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ATMOSPHERIC EVAPORATIVE DEMAND AND WATER DEFICIT ON THE ECOPHYSIOLOGY OF *Rubber seedlings*

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Abstract

The search for genetic materials resistant to adverse weather conditions has been a major focus in studies on species of economic interest. The objective of the present study was to assess the growth and photosynthesis of rubber seedlings clones under two conditions of atmospheric evaporative demand, characterized by fluctuations in temperature (TEMP) and vapor pressure deficit (VPD), associated to two water regimens. Hevea brasiliensis Muell. Arg (RRIM 600 and FX 3864) clones were assessed in two microclimates, at low (TEMP 21.2 °C and VPD 0.29 Kpa) and high (TEMP 26.9 °C and VPD 1.49 Kpa) atmospheric evaporative demand, under two water regimens: water deficit and well-watered. Water deficit 50% water availability was sufficient to reduce the net CO2 assimilation rate, leaf area and total chlorophyll of the clones studied that impacted growth in both microclimates. The effects of water deficit on growth and net carbon assimilation rate were intensified under high atmospheric evaporative demand. However, when comparing the two clones studied, RRIM 600 showed greater growth and photosynthesis without water restriction. The FX 3864 clone, despite the high CO2 assimilation values under high atmospheric demand and without water restriction, showed a reduced growth. The results of this study form an important basis for the selection of genotypes with the potential to develop in adverse climatic conditions. In this sense, the RRIM 600 genotype is recommended as a promising material that would best adapt under adverse climatic conditions.

Keywords: Climate variability. Natural rubber. Photosynthesis rates. Tropical tree species. Water use efficiency.

1. Introduction

Hevea brasiliensis Muell. Arg. is an arboreal Euphorbiaceae, native to a wet and humid tropical climate, that occurs naturally in the Amazon region. It is cropped mainly in Brazil and Asia (Camargo et al. 2003; Sterling et al. 2019). Commonly known as rubber tree, the species is the biggest latex source in the world, being commercially important to natural rubber production. Asia accounts for 91% natural rubber

produced in the world. Despite of being the center of rubber tree origin and genetic diversity, Brazil meets only a third its rubber domestic demand, corresponding 01.4% world production (CONAB 2021).

Rubber tree is originally from Amazon Basin. In 1927, around 3,000 hectares of rubber trees were planted in Amazon region, in lands known as Fordland. The attack of the fungus *Microcyclus ulei* destroyed all the plantations in the Fordland area in 1933 (Lieberei 2007). Since then, the main limiting fact to rubber tree cultivation in Brazil is this fungal disease, South American leaf blight, known as "mal das folhas". After for that, the largest rubber tree cropping areas in Brazil, occur in the states of São Paulo, Bahia and Minas Gerais, regions considered escape areas because of the low leaf blight incidence. In addition, these escape regions present satisfactory heat for its plant development (Camargo et al. 2003). However, the occurrence of intense droughts and extreme temperatures in these regions has affected the rubber tree production and threatened its cultivation. For example, genotypes of rubber tree submitted to challenging high (>35°C) and low (<10°C) temperatures presented a negative net photosynthesis rate after 7 days of intensive exposure to these conditions (Noia Junior et al. 2018). To face these challenging climates, rubber tree breeding programs have used assessments of agronomic parameters for productivity, biomass production, water use efficiency and disease resistance, to domesticate genotypes with high performance and wide adaptability to different agroclimatic conditions (Sterling et al. 2019).

Research on how the variation of climatic variables, such as temperature, water availability, wind and radiation interfere on rubber tree ecophysiology is of great importance for the management and development of adapted genetic material. These climatic variables, affect directly the biochemical metabolism in CO₂ assimilation and plant morphology (Xavier et al. 2018). Water deficit and high vapor pressure deficit affect plants by reducing stomatal conductance and, depending on their intensity, leading to dehydration of the plant tissue and further decreasing CO₂ absorption and, consequently, photosynthesis (Costa et al. 2018; Gama et al. 2020).

In this context, the study of plant ecophysiology, analyzing mainly physiological characteristics, as already carried out for *Pinus* spp (Espinoza 2017), *Eucalyptus* spp (Xavier et al. 2018), rubber tree clones (Sterling et al. 2019) and *Pilocarpus microphyllus* Stapf ex Wardlew (Amaral et al. 2021) enables quick and early selection of genotypes with desirable characteristics. As a result, the productive performance of rubber tree is maximized. Based on the above-mentioned, the objective of the present study was to assess the growth and photosynthesis of ruber seedlings clones under two conditions of atmospheric evaporative demand, characterized by temperature fluctuation and vapor pressure deficit, associated to two water regimens.

2. Material and Methods

Experiment location and material

The study was carried out in the municipality of Jerônimo Monteiro, ES, Brazil (latitude 20°47'25"S and longitude 41°23'48"W, 120 m altitude), from September to November 2014, in a total of 90 days experimentation.

The experiments were carried out simultaneously in two acclimatized greenhouses (Van der Hoeven[®], Artur Nogueira, BR), with controlled temperature and relative air humidity to simulate the microclimatic conditions. The microclimates studied were defined based on the values of the thermal limits favorable for the development of the rubber tree, described in the climatic suitability chart determined by Camargo et al. (2003). Microclimate 1 was characterized by low atmospheric evaporative demand (low temperature and low VPD) and microclimate 2 by high atmospheric evaporative demand (high temperature and high VPD).

The microclimate data inside the greenhouses were registered by an automatic meteorological station, consisting of temperature and relative air humidity probes (Vaisala[®], model CS500). The data were collected by a *datalogger* (Campbell Scientific Inc[®], Model CR-10x, Logan, USA) with readings every 10 seconds and storage every 5 minutes. The VPD was obtained from the difference between the values of saturation vapor pressure (es) and partial vapor pressure (ea) (Pereira et al. 2002). Microclimate1 presented mean daily 6°C heat range, while microclimate 2 registered mean 9.5°C daily heat range (Table 1).

Temperature (^o C)	Microclimate 1	Microclimate 2		
Tmax (°C)	24.9	31.6		
Tmean (°C)	21.2	26.9		
Tmin (°C)	18.9	22.1		
VPDmax (kPa)	0.81	2.49		
VPDmean (kPa)	0.29	1.49		
VPDmin (kPa)	0.05	0.63		

Table 1. Mean, maximum and minimum temperature and vapor pressure deficit in the microclimates. 1 (low atmospheric evaporative demand) and 2 (high atmospheric evaporative demand), from September to November. 2014. in Jerônimo Monteiro. ES. Brazil.

We studied the ecophysiology responses to water deficit and evaporative demand of two rubber seedlings genotypes: RRIM 600 and FX3864. The rubber seedlings were grafted on one-year-old hipobiotos (rootstocks); the genotype RRIM 600 was grafted on a GT1 rootstock and FX 3864 on an IAN 873 rootstock.

The grafted seendlings were selected for uniform height and crown diameter and transplanted to 21 dm³, 30 cm high pots with 37 cm upper diameter and 27 cm lower diameter. The pots had approximately 0.5 cm diameter circular holes in the bottom to percolate excess water. The substrate used to fill the pots consisted of Red-Yellow Latosol (60%) taken from 20 to 40 cm depth, washed sand (30%) and biostabilized pine bark compost (10%).

A water retention curve was made to determine the water availability (WA) in the substrate, according to Teixeira et al. (2017), when the gravimetric moisture (U) was obtained corresponding to tensions of 0.010 MPa for field capacity (FC) and 1.5 MPa for wilt point WP. The WA was 11% (FC = 24%, and WP = 13%), and was calculated using the equation 1 (Centurion and Andreoli 2000):

The seedlings were submitted to regimens with no water deficit, (fully irrigated) and water deficit (50% maximum water retention capacity); the seedlings submitted to water deficit were also frequently irrigated to keep 50% of the water availability. Water replacement was controlled by weighing the pots daily, using scales with 10g precision.

Plant growth assessment

At the end of the experiments, the leaf area and the dry matter of the leaves, stem and roots were determined. The leaf area (LA) was measured by a leaf area scan (LI-COR[®], LI 3100, Nebraska, USA) and the plant dry matter was obtained by drying the leaves, stem, and root in a forced air circulation chamber at 75 °C for 72 hours. Subsequently, the values of Total Dry Matter (TDM) were calculated; Fraction of Dry Leaf Matter (LMF), Fraction of Dry Root Matter (RMF), and Fraction of Dry Matter of the stem (SMF) as described in equations 2 to 5:

$$TDM = \sum Dry matter (leaves, roots and stem) (Eq. 2)$$

$$LMF = Leaf dry matter/MAT (Eq. 3)$$

$$RMF = Root dry matter/MAT (Eq. 4)$$

$$SMF = Dry matter of the aerial part (leaves and stem) / MAT (Eq.5)$$

Gas Exchange assessment

The net CO₂ assimilation rate (*A*), transpiration (*E*), stomata conductance (g_s), and internal CO₂ concentration, (C_i), were calculated in the last week of the experimental period, using a portable infrared gas analyzer (IRGA[®], Li-Cor, model LI-6400). The measurements were done with light sourced fixed at 1500 µmol m⁻² s⁻¹ photosynthetically active radiation intensity and 400 ppm CO₂. The measurements were done in the morning between 9.00 a.m. and 10.30 a.m. on completely opened leaves, located on the upper third of the plant, with five replications per treatment; each replication consisted of one leaf. The gas exchange data

were used to calculate the instantaneous water use efficiency, given by the ratio A/E, and the intrinsic water use efficiency, given by the ratio A/gs.

Photosynthetic pigment contents

The contents of chlorophyll *a*, chlorophyll *b*, and carotenoids were obtained at 66 days of the experiment, from leaves collected on the upper third of the plants, in four replications. After collection, the leaves were placed in aluminum foil bags identified according to treatment, placed immediately in Styrofoam boxes containing liquid nitrogen, and then placed in a freezer until analysis.

The pigments were extracted from 80 mg leaves squashed in 80% acetone and their concentrations were determined by the absorbency of the extracts at different wavelengths (470 nm, 646.8 nm, and 663.2 nm) in a light spectrophotometer. The contents of each pigment in g kg⁻¹ were calculated from these values as described in Licthenthaler (1987), following the equations listed below. The ratios were calculated from the data obtained for chlorophyll a/chlorophyll b, and chlorophyll a+b/carotenoids.

Chlorophyll a = $C_a = 12.25 A_{663.7} - 2.79 A_{646.8}$ (Eq. 6) Chlorophyll b = $C_b = 21.50 A_{663.7} - 5.10 A_{663.7}$ (Eq. 7) Total chlorophyll = $C_{(a+b)} = 7.15 AA_{663.7} + 18.71 A_{646.8}$ (Eq. 8) Carotenoids (xantofilas+carotenos)= $(1000A_{470} - 1.82 Ca - 85.02 Cb)/198(Eq. 9)$

Where, A= absorbancy at the indicted wavelength

Experimental design and data analysis

The growth of the *H. brasiliensis* clones (RRIM 600 and FX 3864) was assessed after 90 days (i.e. at the end of the experimental period) under the different microclimatic conditions and water regimens. Thus, two experiments were set up, one for each clone, in a randomized complete design in a 2 x 2 factorial scheme (two microclimate conditions x two water regimens), with five replications, and each replication consisted of one plant.

The data were submitted for analysis of variance (*F* test) and the means were compared by the Tukey test ($P \le 0.05$) using the ASSISTAT^{*} 7.7 program (Silva and Azevedo 2002).

3. Results

Seedling growth of rubber tree genotypes

In the present study, water deficit hurt gas exchange and, consequently, reduced seedling growth of the rubber tree genotypes RRIM 600 and FX 3864 (Table 2).

The RRIM 600 seedlings, under water deficit, presented lower values of TDM, LA, and LMF and higher values of RMF and SLA, about plants without water deficit, regardless of the microclimate. Plants subjected to water deficit showed lower TDM and LMF and higher SLA in microclimate 2 compared to microclimate 1. However, rubber tree seedlings under treatment without water deficit significantly increased TDM, LA, SLA, and SMF, in microclimate 2 compared to microclimate 1 (Table 2).

For clone FX 3864, under water deficit and regardless of microclimate, there was a decrease in TDM, LA, LMF, and SMF. Plants under microclimate 2 showed lower TDM and LMF and higher SMF compared to microclimate 1, regardless of soil water availability. In addition, plants without water restriction significantly reduced FMR in microclimate 2 (Table 2).

Gas exchanges

For the RRIM 600 clone, water deficit reduced the variables *A*, *gs*, and *E*, and increased the *A/E* value, compared to the treatment with no water deficit, in both microclimates; *Ci* decreased in the treatment with

no water deficit and it occurred only in microclimate 1. Plants submitted to microclimate 2, presented a decrease in A and E in the water deficit treatment, in comparison with microclimate 1. There were no differences for the A/gs among the microclimates (Table 3).

Table 2. Mean values (n=5) of the Total Dry Matter (TDM), Total Leaf Area (LA), Specific Leaf Area (SLA), Leaf Matter Fraction (LMF), Root Matter Fraction (RMF) and Shoot Matter Fraction (SMF) of the RRIM 600 and FX 3864 clones.

Genotype	Growth variable –	Microclimate 1		Microclimate 2	
		w/ no water deficit	w/water deficit	w/ no water deficit	w/water deficit
RRIM 600	TDM (g)	48.70 Ba*	29.13 Ab	57.47 Aa	25.14 Bb
	LA (cm²)	391.14 Ba	261.42 Ab	536.40 Aa	236.74 Ab
	SLA (cm ² g ⁻¹)	44.74 Bb	53.03 Ba	48.64 Ab	81.41 Aa
	LMF (g g⁻¹)	0.21Aa	0.16 Ab	0.19 Aa	0.11Bb
	RMF (g g⁻¹)	0.68 Ab	0.72 Aa	0.63 Ab	0.75 Aa
	SMF (g g⁻¹)	0.32 Ba	0.28 Aa	0.37Aa	0.25 Aa
FX 3864	TDM (g)	40.2 Aa*	32.9 Ab	34.1 Ba	24.4 Bb
	LA (cm²)	271.2 Aa	150.44 Ab	268.48 Aa	144.99 Ab
	SLA (cm ² g ⁻¹)	37.58 Aa	44.23 Aa	30.8 Aa	37.15 Aa
	LMF (g g⁻¹)	0.26Aa	0.16Ab	0.18Ba	0.07Bb
	RMF (g g⁻¹)	0.72Aa	0.73Aa	0.61Bb	0.81Aa
	SMF (g g⁻¹)	0.28Ba	0.17Bb	0.39 Aa	0.19Ab

*For the same variables, between the microclimates, means followed by an uppercase letter are not significantly different under the same water regimen, and means followed by the same lowercase letter are not significantly other among the water regimens within each microclimate. The means were compared by the Tukey test (*P*<0.05).

Table 3. Mean values (n=5) of the net CO₂ assimilation rate (A), stomatal conductance (gs), transpiration (E), water use efficiency (A/E), intrinsic water use efficiency (A/gs), and internal CO₂ concentration (Ci) of the RRIM 600 and FX 3864 clones.

Gas exchange parameters	Microclimate 1		Microclimate 2	
	w/ no water deficit	w/ water deficit	w/ no water deficit	w/ water deficit
A (μmol CO ₂ m ⁻² s ⁻¹)	11.52Ba*	6.60Ab	12.75Aa	4.19Bb
<i>g</i> s (mol H ₂ O m ⁻² s ⁻¹)	0.21Aa	0.09Ab	0.25Aa	0.06Ab
<i>Сі</i> (µmol CO ₂ m ⁻² s ⁻¹)	292.44Ab	325.42Aa	298.14Aa	317.31Aa
<i>E</i> (μmol H ₂ O m ⁻² s ⁻¹)	3.37Ba	1.32Ab	3.74Aa	1.14Bb
A/E (μmol mmol⁻¹)	3.42Ab	5.00Aa	3.40Ab	3.75Aa
<i>A/gs</i> (µmol mmol⁻¹)	54.85 Aa	73.33Aa	51,.3Aa	69.83Aa
A (μmol CO ₂ m ⁻² s ⁻¹)	11.11Ba*	4.55Ab	12.60Aa	4.67Ab
<i>g</i> ₅ (mol H₂O m ⁻² s ⁻¹)	0.21Ba	0.13Ab	0.28Aa	0.09Ab
<i>Сі</i> (µmol CO ₂ m ⁻² s ⁻¹)	295.07Ab	332.17Aa	280.10Aa	292.99Ba
<i>E</i> (μmol H ₂ O m ⁻² s ⁻¹)	2.96Ba	1.85Ab	3.88Aa	1.74Ab
<i>A/E</i> (μmol mmol⁻¹)	3.82Aa	2.69Aa	3.30Aa	2.65Aa
A/gs (µmol mmol⁻¹)	55.11Aa	37.8Ba	44.86Aa	51.35Aa
	Gas exchange parameters A (μ mol CO ₂ m ⁻² s ⁻¹) g_s (mol H ₂ O m ⁻² s ⁻¹) Ci (μ mol CO ₂ m ⁻² s ⁻¹) E (μ mol H ₂ O m ⁻² s ⁻¹) A/E (μ mol mmol ⁻¹) A/gs (μ mol mmol ⁻¹) A (μ mol CO ₂ m ⁻² s ⁻¹) g_s (mol H ₂ O m ⁻² s ⁻¹) Ci (μ mol CO ₂ m ⁻² s ⁻¹) E (μ mol H ₂ O m ⁻² s ⁻¹) A/E (μ mol mmol ⁻¹) A/E (μ mol mmol ⁻¹) A/gs (μ mol mmol ⁻¹)	MicroclirMicroclirW/ no water deficitA (μ mol CO ₂ m ⁻² s ⁻¹)11.52Ba* g_s (mol H ₂ O m ⁻² s ⁻¹)0.21AaCi (μ mol CO ₂ m ⁻² s ⁻¹)292.44AbE (μ mol H ₂ O m ⁻² s ⁻¹)3.37BaA/E (μ mol mmol ⁻¹)3.42AbA/gs (μ mol mmol ⁻¹)54.85 AaA (μ mol CO ₂ m ⁻² s ⁻¹)0.21BaCi (μ mol CO ₂ m ⁻² s ⁻¹)0.21BaCi (μ mol CO ₂ m ⁻² s ⁻¹)295.07AbE (μ mol H ₂ O m ⁻² s ⁻¹)2.96BaA/E (μ mol mmol ⁻¹)3.82AaA/gs (μ mol mmol ⁻¹)55.11Aa	Microclimate 1w/ no water deficitw/ water deficitA (μ mol CO ₂ m ⁻² s ⁻¹)11.52Ba*6.60Abgs (mol H ₂ O m ⁻² s ⁻¹)0.21Aa0.09AbCi (μ mol CO ₂ m ⁻² s ⁻¹)292.44Ab325.42AaE (μ mol H ₂ O m ⁻² s ⁻¹)3.37Ba1.32AbA/E (μ mol mmol ⁻¹)3.42Ab5.00AaA/gs (μ mol mmol ⁻¹)54.85 Aa73.33AaA (μ mol CO ₂ m ⁻² s ⁻¹)0.21Ba0.13AbGi (μ mol CO ₂ m ⁻² s ⁻¹)0.21Ba0.13AbCi (μ mol CO ₂ m ⁻² s ⁻¹)2.96Ba1.85AbA/E (μ mol mmol ⁻¹)3.82Aa2.69AaA/E (μ mol mmol ⁻¹)55.11Aa37.8Ba	Microclimate 1Microclimate 1Microclimate 1Gas exchange parametersw/ no water deficitw/ water deficitw/ no water deficit A (µmol CO2 m ⁻² s ⁻¹)11.52Ba*6.60Ab12.75Aa g_s (mol H20 m ⁻² s ⁻¹)0.21Aa0.09Ab0.25Aa Ci (µmol CO2 m ⁻² s ⁻¹)292.44Ab325.42Aa298.14Aa E (µmol H20 m ⁻² s ⁻¹)3.37Ba1.32Ab3.74Aa A/E (µmol mmol ⁻¹)3.42Ab5.00Aa3.40Ab A/gs (µmol mmol ⁻¹)54.85 Aa73.33Aa51.3Aa A (µmol CO2 m ⁻² s ⁻¹)11.11Ba*4.55Ab12.60Aa g_s (mol H20 m ⁻² s ⁻¹)0.21Ba0.13Ab0.28Aa Ci (µmol CO2 m ⁻² s ⁻¹)295.07Ab332.17Aa280.10Aa E (µmol H20 m ⁻² s ⁻¹)2.96Ba1.85Ab3.88Aa A/E (µmol mmol ⁻¹)3.82Aa2.69Aa3.30Aa A/gs (µmol mmol ⁻¹)5.11Aa37.8Ba44.86Aa

*For the same variables between the microclimates, means followed by the same uppercase letter are not significantly different in the same water regimen, and means followed by the same lowercase letter are not significantly other in the water regimens in each microclimate. The means were compared by the Tukey test (*P*<0.05).

The plants of the FX 3864 clone under water deficit decreased significantly the *A*, *gs*, and *E* rates in the two microclimates. There was a significant increase in the *A*, *gs*, and *E* variables of the seedlings kept in the treatment with no water deficit, in microclimate 2 compared to microclimate 1. Plants under water deficit in microclimate 2 showed a significant increase in *A*/*gs* and reduction in *Ci*. There were no statistical differences in the *A*/*E* between the treatments studied (Table 3).

Photosynthesis pigments

The contents of *Chl a* and *Chl a+b* decreased significantly in the RRIM 600 genotype seedlings under water deficit treatment, regardless of the microclimate. Under water deficit, the plants in microclimate 2 presented lower *Chl b* contents (Table 4).

Table 4. Mean values (n=4) of the pigments chlorophyll a (*Chl a*), chlorophyll b (Chl b), total chlorophyll (*Chl a*+*b*) and carotenoids (*Car*), chlorophyll a and b ratio (Chl a/b) and total chlorophyll and carotenoids ratio (*Chl a*+*b*/*Car*) of the RRIM 600 and FX 3864 clones.

Genotype	Gas exchange parameters	Microclimate 1		Microclimate 2	
		w/ no water deficit	w/ water deficit	w/ no water deficit	w/ water deficit
RRIM 600	Chl a (g Kg⁻¹ MF)	11.38 Aa*	8.48 Ab	11.22 Aa	9.74 Ab
	Chl b (g Kg ⁻¹ MF)	4.54 Aa	3.86 Aa	4.42 Aa	3.16 Ab
	Chl a+b (g Kg ⁻¹ MF)	15.92Aa	12.34Ab	15.65Aa	12.91Ab
	Car (g Kg⁻¹ MF)	3.29 Aa	3.19 Aa	3.11 Aa	2.36 Aa
	Chl a/b	2.57Aa	2.24 Aa	2.56 Aa	3.28 Aa
	Chl a+b/car	5.28 Aa	3.76 Aa	6.72 Aa	4.16 Aa
FX 3864	Chl a (g Kg⁻¹ MF)	12.68 Aa*	8.69 Ab	11.52 Aa	9.36 Ab
	Chl b (g Kg ⁻¹ MF)	3.98 Aa	3.49 Aa	4.15 Aa	3.56 Aa
	Chl a+b (g Kg⁻¹ MF)	17.17Aa	12.69Ab	15.67Aa	13.18Ab
	Car (g Kg⁻¹ MF)	2.66 Aa	3.23 Aa	3.04 Aa	2.78 Aa
	Chl a/b	3.18 Aa	2.53 Aa	2.85 Aa	2.63 Aa
	Chl a+b/car	6.45 Aa	3.92 Ab	5.16 Aa	4.67Aa

*For the same variables, in both microclimates, means followed by an uppercase letter are not significantly different in the same water regimen, and means followed by a lowercase letter are not significantly other in the water regimens in both microclimates. The means were compared by the Tukey test (*P*<0.05).

Plants of the FX 3864 clone, under water deficit, presented, regardless of the microclimate, lower *Chl a* and *Chl a*+*b* contents. The *Chl a*+*b/Car* ratio was significantly smaller, under water deficit only in the plants kept in microclimate 1. However, a comparison of the microclimate conditions showed that the pigment contents in the plants were not significantly different, regardless of the water treatment.

4. Discussion

The water deficit negatively affected the *A*, *gs*, and *E* of both genotypes, regardless of the microclimate. The reduction in the *gs* is the main control mechanism in rubber tree plants under water deficit conditions (Nóia Júnior et al. 2018; Sterling et al. 2019). The stomatal limitation can be considered a strategy of the plant to prevent an increase in transpiration (Sevanto et al. 2014) and to prevent embolism and cavitation in the xylem (Chen et al. 2010). Reduced transpiration rates limit the capacity of the leaves to cool by the latent heat flux (Mathur et al. 2014), one of the most important processes to regulate leaf temperature and controlling water loss from the plant (Nóia Júnior et al. 2018).

The *Ci* values for the two clones tended to increase, even with the decrease in *A* due to water deficit in microclimate 1. The increase in *Ci* under low leaf water potential suggests a decrease in activity in the CO_2 assimilation mechanisms, with decreased carboxylation efficiency (Zymańska et al. 2017).

Under water deficit, the rubber seedlings clones presented decreased leaf area, which can be understood as a survival strategy to decrease the transpiration by reducing the transpiring area and to protect the plants from potential oxidative damage. These alterations could imply low leaf biomass production in deciduous plants, as is the case of the rubber tree (Chen et al. 2010). On the other hand, for the same water deficit condition, the specific leaf area increased only in the RRIM 600, regardless of microclimate. This response may be related to the increase in the number and size of the mesophyll cells per leaf area unit of the clone, and the increase in the palisade parenchyma thickness which is a known plant response to water deficit (Melo et al. 2017).

The combined effect of different atmospheric evaporative demands and water availability levels resulted in alteration of the carbon partitioning in the rubber seedlings clones, caused by a reduction in gas exchanges and an increase in photorespiration (Ataíde et al. 2018). These changes reduced the allocation of photoassimilates to the shoot and directed them to root development, which may be associated with abscisic acid production (ABA), which induces root growth, stimulating the emergence of side roots for better water absorption, supporting leaf growth (Wang et al. 2019).

In microclimate 2, with high VPD, the effects of water deficit were intensified in the two studied clones. The clones showed reduced growth, which can be connected to proportionally smaller values of *A*, *gs*, and *E*. The increase in VPD leads to changes ecophysiological changes in plants (Way et al. 2015). The

increase in VPD usually leads to a reduction in *gs* (Slot and Winter 2017) due to stomatal closure, which avoids excessive loss of water through transpiration under the high atmospheric evaporative demand. The decrease in *A* may be associated with this *gs* reduction. since the stomatal closure causes a reduction in *Ci* for the sub-stomatal chamber (Mathur et al. 2014).

The water deficit established in this study for the two clones was sufficient to significantly reduce the *ChI a* and total chlorophyll contents, regardless of the microclimate. The decrease in photosynthetic pigments is commonly observed in plants under water deficit (Asharaf and Harris 2013) and is a good indicator of oxidative stress on the chloroplast stroma. A decrease in the pigments may be the result of chlorophyll degradation or due to a deficiency in chlorophyll synthesis together with the alterations in the thylakoid membrane structure (Tamary et al. 2019). This damage to the thylakoid membrane is evident due to reduced *ChI a*, responsible for capturing light energy and transferring energy to the reaction centers (photochemical) (Lin et al. 2018), affecting the photosynthetic quantic yield, due to the reduction in the light absorption efficiency in photosystem I and II (Asharaf and Harris 2013).

The RRIM 600 clone, with no water deficit, presented higher *A* and *gs* in microclimate 2 than microclimate 1, resulting in high shoot growth. According to Krishna et al. (1991), rubber seedlings have high assimilatory CO_2 rates, even under high temperatures, due to their big cuticular transpiration. In this sense, this is what reinforces the performance of the RRIM 600 clone at higher temperatures, exactly the adaptability of this genotype to the dryer and warmer climates, resistant to water deficit (Nascimento et al. 2019). In addition, it suggests that RRIM 600 showed a strong regulation of photosynthetic metabolism to maintain the difference between the water potential of the leaf and the constant potential of the soil in a dry atmosphere (Kumagai et al. 2015). This may be related to the iso-hydric performance of the rubber tree, as the species has an efficient stomatal control (Ayutthaya et al. 2011). Without water deficit, clone FX 3864 showed a greater accumulation of dry matter under microclimate 1, compared to microclimate 2. This fact suggests that rubber tree seedlings adapt well to environments with mild temperatures. According to Gasparotto et al. (2012), FX 3864 clones are suitable for regions considered to have phytosanitary restrictions on rubber tree cultivation in Brazil, with temperatures $\leq 20^{\circ}$ C, as they change leaves uniformly in the dry season and at low temperatures, remaining for a long time. The period in hibernation.

The reduction in the growth of FX 3864 seedlings was mainly due to the lower accumulation of root dry matter. However, there was the greater allocation of carbohydrates to the aerial part of the seedlings. Carbohydrate allocation in the shoots of plants under high atmospheric demand is a strategy to increase plant leaf area and compensate for photosynthetic efficiency at the expense of roots (Szymańska et al. 2017).

5. Conclusions

The seedlings of rubber tree clones RRIM 600 and FX 3864 were very susceptible to water deficit. Treatment with 50% water availability reduced net CO2 assimilation, leaf area, total chlorophyll, and total dry matter in both clones.

The effects of water deficit on growth and photosynthetic rates in the two clones were intensified when submitted to high atmospheric evaporative demand. However, without water deficit, the RRIM 600 clone showed higher performance under high atmospheric evaporative demand, with high dry matter and photosynthesis rates. On the other hand, clone FX 3464 showed better results under low atmospheric evaporative demand.

The results of the present study form an important basis for the choice of rubber tree genotypes potentially adapted to adverse environmental conditions, that is, outside the limits of favorable climatic ideas for their development, provided that strategies to mitigate water deficit are adopted.

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