

Eco-physiological behavior and carbon metabolism in young plants of balsa wood (*Ochroma pyramidale*) under three different water regimes

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Abstract

The aim of this research was to evaluate the eco-physiological and biochemical responses in balsa wood plants (*Ochroma pyramidale*) under different water regimes. For this purpose, an experiment was conducted in a greenhouse in July 2013. The experimental design was completely randomized in a factorial design (3×4) with three water conditions: control (irrigated), water deficit and flooding in four periods and 5 replications. The physiological parameters evaluated were (predawn leaf water potential (Ψ_{am}), stomatal conductance (g_s) and transpiration (E)). The biochemical parameters were (abscisic acid, starch concentrations, total soluble carbohydrate and sucrose). The predawn water potential and the variables of gas exchange were significantly decreased under water deficit and flooding. The abscisic acid concentrations showed increases in the same treatments over time. The starch concentrations decreased significantly in plants under water deficit, representing a decrease of 2.06 times. In plants under flooding stress the starch concentrations decreased 8 times, when compared to the control plants. The contents of total soluble carbohydrate and sucrose increased around 1.65 and 1.54 times in plants under water deficit, respectively. On the other hand, the total soluble carbohydrate was decrease more than 2 times in flooding, when compared to control plants. The water deficit and flooding for twelve days was enough to change the physiological and metabolic processes of balsa wood plants.

Keywords: Balsa wood; Flooding; Stomatal Conductance; Water Deficit.

Abbreviations: In the abbreviations were inserted as follows words: AA_aminoacids, ADP_Adenosine diphosphate, ATP_Adenosine triphosphate, DM_Seedling dry matter, EBPs_Biodiversity Studies Laboratory in Higher Plants, Gb_GlycineBetaine levels, N_Nitrogen, NH_4^+ _Ammonium, NO_3^- _Nitrate, Nra_Nitrate Reductase Activity, MPa_Mega Pascal, SAS_Statistical Analysis System, UFRA_Universidade Federal Rural da Amazônia, Ψ_{am} _Predawn Water Potential, stomatal conductance (g_s) and transpiration (E)).

Introduction

The Amazon is known worldwide for its variety of ecosystems, rich in biodiversity as well as for its great water availability. However, all these attributes that are essential to human life, fauna and flora, have been lost considerably in

recent decades because of deforestation and land cover change (Roos et al. 2011). According to Neiman, (2012) we are currently living in a time of great ecological concerns, because of the picture of environmental degradation on the

planet. Mortari, (2012) warned that ecosystem degradation brings droughts in some regions and permanent flooding in others, influencing agriculture, forestry and cultural practices in different parts of the world.

Concerns over climate changes and the behavioral changes of plants under abiotic stresses have increased interests on the physiology of native tree species. The deepening on this subject allows the characterization of tolerance or sensitivity of these species (Gorai et al. 2010; Silva et al. 2010; Liu et al. 2011; Maraghni et al. 2011; Nascimento et al. 2011).

One of the species with little knowledge in this line of research is *Ochroma pyramidale* (Cav. Ex Lam) Urb., commonly known as balsa wood with potential for recovery of degraded areas and reforestation due to its rapid growth and tolerance to direct light (Lorenzi, 1992). Its wood is soft and easy to handle and can be used for the manufacture of cellulose and paper, making craft toys, model airplanes (Varela and Ferraz, 1991).

Water deficit is one of the main limiting factors of development and growth of plants. Only the species with drought resistance mechanisms may develop in environments with reduced amount of water (González et al. 2012). Plants can present multiple responses to water deficit, among them, the physiological responses which are able to identify water conditions that the plants have. Some indicators may be useful in improvement program aimed at the selection of resistant species to water deficit, the main indicators are stomatal closure (Ashraf, 2010; Dalberto, 2012) and the reduction in stomatal conductance (gs) (Lawlor and Tezara, 2009).

The accumulation of compatible solutes in plants under water deficit is not harmful to cellular metabolism and performs some important functions such as increases in osmotic pressure inside the cell, maintaining the water absorption and cell turgor pressure, which contributes to the continuity of physiological processes, even at lower levels (Marijuan and Bosch, 2013).

The accumulation of organic solutes in water stress conditions may be related both to the osmotic adjustment and osmoprotectors agent in cellular dehydration (Pereira, 2012). Studies have shown that the flooding condition eliminates the air from porous spaces of the soil forming a hypoxic or anoxic environment, and reducing aeration limiting breathing air (Bailey-Serres and Voesenek, 2008). This promotes the inhibition of photosynthesis and the transport of carbohydrates to increase concentrations of abscisic acid (Chen et al., 2010). Under these conditions the changes vary at morphophysiological, anatomical, (Parolin et al. 2004) metabolic (Kozlowski, 1997) and biochemical levels (Ashraf, 2003).

The aim of this research was to determine the physiological and biochemical mechanisms of adaptation to water deficit and flooding in young plants of balsawood with emphasis on stomatal behavior, water relations and organic solutes.

Results

Predawn leaf water potential

The Predawn leaf water potential (Ψ_{am}) reduced significantly in plants under water deficit (-0.36; -1.21; -2.39 and -3.35 MPa) and flooding (-0.30; -1.76; -3.07 and -3.85 MPa.) (Fig 1A), representing a decrease of about 10 and 12 times lower, respectively, when compared to control plants (-0.31MPa). The statistical differences between all water conditions occurred from four days of evaluation; however, only in plants under water deficit and flooding the statistical differences between evaluation periods observed (Fig 1A).

Transpiration

The stomatal activities considerably decreased in plants under water deficit (10.94; 6.42; 3.96 and 1.8 $\text{mmol m}^{-2} \text{s}^{-1}$) and flooding (11.08; 3.05; 1.14 and 0.15 $\text{mmol m}^{-2} \text{s}^{-1}$). When compared to control plants (10.85 $\text{mmol m}^{-2} \text{s}^{-1}$), these results show a representative fall of their activities around 6 and 72 times, respectively. Statistical differences between the water conditions occurred from the 4th day of the experiment. However, it can be seen that the plants under water deficit differ significantly between the periods of evaluation throughout the experimental period, which did not occur in plants under flooding where noted differences only in the first three periods of the experiment (0, 4 and 8 days) (Fig 1B).

Stomatal conductance

The stomatal conductance shows similarity with transpiration data because there is a direct relationship between these two parameters. The values found in water deficit condition were: 591; 251; 158 and 92 $\text{mmol.m}^{-2}.\text{s}^{-1}$ and flooding condition: 574; 154; 81 and 11 $\text{mmol.m}^{-2}.\text{s}^{-1}$, showing a decrease significantly over the evaluation period, this represented a decrease of 6.73 and 56 times, respectively, when compared to the control plants (620 $\text{mmol.m}^{-2}.\text{s}^{-1}$). The statistical differences between the water conditions made themselves from the 4th day of the experiment (Fig 1C). Statistical differences between the water conditions occurred from the 4th day of the experiment (Fig 1C). In significant relationship between the evaluation periods, the plants under water deficit differed in the first three periods (0, 4 and 8 days), plants flooded the differences occurred in the periods 0, 4 and 12 days.

Concentrations of abscisic acid (ABA)

The significant increases were occurred in abscisic acid concentrations in plants under water deficit and flooding over the days of the experiment (Fig 1D). The values presented were respectively: 22.85; 72.05; 93.25 110.01 $\text{ng g}^{-1} \text{DM}$ and 20.48; 67.2; 100.2 and 126.25 $\text{ng g}^{-1} \text{DM}$. However, concentrations of plant hormone represented increases of 4.73 and 5.43 times, respectively, when compared to the control plants (23.25 $\text{ng g}^{-1} \text{DM}$). Plants under water deficit and flooding differed on the 4th day of the experiment. However, plants differed only on the last day of the experiment, when these treatments were compared to control.

Starch concentration

Concentrations of starch in the balsawood leaves decreased in all treatments under water deficit, compared to the control plants (Fig 2A), being more pronounced in flooding conditions. The values found under water deficit were: 0.61; 0.41; 0.34 and 0.31 mmol GLU/g of residue and under flooding the values were: 0.59; 0.30; 0.11 and 0.08 GLU mmol g of residue, representing a decrease of 2.06 and 8 times lower, respectively, compared to control plants (GLU 0.64 mmol/g of residue). Statistical differences between the water conditions occurred from the 4th day of evaluation remaining until the end of the experiment. For periods of evaluation, the statistical differences in water deficit condition occurred only in the first period (0 day) than the other, on flooding condition this difference occurred in the first three periods (0,4th and 8th day).

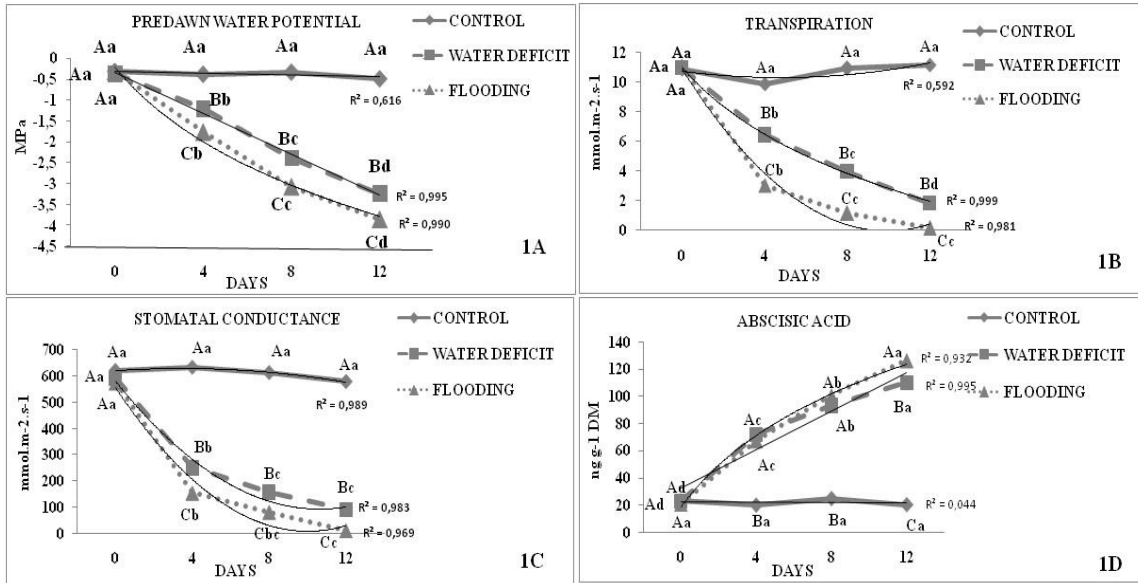


Fig 1. Predawn water potential (Ψ_{am} , 1A), transpiration (E, 1B), stomatal conductance (gs, 1C), and abscisic acid (1D), in young plants of *Ochroma pyramidale* (Cav. Ex Lam) Urb. Means followed by the same uppercase or lowercase, they do not differ by Tukey's test at 5% probability. Uppercase statistically compare the water conditions each other and lowercase letters compare evaluation periods with each other, within each treatment.

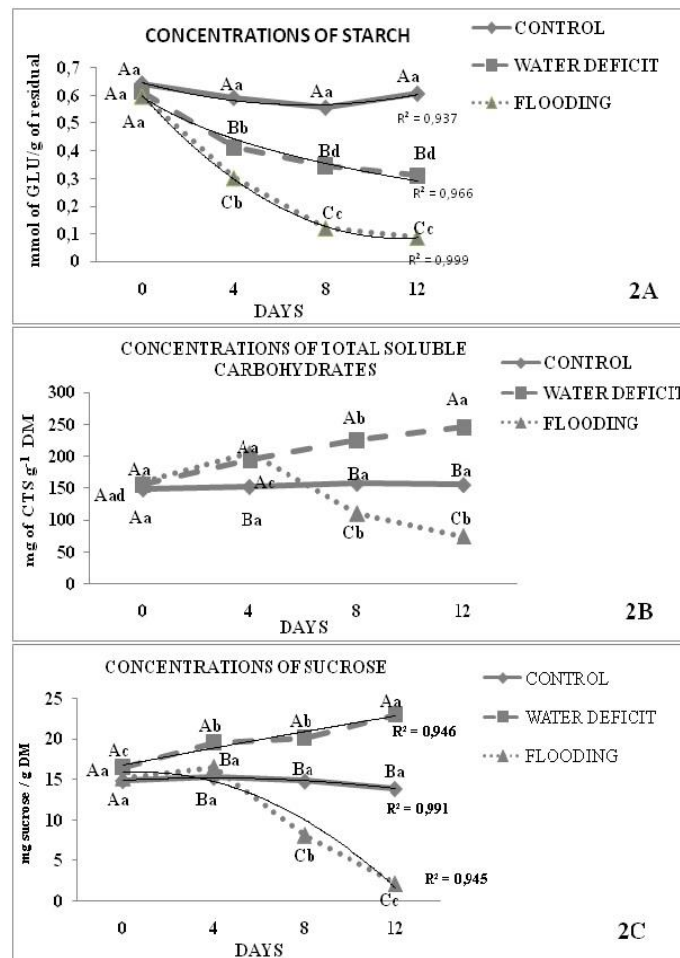


Fig 2. Concentrations of starch (2A), Concentraions of otal soluble carbohydrates (2B) and concentrations of sucrose (2C) in Young plants of *Ochroma pyramidale* (Cav. Ex Lam) Urb. Means followed by the same uppercase or lowercase, they do not differ by Tukey test at 5% probability. Uppercase statistically compare the water conditions each other and lowercase letters compare evaluation periods with each other, within each treatment.

Total soluble carbohydrate concentrations

The results presented for this parameter in water deficit condition showed increase over time (Fig 2B) representing an increase of 1.65 times higher, compared to control plants (148 mg g⁻¹ DM), which did not occur in plants under flooding. This observed significantly from the 4th day of the experiment, culminating in reduction of around 2 times lower, compared to the control plants (Fig 2B). The values obtained for the leaf under water deficit were: 156, 195, 225 and 245 mg g⁻¹ DM and under flooding were: 160, 205, 110 and 74 mg g⁻¹ DM. This represented statistical differences between the water conditions on the 8th day of the experiment. For periods of evaluation, the differences occurred on water deficit condition in 0 and 8th day. In flooding condition this difference occurred in the 4th and 8th day.

Sucrose concentrations

The sucrose concentrations were similar to the total soluble carbohydrates in plants under deficit and flooding conditions (Fig 2C). The sucrose concentrations increased gradually in plants under water deficit (16.48; 19.54; 20.12 and 23.01mg sucrose/g DM), obtaining an increase of 1.54 times higher, compared to control plants (14.85 mg sucrose/g DM). In plants under flooding, the results were (15.14; 16.45, 8.1 and 2.14 mg sucrose/g DM), showing a decrease from the 4th day. This drop was around 6.93 times lower than the control plants. Statistical differences between the water conditions occurred on the 8th day. In the relationship between the evaluation periods, the differences occurred on days 0 and 12th for water deficit, while for flooding condition it was occurred in three periods (4th, 8th and 12th day).

Discussion

The reduction of water potential in plants under water deficit is due to lower availability of water in the soil. The decrease in water availability in the soil causes drop in water potential in leaves, leading to loss of turgor and reduction in stomatal conductance (Fig 1C), resulting in a decrease of transpiration (Fig 1B). Cordeiro (2012) working with seedlings of three tree species found significant reductions in water potential during the dry season at conditions in the municipality of Igarapé-Açú / Pará.

In flooding condition, there was a low concentration of O₂, due to the change in respiratory route of the root system, altering aerobic to anaerobic, consequently reducing their energy efficiency and changing its pattern of growth and development (Oliveira Neto, 2010). However, according to this author this reduction is probably due to a change in the metabolic behavior of the plant, such as oxidative phosphorylation to the phosphorylation of ADP to ATP reactions that occur exclusively in glycolysis and fermentation. Presenting similarities with this work, Alves (2010) studying plant yellow ipe showed significant decreases in plants under water deficit (1050%) and flooding (1350%), when compared to control plants.

The low water supply in the soil partly explains the decreased transpiration under conditions of water deficit and flooding (Fig 1B), as well as to decrease the hydraulic conductivity of roots, or by reducing the growth and finally death, leading to decrease in leaves water potential because of lower water absorption (Franco and Lüttge, 2004). This fallen potential may be temporary due to stomatal closure that prevents the loss of more water through transpiration (Alves

et al. 2012). Other processes can be attributed to stomatal closure, such as increase in production of abscisic acid (Fig 1D). This increase in ABA concentration did not lead to complete stomatal closure, because the leaves still had a low transpiration rate (Fig 2B). Cerqueira, (2011) showed an increase abscisic acid concentration in the leaves of *Vitis vinifera* L. plants, they when subjected to water deficit for 90 days.

Reduction in starch concentrations in the treatment under water deficit is possibly related to the decrease in gas exchange (Fig 1B and 1C). This decrease can be related to the decrease in photosynthetic rate or due to lower entry of CO₂, main component for starch production in the C3 cycle. Another possible response is to increase the activity of enzymes α and β amylase acting on starch degradation under stress conditions (Fig 2C). Another factor that has possibly contributed to reduction of this sugar is in the inactivation of key enzyme in starch synthesis (ADP-glucose pyrophosphorylase) mainly under stress conditions; thus, considerably reducing their level in balsawood leaves.

Paula et al. (2013) worked on African mahogany plants and found decreases in starch concentrations in both water deficit and flooding conditions. Gimeno et al. (2012) studied *Jatropha curcas* plants and found similar results in flooding conditions.

The increase of total soluble carbohydrates in water deficit condition (Fig 2B) is primarily due to plant's response to water stress, causing the osmotic adjustment in the metabolism of these plants, reducing their osmotic potential. The gradient differences keep the cells turgor pressure constant and consequently delay dehydration in plant tissues. This osmotic adjustment protects biomembranes that can consequently be degraded by the lack of water in the cytosol to increase ionic substances, besides triggering several inactive enzymes in the cytosol.

In the case of plants under flooding, the decrease of total soluble carbohydrates should probably be related to a carbohydrate offset to the root system from to para through the xylem with purpose of energy investment in the roots; thus, functioning as an initial adaptive strategy of the plant, causing increase the fermentation process of carbohydrate to sustain ATP production in anoxia and reoxidation of essential reducing agents to maintain glycolysis. In this stress condition, low energy production by flooding speeds up the process of glycolysis to satisfy the demands for ATP to keep the plant metabolism up (Larré, 2011).

Similar results were found by Alves et al. (2012), working on *Tabebuia serratifolia* species. The results obtained by Rivas et al. (2013) showed increases in concentration of carbohydrates in *Moringa oleifera* leaves under water deficit for 10 days. The possible explanation to the increase of sucrose in plants under water deficit (Fig 2C) is that like other sugars, sucrose is hydrolyzed to release hexoses which are used in the osmotic adjustment processes. It can bind to water molecules in the leaf with the purpose of keeping the water level in the leaf organ. This defense mechanism is established through the accumulation in the vacuole or in the cytosol of compatible solutes such as sucrose, which contribute to the maintaining of water balance and preserving the integrity of proteins, enzymes and cell membranes (Ashraf et al. 2011).

The low production of energy in the root system is due to anaerobic respiratory running, which contributes to stomatal closure (Fig 1C) and the decline of photosynthetic process (Kreuz wieser et al. 2004). Therefore, a decrease in starch content (Fig 2A) is occurred and concentration of sucrose in the leaves of plants is increased under flooded condition until

the fourth day. From that point, there is a marked decrease of this sugar. Similar results were found by Oliveira Neto (2010) working on jatoba (*Hymenaea courbaril* L.) plant, where he obtained a sucrose increase in leaves under water deficit and decrease in flooded plants.

Materials and Methods

Study location and plant materials

The experiment was carried out in a greenhouse at the Universidade Federal Rural of Amazônia, Capitão Poço campus\PA (Latitude 01° 44 '47' 'and longitude 47 03'34' '), in July 2013. The *Ochroma pyramidal* (Cav. Ex Lam) Urb. Seedlings with approximately 15 cm in height were used.

The seedlings were kept in 10L pots. The substrate was a dystrophic yellow latosol and cattle manure in the ratio of 3: 1 respectively, on a layer of 0.02 m of crushed stone to facilitate water drainage.

Three months before the experiment, 600 mL of complete nutrient solution according to Hoagland and Arnon (1950) were applied, as 200 mL of solution for each month. Daily irrigations were made to keep the soil at field capacity. The weighting of whole pots/plant was done periodically to deposit the transpired water based on weight difference. The field capacity of the substrate was estimated in the laboratory, according to the soil column method according to Sykes and Fernandes (1968). The plants were subjected to three water regimes: irrigated (control), water deficit (total suspension of irrigation at the beginning of the experiment) and flooding (maintained a blade 5 cm of water above ground) in an interval of twelve days.

The plants were subjected to three water regimes: Control: these were maintained in daily irrigation system not suffering any stress; Drought: consisted of full suspension of irrigation from beginning to end of the experiment; Flooding: a sheet of 5 cm of water above the soil was maintained from the beginning to the end of the experiment. During the experimental period, the control plants were irrigated daily to replace the lost water, being made individually for each pots, taking into consideration the daily weightings whole (vase+ plant+soil). For biochemical analysis, leaf samples were collected (second or third pair of mature leaves from the apex), between 9:00 am and 11:00 am, and immediately frozen (approximately -20 °C).

Experimental design

The experimental design was completely randomized in a factorial scheme (3×4), represented by three water conditions: (control, water deficit and flooding) and periods of evaluation (0, 4th, 8th and 12thday), with 5 repetitions, totaling 60 experimental units, each unit being composed of a plant/vase.

Predawn leaf water potential

It was determined between 4:30 and 5:30 am, as described by Pinheiro et al. (2007) via a pressure pump Scholander type (m670, Pms Instrument Co., Albany, USA).

Gas exchange

And stomatal conductance to water vapor (gs), transpiration (E) and leaf temperature (Tfol) were determined by a portable porometer dynamic equilibrium (mod. Li 1600, LiCor, Nebraska, USA) and the flow of photosynthetically active

radiation (PAR) was determined using a quantum sensor coupled to porometer. The measurements were conducted under light conditions and CO₂ environment, between 09:30 and 10:30 (Costa and Marengo, 2007).

Concentrations of abscisic acid (ABA)

The Ross et al. (2004) method was adopted with some modifications. A 50 mg of dried leaf tissues in a forced air circulation glasshouse, were macerated in liquid N with PolivinilPolipirrolidona 100% (PVPP). Then, it was placed in 1.5 mL of solvent extraction (acetone/H₂O/acetic acid: 80/19/1). The extract was transferred into another tube. The mortar was washed with 500 µL of solvent extraction (acetone/H₂O/acetic acid: 80/19/1) and transferred to the same tube, adding 40 mg of (-) -5,8',8',8',-d4-ABA deuterated. Next, the supernatant was transferred to the injector. The analysis was made by liquid chromatography coupled to mass spectrometry in ionization mode negative electrospray (HPLC/MS/ESI-). The detection and quantification of ABA in the samples were made by multiple reaction monitoring (MRM) via mass specific transition selecting of the molecule of interest (for ABA, 263 → 153 and, for ABA d4, 267 → 156).

Starch concentration

For determination of starch 50 mg of powder was incubated with 5 mL of ethanol at 80°C for 30 min, centrifuged at 2.000 g for 10 min at 25°C, and the supernatant was removed. In addition, a second extraction was carried out with the same powder incubated with 5 mL of 30% HClO₄ at 25°C for 30 min and centrifuged in conditions previously described. The supernatants of the two extractions were mixed. The quantifications of the total soluble carbohydrates and starch were carried out at 490 nm using the method of Dubois et al. (1956), using glucose (Sigma Chemicals) as standard.

Total soluble carbohydrates concentrations

To determine the quantity of total soluble carbohydrates, 20 mg of leaf powder was incubated with 2.0 µL of 80% ethanol at 95°C for 20 min and centrifuged for 5 min at 5.0 g and 20°C. The supernatant was then removed and the quantification of the total soluble carbohydrates was performed in reactions containing 1.250 µL of 100% H₂SO₄, 70 µL of 15% phenol, 580 µL H₂O, and 100 µL of extract for a total volume of 2.0 µL. Measurements were taken at 490 nm (Dubois et al. 1956) using glucose (Sigma chemicals, São Paulo, Brazil) as a standard.

Sucrose concentrations

The determination of sucrose was carried out with 50 mg of leaf powder incubated with 1.5 mL of solution MCW (methanol, chloroform and water) in the proportion of 12:5:3 (v/v) at 20°C by 30 minutes under agitation, centrifuged at 10,000g for 10 minutes at 20°C and the supernatant was removed. The sucrose quantification was carried out at 620 nm, in agreement with Van Handel (1968), using sucrose (Sigma Chemicals) as standard.

Statistical analysis of the data

The experimental results were submitted to analysis of variance (ANOVA), and when significant differences observed, the means were compared using Tukey's test at 5%

significance level. Regression analyzes were performed on variables, whose significance was determined by F-test ($P \leq 0.05$). The SWNTIA program was used for statistical analysis (EMBRAPA, Campinas-SP, 1995). The regression models that best represented the behavior of the variables were polynomial models of 2nd order and logarithmic function.

Conclusions

The water deficit conditions and flooding in young plants of balsawood strongly reduced the predawn water potential, stomatal conductance and transpiration indicating an efficient stomatal control of transpiration in this species. Increases in levels of total soluble carbohydrates and sucrose were higher in plants under water deficit, indicating a possible occurrence of osmotic adjustment in this species. Plants under flooding showed to be more sensitive in relation to plants under water deficit.

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