarce environments. Understanding the physiological and biochemical responses of plants to water stress is critical to improving productivity in planted forests, natural ecosystems conservation, and facing the challenges arising from climate change.

Declarations

Data citation

All data supporting the findings of this study are available in the paper and its Supplementary Information. Additional data will soon be made available in the NGEE-Tropics archive: <https://ngt-data.lbl.gov/>.

Plant source

The plant seedlings used in the present study were produced by the company Symbiosis Investimentos e Participações S.A., where the field experiment was conducted.

Ethics approval and consent to participate

The three plant species used in this study were acquired from the company Symbiosis Investimentos e Participações S.A., which provided all the necessary plant material. The collection and handling of the

samples were conducted following current research ethical guidelines, ensuring compliance with all applicable standards for the preservation and study of genetic resources. Symbioses is duly authorized to supply plant material for scientific research, and all samples were appropriately documented and characterized for this study. Research and development actions related to the restoration supply chain in the region are conducted in collaboration with the Federal University of Southern Bahia (UFSB) and are part of technological action plans for sectors such as energy systems, agriculture, forestry, and land use, under the Ministry of Science, Technology, and Innovations, and the United Nations Environment Programme, 2021. Additionally, various research projects focusing on restoration are being developed through these collaborations.

Ethical declaration

The authors confirm that all methods in this study were carried out by relevant guidelines and regulations of Symbiosis Investimentos e Participações S.A. The experimental protocols were approved by the Symbiosis Investimentos e Participações S.A. The collection of the plants used in the study complies with local or national guidelines with no need for further affirmation.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This material is based upon work supported as part of the Next Generation Ecosystem Experiments-Tropics (NGEE-Tropics), as part of DOE's Terrestrial Ecosystem Science Program – Contract No. DE-AC02-05CH11231. Additional funding for this research was provided by the Coordination for the Improvement of Higher Education Personnel (CAPES), Fundo Brasileiro para a Biodiversidade (Funbio) and Instituto humanize (HI) with Bolsa Funbio Conservando o Futuro.

Author Contribution

IJSF, KJJ, BOG, performed the experiments and analyzed the data. JQC, NH, and KJJ planned and designed the experiments. IJSF, CASS, APS and DCS wrote the manuscript text and IJSF and KJJ prepared all figures. All authors reviewed the manuscript.

Acknowledgement

The authors are thankful for the logistical and scientific support provided by the Laboratório de Manejo Florestal (LMF) at the National Institute of Amazonian Research (INPA) and the UESC.

Data Availability

All data supporting the findings of this study are available in the paper and its Supplementary Information. Additional data will soon be made available in the NGEE-Tropics archive: <https://ngt-data.lbl.gov/>.

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75. Supplementary Figure
76. **SFigure 1** Daily evolution of abiotic variable during the experiment. a) Soil volumetric water content (VWC, %), b) Leaf vapor pressure deficit (VPD, Kpa) and c) leaf relative humidity (%) in the treatment without irrigation (Drought) until the 4th day of the experiment. The species are represented by the colors *A. fraxinifolium* (purple), *C. legalis* (green) and *H. serratifolius* (red). The shadows represent the confidence interval (95%). Partial recovery on the 5th day after rehydration is also represented.
77. **SFigure 2** Daily evolution of the responses of the variables in the treatment without irrigation (Drought) over 5 days of experiment. a) Maximum quantum efficiency of photosystem II (Fv/Fm) and b) Apparent electron transport rate (ETR). The species are represented by the colors *A. fraxinifolium* (purple), *C. legalis* (green) and *H. serratifolius* (red). The shadows represent the confidence interval (95%).
78. Supplementary Table
79. **Stable 1.** Descriptive analysis of physiological variables measured during the experiment. Treatment (TR) with Well-watered – Control (C) and without irrigation - Drought (D). The Species (Sp) *A. fraxinifolium* (AF), *C. legalis* (CL) and *H. serratifolius* (HS). Average values with confidence interval (95%) and minimum and maximum values (Min and Max) for the variables are presented. The physiological variables are photosynthesis (A_{net} in $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); stomatal conductance (gs in

mol H₂O.m⁻².s⁻¹); transpiration (E in mmol H₂O.m⁻².s⁻¹); water use efficiency (WUE - A_{net}/E in μmol CO₂. mmol H₂O m⁻².s⁻¹) and intrinsic water use efficiency (WUEi - A_{net}/gs in μmol CO₂. mmol H₂O m⁻². s⁻¹).

Figures



Figure 1

Porto Seguro City; Pau Brazil National Park; Study area. The site where the experiment was conducted is between the Pau Brazil National Park (PNPB) and the southern coast

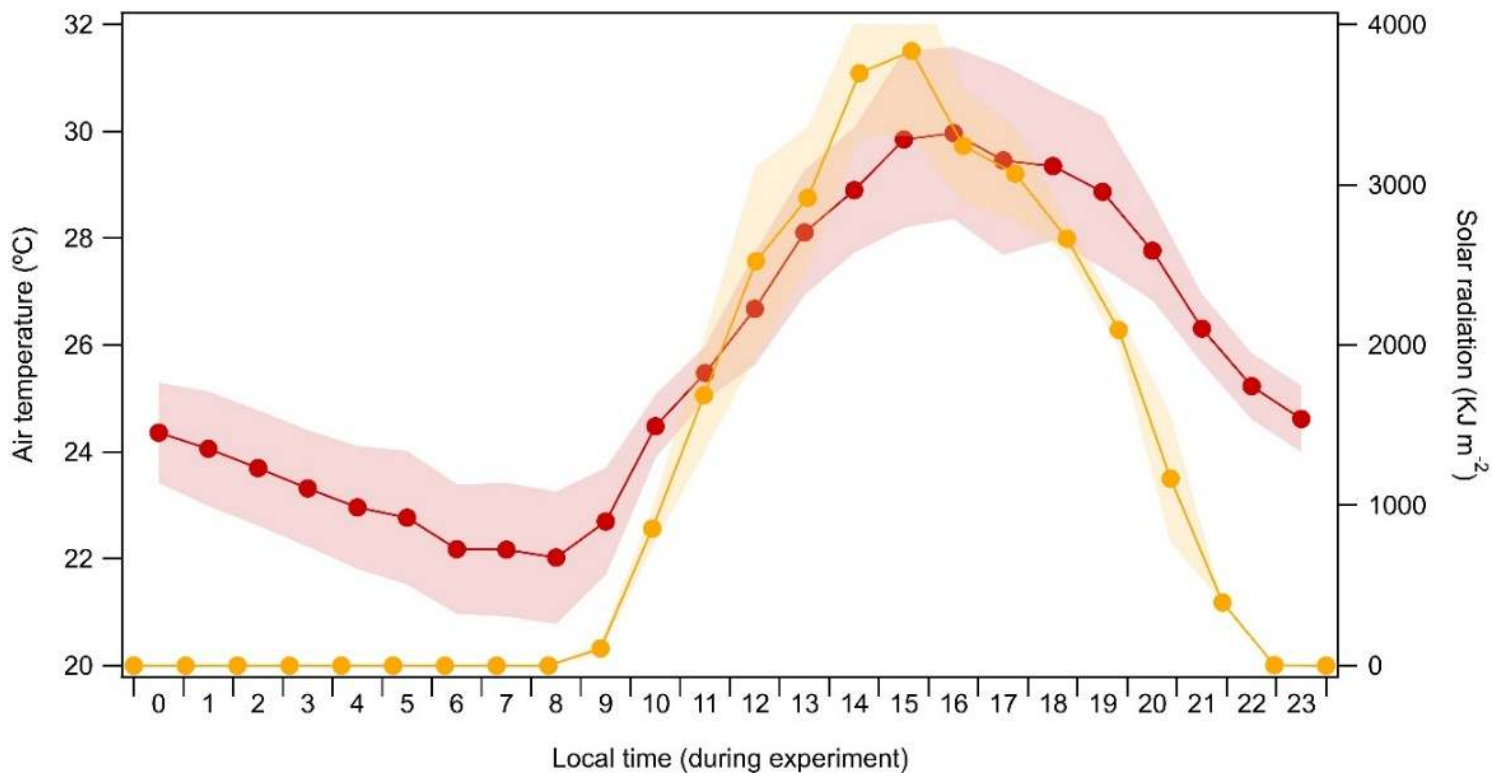


Figure 2

Hourly variations of air temperature and solar radiation during the days of the experiment (from January 04th to 11th, 2018. Source: Meteorological data for the municipality of Porto Seguro, BA, INMET, accessed at <http://www.inmet>

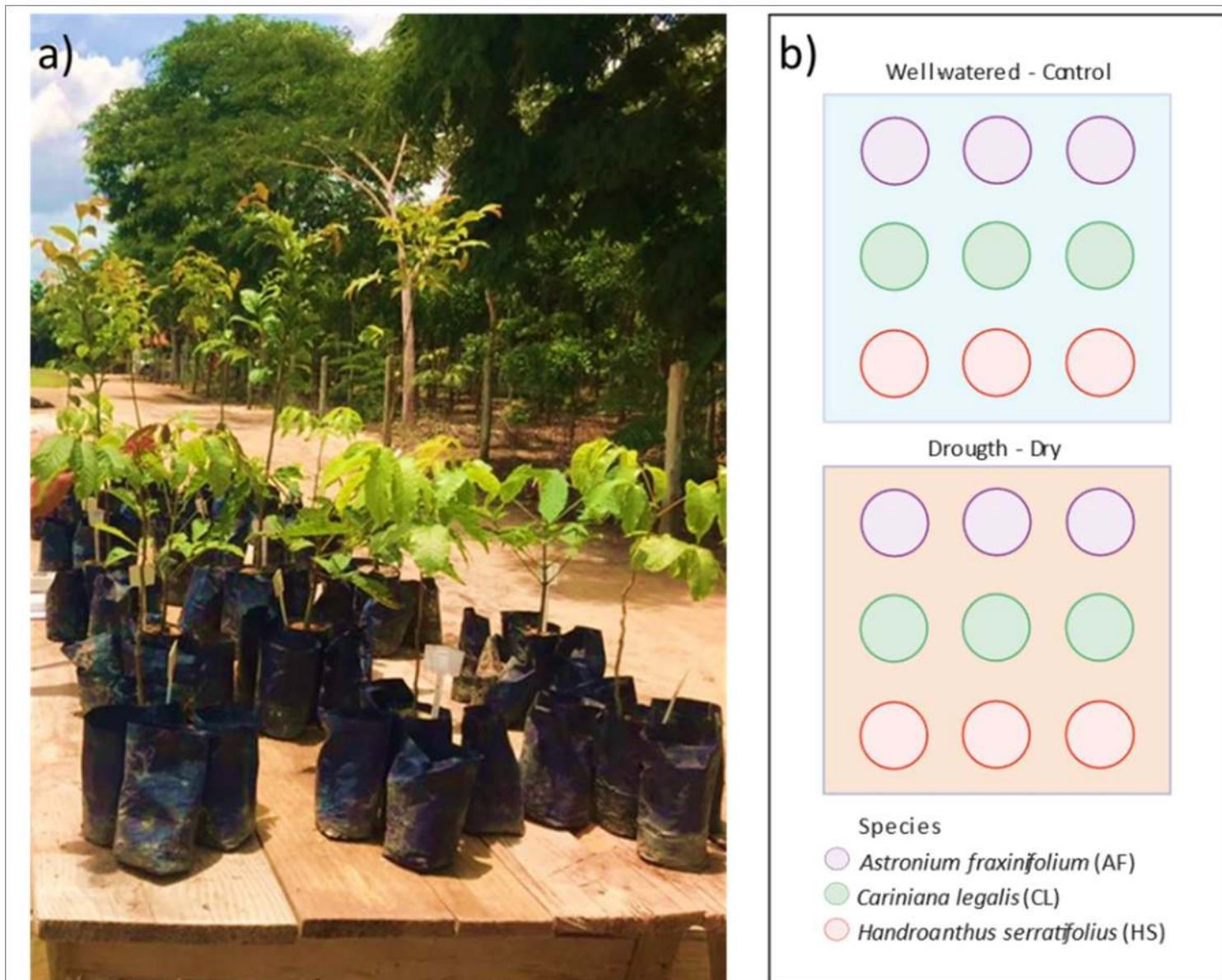


Figure 3

Experimental research design. a) Selection and arrangement of species in the bed, all with a healthy stage without signs of herbivory and b) Experimental design of the species Well-watered – Control group and without irrigation - Drought group during the experiment

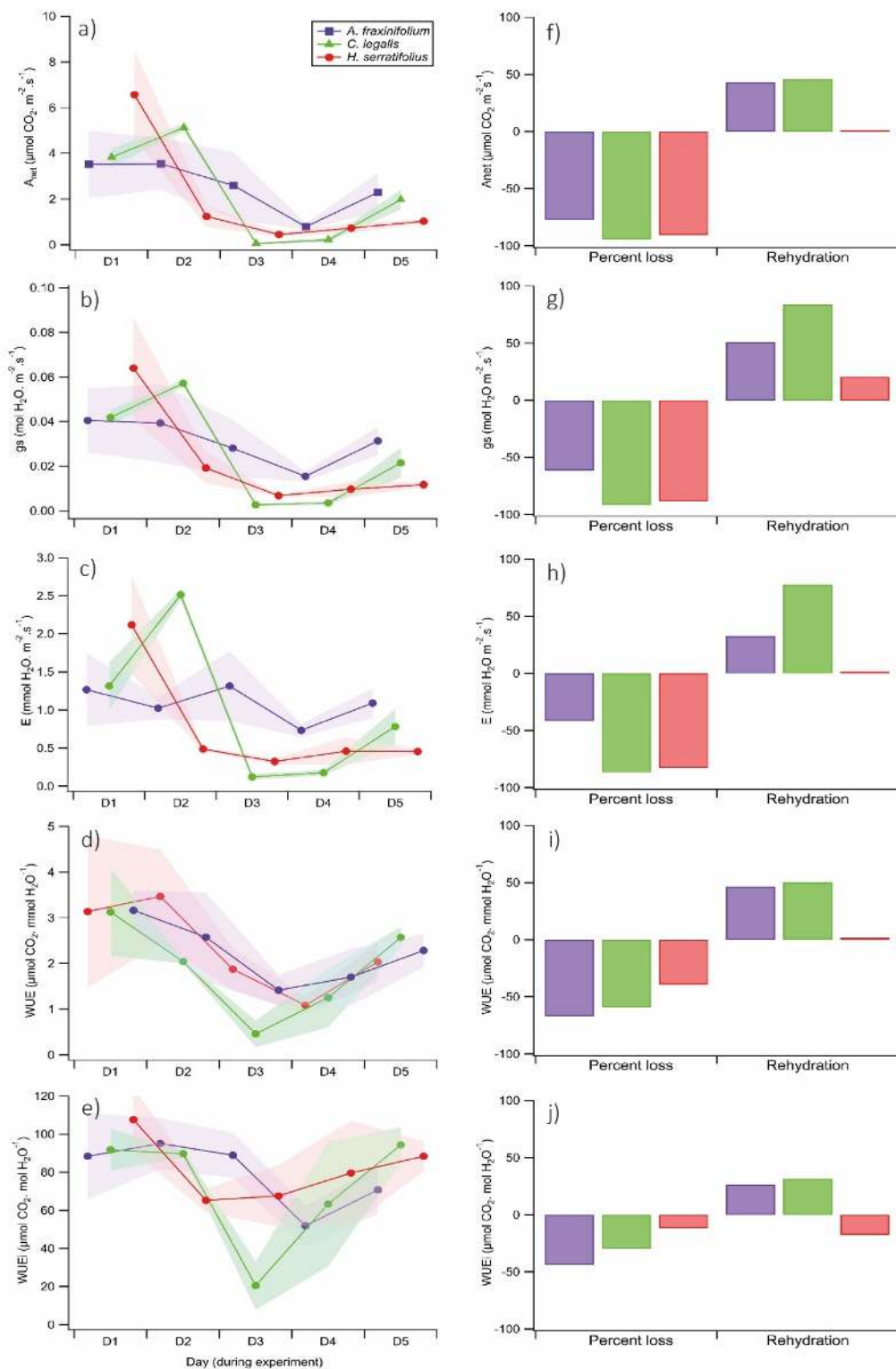


Figure 4

Daily progression of physiological variables responses to drought treatment (without irrigation). The species are represented by the colors *A. fraxinifolium* (purple), *C. legalis* (green) and *H. serratifolius* (red). On the left panels are presented the physiological variables across the Days (D) of experiment a) Photosynthesis (A_{net} in $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), b) stomatal conductance (g_s in $\text{mol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), c)

transpiration (E in $\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); d) water use efficiency ($\text{WUE} - A_{\text{net}}/E$ in $\mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$) and e) intrinsic water use efficiency ($\text{WUEi} - A_{\text{net}}/g_s$ in $\mu\text{mol CO}_2 \cdot \text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$). The shaded areas represent the confidence interval (95%). The right panels represent the loss rates before and after the experiment until the 4th day, followed by the recovery rates after rehydration

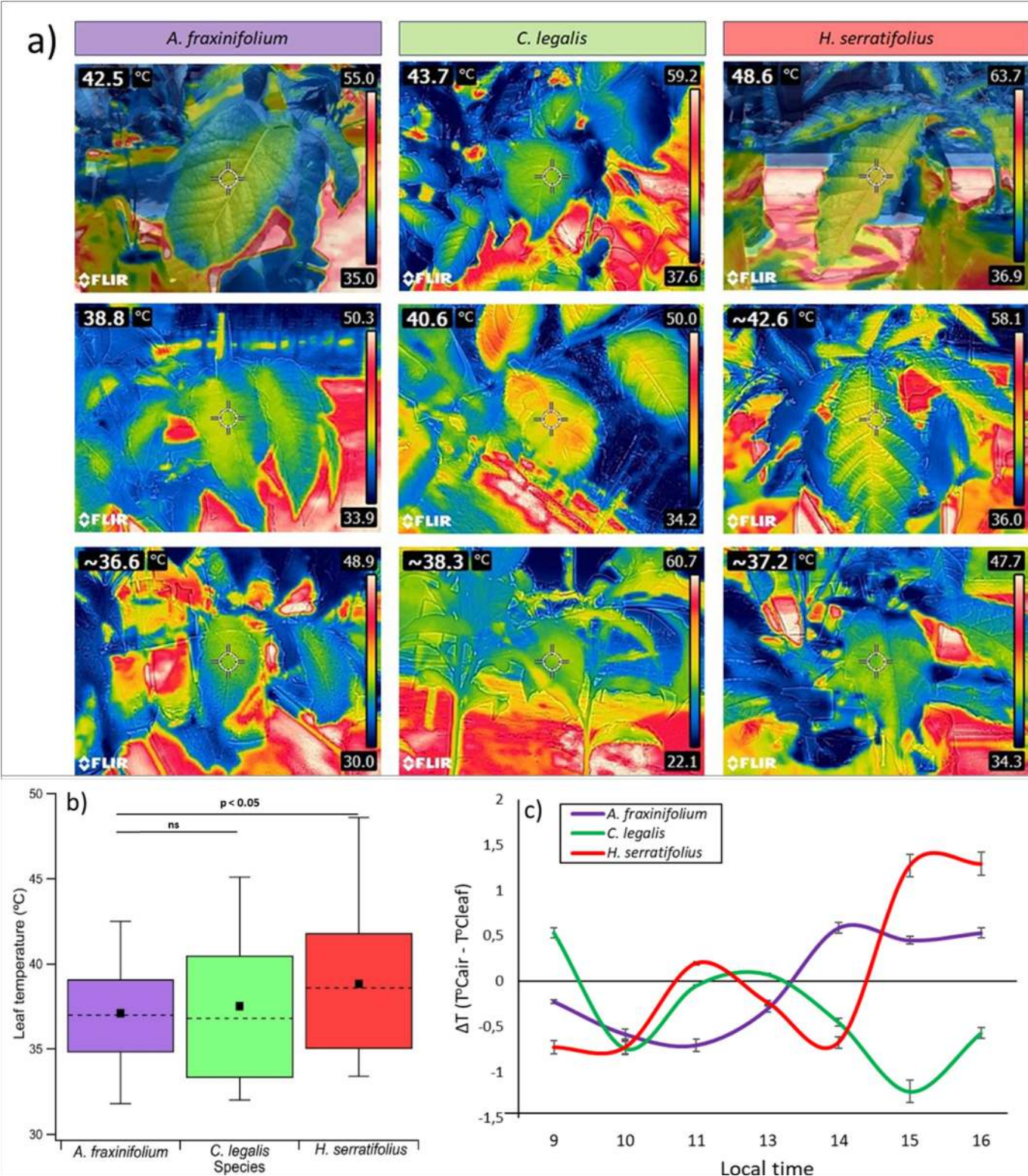


Figure 5

Contrasts in leaf temperature between AF, CL and HS species during the experiment. (a) shows thermophotographs with the maximum temperatures observed for each species (*A. fraxinifolium*, *C. legalis* and *H. serratifolius*, respectively), (b) Box and whisker plots showing median, 25th and 75th percentiles (box plots) and 10th and 90th percentiles (error bars) of leaf surface temperatures (T_{leaf}), t- test significance with $p < 0.05$ and ns = not significant, c) Daily course of the ΔT temperature difference between T_{air} and T_{foliar} each point represents the mean and SD bars

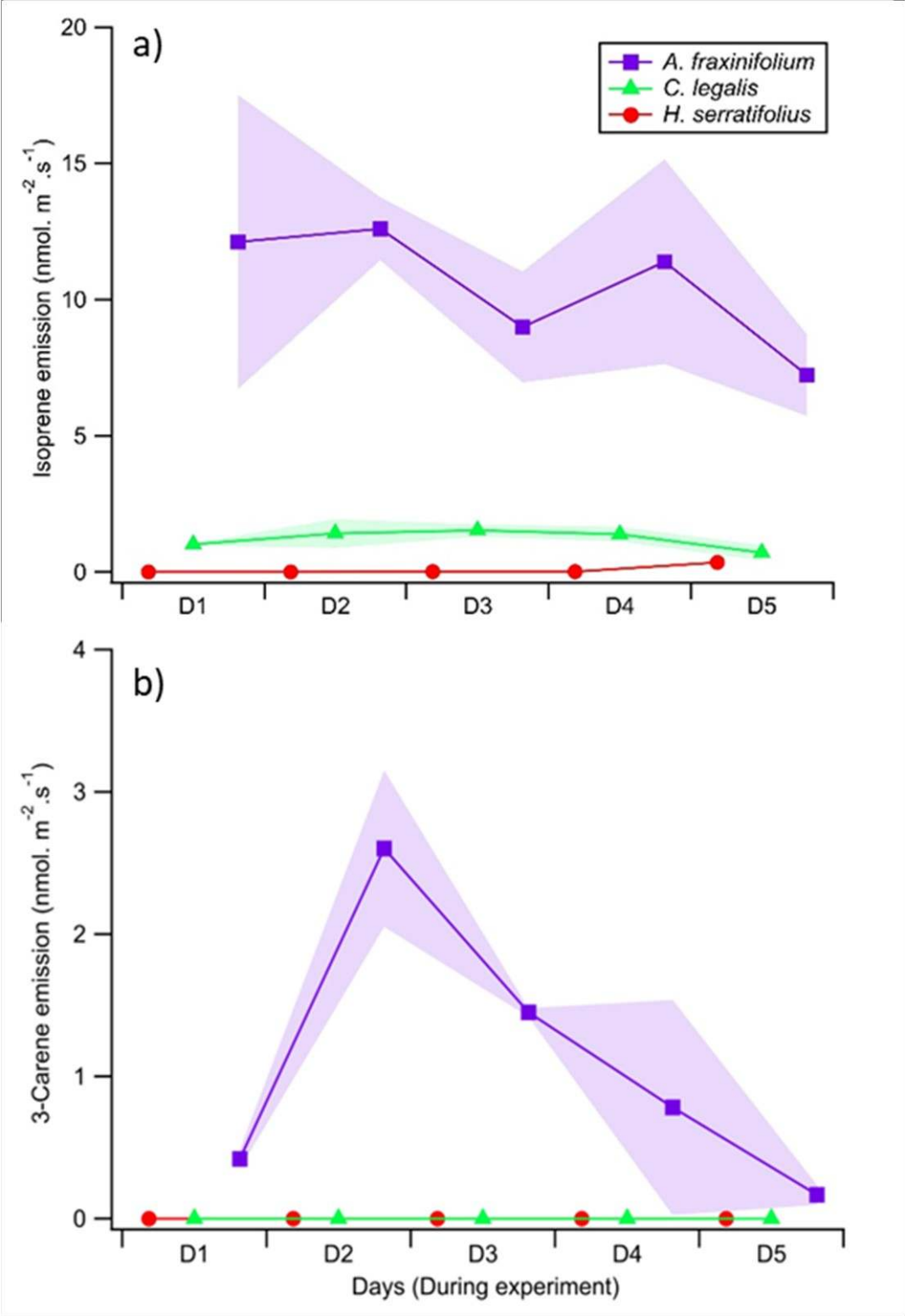


Figure 6

Daily progression of VOC's emission responses from treatments without irrigation (Drought). a) isoprene emission and b) 3-Carene emission. The species are represented by the colors *A. fraxinifolium* (purple), *C. legalis* (green) and *H. serratifolius* (red). The shadows represent the confidence interval (95%)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

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