Physiological behavior of *Annona muricata*, *Dipteryx odorata* and *Copaifera langsdorffii* in response to water and light stress

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**Abstract**

The Amazon is a biome where in recent decades climate change has caused the increase in physiological plasticity of certain plant species. This plasticity characterized as resilience strategy of these species to environmental stress conditions such as drought stress. In this context, understanding the physiological adaptation mechanisms of plants that has ethnobotanical interest to Amazonian traditional communities is new and of great importance for the socio-economic sustainability. Thus, the aim of this study was to analyze experimentally the physiological behavior of *Annona muricata*, *Dipteryx odorata* and *Copaifera langsdorffii* in response to water and light stress. The variables analyzed were photosynthesis (μmol m⁻² s⁻¹), stomatal conductance (mol m⁻² s⁻¹), transpiration rates (mmol m⁻² s⁻¹) and leaf temperature (°C) in leaf tissues subjected to four treatments. The equipment used for the measurement was an Infrared gas analyzer (IRGA). The water and light stress affected more *Dipteryx odorata*, while *Copaifera langsdorffii* and *Annona muricata* were more tolerant to water stress because they have drastically reduced the stomatal conductance and transpiration rates. In function of the ethnobotany´s importance that such species have to Amazonian populations, physiological responses found here, suggest different ways of cultivation and water´s supply.

**Keywords:** Amazon, Annona, Copaifera, water stress

1. **Introduction**

Global and local climate change in the Brazilian Amazon been accentuated in recent decades as IPCC data (IPCC 2014) [15]. Thus, the physiological behavior of woody plants in the different ecosystems in the Amazon also been modified in an attempt to increase their resilience to adverse weather conditions characterized by severe droughts interspersed with periods of heavy rain. Thus, plant species have evolved and adapted physiology at constant environmental stress (Lourenço et al 2014) [23].

Water stress, typical of dry periods in the Amazon characterized by reduced rainfall between the months of July to November, have worsened in recent years, where in 2015 for example in the municipality of Santarém, state of Pará, were observed 150 days without rain. This scenario results in the adoption of physiological strategies by plants to tolerate very negative potential water in Amazonian soils. These strategies range from the reduction in stomatal conductance (Santos Filho 1984) [36], reducing transpiration rates and photosynthetic rates, osmotic adjustments and changes to the genetic level, as reported Noorka1.

In this context, species of high ethnobotanical value to traditional communities in the Amazon as Soursop (*Annona muricata* L.), Cumaru (*Dipteryx odorata* (Aubl.) Willd) and Copaiba (*Copaifera langsdorffii* Desf.) are of great importance for research on the physiology of drought stress.

The genus *Copaifera* (*Leguminosae – Caesalpinioidae*) occurs in Africa (4 spp.), Central America (4 spp.), South America (about 37 spp.) and probably in Asia (1 sp.). The follow species recognized within the Brazilian Amazon: *Copaifera duciei*, *C. glycyrrhiza*, *C. guyanensis*, *C. martii*, *C. multiflora*, *C. paupera*, *C. piresii*, and *C. pubiflora e C. reticulate and C. langsdorffii*. Their habit varies between shrubby and tree-like, with some species forming 40 m tall trees. As sour soup (*Annona muricata*) belongs to Angiospermae, Magnoliidae, order. When the tree is mature, it can reach up to 8 meters high, with single trunk and asymmetric branch. As *Dipteryx, odorata* has the follow taxonomy: Angiospermae, Eurosidaes I, Fabaceae, and Faboideae. This specie is part of a successional group considered climax or climax in demanding light. It is a tree with ever- green behavior.
The largest trees reach dimensions close to 40 m tall and 150 cm in diameter. The choice of physiological characters for species selection could boost genetic improvement in order to obtain varieties tolerant to water stress conditions, including the periods of low rainfall and high vapor pressure deficits (DPV) in the Amazon, with a view to sustainable agriculture and local extraction. Several parameters were studied to assess the response of plant species to water stress and/or light, water potential, photosynthetic activity, stomatal conductance, transpiration, and leaf temperature (Nogueira et al. 2001) [31]. However, little known about the physiological behavior of young plants of Cumaru (Dipteryx odorata) and Copaiba (Copaifera langsdorffii), which are species of high economic value on Amazon. They can be used in plantations for reforestation and forest production (Clay et al. 2000; Tonini et al. 2008 and Batista et al. 2003) [5, 44, 31]; beyond Soursop (Gonçalves et al. 2010) [15], which is an important local fruit for economy of family agriculture, which support a short periods of drought.

As for Annona muricata for example, it is currently presented as an alternative to cancer treatments (Astriini et al. 2013) [1] because it has three compounds are potential anti-cancer, are mono-tetrahydrofuran acetogenins namely, muricin H, muricin I, and cis-annomontacin. Thus, the use of water stress potentials should be analyzed, considering that in flooded soils there is a reduction in photosynthetic rates according to Peng et al. 2012) [49], and therefore the synthesis of compounds of interest. As to gender Dipteryx, it has high importance to ethnobotany for communities in remote areas in forest ecosystems and savannahs in the Amazon due to its anti-ophidic action. According to (Yoshida et al. 2015) [45], the phenolic compounds from Dipteryx alata Vogel assayed against the in vitro neurotoxic effect induced by Bothrops jararacussu (Bjssu) venom. Therefore, knowing the physiological responses of Dipteryx odorata as the efficient use of water and strategies to water stress is important for the management of the species and consequent production of anti-ophidic substances. Other works about fertilization methods for growing Cumaru, such as (Jaqueti et al. 2014) [14], the results suggested the combination of green and chemical fertilization was more effective in improving the photosynthetic performance and growth of Dipteryx odorata during initial establishment in a degraded area than either treatment (alone fertilization). However, little known about the physiological behavior of Cumaru under low potential water of soil.

As for C. langsdorffii, studies have shown that this kind functions as phytoremediation for heavy metals. According to work (Soares et al. 2001) [41], the group of plants only slightly affected by soil contamination such as Acacia mangium, Copaifera langsdorffii and Cedrela fissilis who accumulated more Zn and Cd in the roots than in the shoots, therefore indicating reduced translocation is involved in their tolerance to the excess of heavy metal in soil. Similarly, there is a lack of studies on the physiological responses of Copaiba under water stress.

The objective of this research was to analyze the physiological behavior of A. muricata, D. odorata and C. langsdorffii in response to different water and light levels.

2. Materials and Methods
The experiment was conducted in the greenhouse at Universidade Federal do Oeste do Pará - UFOPA, whose coordinates are 2° 25’ 04.71” S and 54° 44’ 26.61” W in the municipality of Santarém-Pará-Brazil. According to IBAMA (2004) [17], the climate is Ami according to Köppen. Santarém has a small annual temperature variation with average ranging from 25.4 °C to 30.1 °C. The relative humidity values of air are high throughout the year, with an average of 86.7 % and rainfall with annual averages hovering around 1920 mm (INMET 2011) [38]. The study carried with 20 individuals (six months age) of each specie (Annona muricata L., Dipteryx odorata (Aubl. Willd. and Copaifera langsdorffii Desj). Herbarium of EMBRAPA took taxonomic identification of the species. The plants were on black polyethylene bag, containing about 1.5 kg of organic substrate. The chemical composition and granulometry was: coarse sand - 153 g kg⁻¹, sand - 85 g kg⁻¹, silt - 483 g kg⁻¹ and clay Total - 280 g kg⁻¹; pH (H₂O) - 5.3 ; Total Nitrogen - 1.12%; phosphorus - 150 mg dm⁻³; potassium - 108 mg dm⁻³, calcium - 9.2 cmol dm⁻³, magnesium - 12.6 cmol dm⁻³ and aluminium - 0.1 cmol dm⁻³ (EMBRAPA 2011) [39].

The four treatments were T1: normal light conditions (100% light) and water (240 ml dia⁻¹); T2: normal light conditions (100% light = 1 500 µmol m⁻² s⁻¹) and water stress (without H₂O); T3: normal water conditions (240 ml dia⁻¹) and light stress (80% shading = 800 µmol m⁻² s⁻¹) and T4: water stress (without H₂O) and light (80% shading). The shading done on Photosynthetic Active Radiation (PAR) for seven days, as well as the irrigation was suspended. The determination of the optimal amount of irrigation in the seedlings pots (240 ml dia⁻¹) was determined by calculating the capacity of field for the specific size of seedlings pots obtained from the irrigation process until the leakage limit of the water in the pots. The photoperiod in the region is 12 hours a day.

The collected data made on two consecutive days of June. The variables measured in mature leaves fully expanded, intact and asymptomatic of health or nutritional deficiencies were Total Nitrogen - 1.12%; phosphorus - 150 mg dm⁻³; potassium - 108 mg dm⁻³, calcium - 9.2 cmol dm⁻³, magnesium - 12.6 cmol dm⁻³ and aluminium - 0.1 cmol dm⁻³ (EMBRAPA 2011) [39]. Measurements determined by a portable equipment, an IRGA (Infrared Gas Analyzer), LCPro mode + ADC and carried out between 08:30 to 10:30 hours. The conditions was under the following conditions: Scattering environment around the leaf tissues 1500 µmol m⁻² s⁻¹ and radiation on leaves in the shading around 800 µmol m⁻² s⁻¹; atmospheric pressure of 1.012 mbar, photosynthetically active radiation (PAR) 1 500 µmol m⁻² s⁻¹ (constant) and reference carbon around 406 ppm. Statistical analyzes were determined by BioEstat program. Version 5.0 through the Statistical Analysis Descriptive, Multivariate analysis using Hotelling test, ANOVA (Tukey test) and Factorial (a x b x c).
important physiological variables needed for growth, maintenance and plant development, mainly due to the low supply of water and light for short period. For the specie *D. odorata*, the rates photosynthetic between treatments (T1, T2 and T3) had a tendency to be forthcoming, however, treatment with water and light stress (T4) was greatly reduced photosynthesis (Figure 1). The stomatal conductance and transpiration rates for T1 and T2 have similar values, but there was a considerable reduction to the T3 and T4 (Figures 2 and 3). Leaf temperature for the treatment T2 was reduced sharply compared with the treatments (T1, T3 and T4), which had average similar (Figure 4). These results were similar to those reported by Costa Azevedo (2014) studying the *B. excelsa* behavior and andiroba (*C. guianensis*). He found that *B. excelsa* were sensitive to moderate water stress associated with higher photosynthetic rates and faster growth of *C. guianensis* under full sun and moderate water stress make this species a promissory candidate to experiment in reforestation programs. The results showed that the interaction of water and light stress influenced the photosynthesis, stomatal conductance and transpiration of young plants of Tonka bean and the light stress can impair the behavior of the species by influence Gs, E and Tf. Thus, the light is decisive factor in the physiology of the species that showed very sensitive when subjected to light restraint, resulting in less force for the plants during the early stages of development. According to Marenco et al 2003 [26], the rate of electron transport (J), A, and water-use efficiency (WUE) increased consistently at increasing internal CO₂ concentration (Ci). Conversely, increasing [CO₂] decreased Gs, E, and photorespiration (Pr) at *Ochroma pyramidal* leaf tissues. Copaiba had a tendency to similar photosynthetic and stomatal conductance rates between treatments (T1 and T3) (Figures 1 and 2). However, treatments (T2 and T4) for the variables A and Tf reduced when compared with other treatments (Figures 1 and 4). For sweating there were considerable decreases in treatment (T2 and T4) when compared to the T1 (Figure 3), while leaf temperature was reduced to the treatments (T3 and T4) (Figure 4). The results show that in general way was no relationship between A, Gs and E, observed under water stress (T2) and bright stress (T4). So, the stress from lack of water supply may have caused increase in temperature leaf due to the closure of stomata reducing the transpiration of water vapor, necessary for cooling of the leaf surface resulting in lower CO₂ assimilation.

**Fig 1:** Photosynthetic rates (umol CO₂·m⁻²·s⁻¹) of young plants of soursop (graviola), tonka bean (cumaru) and copaiba subjected to four treatments (T1: normal light conditions (100% light) and water (240 ml dia⁻¹), T2: normal light (100% light) and water stress (without H₂O), T3: normal water conditions (240 ml dia⁻¹) and light stress (80% shading) and T4 water stress (without H₂O), and light (80% shading).

**Fig 2:** Stomatal conductance (mol m⁻²·s⁻¹) of young plants of soursop (graviola), tonka bean (cumaru) and copaiba subjected to four treatments (T1: normal light conditions (100% light) and water (240 ml dia⁻¹), T2: normal light (100% light) and water stress (without H₂O) and light (80% shading).
The results of comparing the photosynthetic rates of young plants of Soursop, Cumaru and Copaiba over a week under four treatments showed significant difference ($p = 0.0389$) between the photosynthesis of the species under conditions of water and light stress as factorial analysis (Table 1). These results eventually differing photosynthetic responses of species over a week and thus demonstrating that under unfavorable conditions the species utilize different strategies in relation to the assimilation of CO$_2$, resulting in different growth in the field can determine a successful and extinction another species.

The light intensity and soil moisture are factors that affect the photosynthetic activity of plants (Marenco et al 2006) [25]. The process of opening and closing of stomata is mainly related to the intensity of light and the hydration status of the sheet. Thus, the functioning of stomata and leaf area influence the productivity of the plant. The first factor because it controls the absorption of CO$_2$ and the second because it determines the interception of light. Variations in leaf water potential can affect the assimilation of plant carbon (Hisao 1973) [14]. This is because, if the plant loses water at a higher rate than their capacity to absorb and transport, the leaf water potential decreases, leading to stomatal closure and reduction of photosynthesis (Oren 1999) [3].

According to Koslowski 1962 [21], increased shadowing decreases photosynthesis and, consequently, the production of assimilates and growth regulators, causing reduction in stem diameter. The author considers that photosynthesis apparently keeps a more direct relationship with the growth in diameter than the height of the plant.

However, the results for (Gs, E and Tf) along one week experiment (Table 1) indicated no significant difference in the physiological variables when comparing water and light treatments. These results standardizing physiological responses for the variables in question and therefore the assimilation of CO$_2$ and H$_2$O vapor transpiration into the atmosphere under different conditions. Thus, it is not surprising to find a low correlation between $A$ and $G_s$ in natural conditions and stresses of light and humidity. Furthermore, a poor correlation between Photosynthesis and stomatal conductance can indicate lack of uniformity in the opening of the stomata in the leaf surface (stomatal spots), as shown by Marenco 2006 [25]. The cause can be attributed also to $G_s$, $E$ and $T_f$ that were similar in treatments imposed to species. The increase or reduction in stomatal density can occur with the increase or decrease of the irradiance and a response is found in young plants of many species (Holmes & Cowling 1993) [13].

However, other authors found results where stress conditions influence on physiological variables. In accordance to Gonçalves et al 2005 [11] when submitting saplings of *Aniba roseodora* to different light intensities, observed reductions in transpiratory rate and stomatal conductance. Second Nogueira et al 2001, [31] studying relations between cherry plants subjected to water deficit consisted that increasing periods of water stress caused decreases in plant transpiration and total or partial closing of the stomata also occurred because of such stress extension, the leaf temperature, and the loss of latent heat by transpiration.
Over seven days of observation, the physiological behavior of Graviola (soursop) specie, in different treatments was significant differences (p<0.01) for the variable photosynthesis between treatments (T1 and T4, T2 and T4), identified by analysis of variance (Tukey test). To the variable stomatal conductance were no differences between treatments (T1 and T2, T1 and T3, T1 and T4). Transpiration also showed differences between the same treatments for stomatal conductance and more between treatments (T2 and T4) and leaf temperature were no differences between all treatments. For Cumaru specie was no differences (p>0.01) for most of the treatments. And for Copaiba specie was no differences (p>0.01) among treatments in photosynthesis (T1 and T4, T3 and T4), stomatal conductance and transpiration rates (T1 and T3, T4 and T1, T2 and T3; T2 and T4) and leaf temperature (p>0.01) for most of the treatments. According to Ronquim et al 2008, [22] studying the growth of Caesalpinia ferrea Mart. ex Tul. (Leguminosae, Caesalpinoideae) found that this specie had limitations imposed on light conditions (50% and 70% shading), reducing biometric variables that affect better growth. However, Siebeneichler et al. (2008) [40] found that the growth of Hymenea and E. contortisiliquum under 80% shade level as well as 30, 50% favors the formation of stronger seedlings. According to Costa e Marenco 2007 [7], they found a low correlation between A and Gs and between Gs and Ψ, confirming that both photosynthesis and stomatal conductance are parameters of the plant simultaneously respond to a set of factors that interact in a coordinated manner, but highly complex. While the low concentration of CO2 and high irradiance directly stimulate the opening of the stomata, low humidity and excessive sweating cause decrease in leaf water potential, which can lead to the closing of the stomata (Marenco et al 2006) [23].

According to Medina et al 1999, studying photosynthesis, stomatal conductance and transpiration in Valencia Orange under water stress observed that photosynthesis increased around 9:00 am, when it reached a maximum of 7-8 mmol of CO2 m-2 s-1, then decreasing and remaining virtually stable until 16:00. Stomatal conductance also decreased after 9:00 with averages were 0.08 mol H2O m-2 s-1 to values close to zero and at 8:00 am sweating were close to zero and along the day increased reaching 6 mmol H2O m-2 s-1.

According to Ronquim et al 2009, [35] studying the growth and photosynthesis of young plant leaves of C. langsdorffii under different irradiance, found photo inhibition in the leaves during the dry season and in the shadow associated with drought in the leaves, where the largest means of photosynthesis were around 1-5 umol CO2m-2s-1.

Therefore, the carbon absorption is made by leaves while the water and mineral nutrients is made by the roots, resulting in fostering biomass allocation in leaves when light limitation

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### Table 1: Factor Analyses (a x b x c) to compare interactions of photosynthetic rates (A µmol CO2 m-2 s-1), stomatal conductance (GS mol H2O m-2 s-1) and leaf temperature (ºC) of young plants of Soursop, Tonka bean and Copaiba under 4 treatments (T)*.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>A</th>
<th>Gs</th>
<th>E</th>
<th>T</th>
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<tr>
<td></td>
<td>p</td>
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<tr>
<td>Factor A= species</td>
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<tr>
<td>Factor B= Light</td>
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<tr>
<td>Factor C= water</td>
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<tr>
<td>Interaction A x B</td>
<td>0.1102</td>
<td>0.1057</td>
<td>0.1828</td>
<td>0.6010</td>
</tr>
<tr>
<td>Interaction A x C</td>
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<td>0.5910</td>
<td>0.1217</td>
<td>0.5050</td>
</tr>
<tr>
<td>Interaction B x C</td>
<td>0.0389</td>
<td>0.5599</td>
<td>0.9432</td>
<td>0.2132</td>
</tr>
</tbody>
</table>

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* T1: 100% light + 240 ml H2O week⁻¹, T2: 100% light +0 ml H2O week⁻¹, T3: 80% shading + 240 ml H2O week⁻¹ and T4: 80% shading + 0 ml H2O week⁻¹
and in favor of roots where mineral nutrients become limiting for growth (Shipley 2002) [19].

The growth and primary productivity of ecosystems are dependent mainly on the process of photosynthesis. The chemical energy stored in the oxidation reaction processed through the electron transport chain in the thylakoid of chloroplasts by a series of clips to produce NADPH and high-energy molecules (ATP) to use in reduction reactions of carbon (Melis 1999) [28]. Thus, the species can accumulate carbon under stress conditions (high light intensity and low nutrient availability, for example) and carbon stocks can sustain growth when the stress is relieved (Martin & Smith 1995) [27]. Our results showed different levels of physiological plasticity among the three species studied before light and water stress, thus daytime photosynthesis regulatory processes are essential to maintain a positive internal balance for the various processes involved (physical components, photochemical and biochemical) while responding to environmental conditions (Geiger & Servaites 1994) [10].

News studies are needed for understanding all physiological behavior of A. muricata, D. odorata and C. langsdorffii, like studies with osmotic adjustment and fluorescence.

4. Acknowledgments

The interaction of water and light stress can influence the development of Amazonian species such as A. muricata, D. odorata and C. langsdorffii during the early stages of growth, especially in photosynthetic capacity and transpiration, stomatal conductance and leaf temperature plants.

The water and light stress affected more D. odorata, because it is a complaining sort of light and rapid growth, while C. langsdorffii and Annona muricata were more tolerant to water stress because they have drastically reduced the stomatal conductance and transpiration rates. In function of the ethnobotany’s importance that such species have to conductance and transpiration rates. In function of the stress because they have drastically reduced the stomatal conductance and leaf temperature plants.

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