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Amazonian medicinal herbs: Physiology of stress

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Abstract

The use of medicinal herbs by traditional populations along the continents is a reality not only cultural, but also a question of resilience. The community's resilience to response to precarious public health infrastructures in remote rural areas is a signal of disturbance on local development. Thus, the study of the physiological behavior of three medicinal herbs used by agroextractivists communities in the territory of the Lower Amazon, in Amazon biome, under water and light stress conditions was the objective of this work aiming to improve the means of cultivation and quality of herbal medicines production in contexts of local and global climate change. The medicinal herbs studied were *Lippia alba* (Mill.) N.E. Brown (*Verbenaceae*), *Justicia pectoralis* Jacq. (*Acanthaceae*) and *Cymbopogon citratus* (DC) Stapf (*Poaceae*). The experiment conducted in a greenhouse, where the plants submitted to variations of light and water. The variables studied were stomatal conductance, specific leaf area and biomass partitioning. The results showed that the medicinal herbs presented different physiological strategies to survive with different water and light stresses. Cumaruzinho (*Justicia pectoralis*) was the least efficient species in the conversion of CO₂ to leaf dry matter, given its low values of Specific Leaf Area. However, Capim Santo (*Cymbopogon citratus*) was the most efficient species in the production of foliar mass, because smaller Specific Leaf Area obtained. *Lippia alba* did stomatal control under water stress situations. The physiological behavior of the species studied here, suggests different ways of planting for potential production of metabolites that interest to phytotherapy and Ayurveda medicine.

Keywords: stomatal conductance, phytotherapy, water stress.

1. Introduction

The study of physiological behavior of medicinal herbs of interest to phytotherapy and ayurvedic medicine, in face of the edaphoclimatic variations in the productive ecosystems, is essential for understanding the mechanisms of species adaptation (HOFFMANN; FRANCO, 2008) [36]. This knowledge is particularly necessary in the face of local and global climate change (Barbosa *et al.* 2012) [6]. aiming at conservation of these species, new forms of management and increase the production of herbal medicines from medicinal herbs. The physiology of stress can increase the production of active principles by certain medicinal herbs increasing the value of the final product as well as the efficiency of the vegetal drugs in contexts of herbal medicine and Ayurveda. Changes in the primary metabolism and total biosynthesis of natural products with potential for the development of new drugs in response to water stress observed by Martim (2013) [50]. The same happened in a study on peppers and medicinal plants that grew under water stress (lack of water), which presented high concentrations of active compounds compared to plants that grew without water stress (Selmar; Kleinwächter, 2013) [67].

Water stress also negatively affects many aspects of plant physiology, especially the photosynthetic rate (Osakabe *et al.* 2014) [61]. Thus, the physiological behavior of plant species in face of water stress is expressed by complex regulatory events mediated by Abscisic acid (ABA), ion transport, and the activities of transcription factors (TF) involved in the regulation of stomatal responses (Mcadam, Brodribb, 2014) [53]. The overexpression of the ZAT10 and ZAT12 genes is highly induced by several genes related to water stress in plants.

Considering that pharmacognosy consists in the extraction of a wide range of substances such as essential oils and alkaloids from medicinal herbs in order to identify such active principles (KOROLKOVAS, 1996) [43], once it is associated with physiology of stress research in order to increase the active principles, we would then have an interdisciplinary research. This research could capable of improving ways of water managing in productive herbal ecosystems, as well as the production of more potent plant drugs.

In Brazil, mainly in the Amazon region, there is a high diversity of plants with medicinal use by traditional populations (indigenous and quilombolas) that still do not have complete pharmacognostic evaluation like Erva Cidreira (*Lippia alba*); Capim Santo (*Cymbopogon citratus*) and Cumaruzinho (*Justicia pectoralis*). *Lippia alba* (Mill) NE Brown is a plant belonging to Verbenaceae family, commonly known as a lemongrass. It is characterized as a shrub up to 1.50 m high, with thin, whitish and brittle branches (HENNEBELLE *et al.* 2008) [34]. *L. alba* has several chemotypes with morphological and chemical divergences regarding the contents of its essential oils varying according to climatic conditions and regions (BARBOSA; BARBOSA; MELO, 2006). This fact raises the possibility that the variations in the chemotypes would be a consequence of the influence of environmental factors (TAVARES; JULIÃO; LOPES, 2005) [72]. (BIASI, 2003) [8]. The compounds most commonly found in its essential oil are carvone, citral, limonene, linalool and myrcene (SOARES, 2001). Recently, beta-copaene, gamma-morphine and cis-beta-guaiane have also been identified (PARRA-GARCÉS *et al.* 2010). However, genotypes with larger leaf areas and longer stem length tended to have higher oil contents and higher concentration of Linalol. The concentration of oil was inversely proportional to the production of dry leaf mass (JANNUZZI *et al.* 2010).

Lippia Alba is popularly used as sedative, spasmolytic, expectorant and antiseptic (ANDRIGHETTI-FRÖHNER *et al.* 2005) [3], in the treatment of hemorrhoids, pain in the teeth and in hepatic affections (AGUIAR, COSTA, 2005), as well as antihypertensive agents (CARMONA *et al.* 2013), antipyretic and emenagogic, in case of dysentery and antirheumatic (OLIVEIRA *et al.* 2006). The most recent studies detected anxiolytic and sedative activity in the essential oil components of *Lippia alba* (VALE *et al.* 1999), (DO VALE *et al.* 2002). Another study suggests that *L. alba* exerts anxiolytic effects in generalized anxiety disorder, and suggests that Carvone is one of its constituents responsible for its soothing action (HATANO *et al.* 2012). Regarding its antiviral activity, *L. alba* extract showed inhibition of Herpes Simplex virus type 1, resistant strain 29R / aciclovir. In addition, *L. alba* extract in ethyl acetate showed antipoliiovirus activity (ANDRIGHETTI-FRÖHNER *et al.* 2005) [3]. As well as the inhibitory effect of the essential oil seems to cause direct inactivation of the dengue virus before adsorption in the host cell (OCAZONEZ *et al.* 2010). In a study conducted to demonstrate the antifungal and antibacterial activity of *L. alba* extracts, antioxidant and antibacterial activity was demonstrated (HENNEBELLE *et al.* 2008) [35]. (FABRI *et al.* 2011) [24], against four pathogenic gram-positive bacteria and six bacteria Gram-negative pathogens. However, little is known about the physiology of *Lippia alba*, such as its water use efficient, stomatal conductance, photosynthesis and whether it increases oil contents in the face of the imposed water stress.

As for grass (*Cymbopogon citratus*), it is an herbaceous grass, whose leaves meet at the base, being 100 cm long and 1.5 to 2.0 cm wide (DUARTE, 2004) [23]. The leaves of *C. citratus* contain about 60% carbohydrates, protein (20%), fat (5%), ash (4%) and moisture (9%). Phytochemical screening revealed the presence of tannins, flavonoids and terpenoids in all extracts. In its essential oil are present as major constituents: Geranial, Nerol, B-Pinene, Cis-Geraniol, Cis-Verbenol and Geranyl Acetate. The mixture of two geometric isomers, Geranial and Nerol constitutive of Citral, is

responsible for about 75% of the total oil (KPROVIESSI *et al.* 2014) [44]. The plant also contains phytochemicals such as flavonoids and phenolic compounds, which consist of Luteolin, Quercetin, Kaempferol and Apiginine (SHAH; SHRI; PANCHAL, 2011) [68]. The species *C. citratus* is a medicinal plant traditionally cultivated and used in several places in the world. It originates in Southeast Asia and now well distributed among tropical and subtropical regions. In Malaysia it is used for high fever, stomach, intestinal problems and headache (KOH; MOKHAR, IQBAL, 2012) [42]. In Cuba as antihypertensive and anti-inflammatory (CARBAJAL; CASACO, 1989) [14]. In Brazil as anxiolytic, hypnotic and anticonvulsive (BLANCO *et al.* 2009) [10]. Research has demonstrated the antioxidant power of lemongrass (ALVIS; MARTÍNEZ; ARRAZOLA, 2012), which can prevent endothelial dysfunction (CAMPOS *et al.* 2014). In addition, it revealed that lemongrass is cardio protective and anti-oxidative lipids being able to increase several antioxidants at the dose of 200mg / kg, which is comparable to vitamin E (GAYATHRI *et al.* 2011). This antioxidant power has also been investigated and reported as beneficial for its liver function. Studies have shown that the extract of leaves of lemongrass presents inhibitory activity of α -amylase and glycosidase- α enzymes, which may contribute to the management of Type II diabetes mellitus (BOADUO *et al.* 2014) and it can be safely used to reduce blood cholesterol levels (COSTA *et al.* 2011) [19]. Its efficacy as anxiolytic, hypnotic and anticonvulsant of low toxicity has been demonstrated (BLANCO *et al.* 2009) [10]. The anxiolytic potential of the essential oil is due to interaction with the benzodiazepine GABA receptor complex (COSTA *et al.* 2011) [19].

Research confirms that *C. citratus*, in particular its polyphenolic compounds, may be a natural source of a novel and safe anti-inflammatory drug (FRANCISCO *et al.* 2011) [28]. In a study carried out to determine the scientific basis for the therapeutic use with *C. citratus*, it was demonstrated its efficiency for several pathogens very prevalent in the Amazon region such as *Entamoeba histolytica*; Bacteria very common in the Amazon region. The essential oil of fresh leaves has an anti-ascaris activity (SHAH; SHRI; PANCHAL, 2011) [68]. In relation to Malaria, essential oils of *C. citratus* produced 86.6% suppression of *Plasmodium berghei* growth when compared to chloroquine (TCHOUMBOUNANG *et al.* 2005) [73].

As for Cumaruzinho (*Justicia pectoralis*) is an herb with approximately 30 cm in height. According to FONSECA; SILVA; LEAL, (2010) [27], coumarin are the major secondary metabolites of the species and responsible for the therapeutic action. The distribution of these secondary metabolites in the *Acanthaceae* family is restricted to a few species, and *J. pectoralis* is the only member of its genus to produce them, which gives it great ethno pharmacological importance (MURRAY; MENDEZ; BROWN 1982) [75]. Previous phytochemical studies with *J. Pectoralis* have demonstrated the presence of several components including coumarin (1, 2-benzopyrone) and umbelliferone (7-hydroxycoumarin) that are related to estrogenic, progesterogenic and anti-inflammatory activities, justifying its popular use for pre-menstrual and menopause (CORRÊA; ALCÂNTARA, 2011) [18]. Ortho-Methoxylate Glycosideoflavones and polyphenolic compounds that related to their antioxidant capacity (ARTEAGA *et al.* 2010) [4], besides *Justicidin B*, a bioactive lignan with cytotoxic action power in leukemia and solid tumors. (HUI *et al.* 1986) [73]. (JOSEPH *et al.* 1988) [40]. It has

also been reported (LINO, 1995) [47]. the presence of caffeic acid, glycosides and essential oils in addition to suggesting the presence of N, N-dimethyl tryptamine in the dry leaves of plant. Its yield in terms of essential oil showed a maximum of 0.5% tending to decrease when the amount of soil fertilizer increased (BEZERRA, 2008) [7]. It is a perennial herbaceous plant, often found in Mexico, Venezuela, Cuba, Jamaica, West of Ecuador, and North and Northeast regions of Brazil where they popularly known as chambá, clover-cumarú, clover and cumaruzinho, (Oliveira and Andrade 2000) [60]. It commonly used in folk medicine for pain, inflammation and respiratory problems. Despite their traditional use in hallucinogenic snuff by Brazilian natives, studies have not demonstrated the presence of hallucinogenic alkaloids in their composition (MACRAE; TOWERS, 1984) [48]. (OLIVEIRA; ANDRADE, 2000) [59]. However, little known about the physiology of *J. pectoralis*, *Lippia alba* and *C. citratus*.

Thus, this research aimed to study the physiological behavior of 3 medicinal herbs; Erva Cidreira (*Lippia alba*); Capim Santo (*Cymbopogon citratus*) and Cumaruzinho (*Justicia pectoralis*) of interest to Amazonian communities under experimental conditions, in order to diagnose how such medicinal herbs would respond to water stress (lack of water). The species studied listed in the List of Vegetable Drugs approved by the Brazilian Unified Health System and therefore important to Brazilian herbal medicine.

2. Material and Methods

2.1. Description of study area

The Eixo-Forte Agro-Extractive Settlement Project (PAE) was created by the National Institute of Colonization and Agrarian Reform (INCRA) in 2005 and has an area of 12,689 hectares and consists of sixteen communities (PINTO, 2013) [66]. which plant medicinal herbs for their own use. The Eixo-Forte region is located along the highway PA-457 corresponding to the coordinates (2° 24'S, 54° 58'W) and (2° 32'S and 54° 46'W). The presence of savannas that occur in small extensions along the highway, generally isolated, forming mosaics of vegetation, ranging from forests open to savannas, characterize this region. The average annual precipitation in the region is 1,950 mm with a pronounced dry season between the months of June and December, and a rainy season from January to May. The dominant climate in the region is hot and humid, with annual average temperatures varying between 25° and 28°C. According to the climatic classification of Köppen, Santarém is in the climatic type Am, that is, the climate is humid equatorial with a well-defined dry season and another with high rainfall indexes.

2.2 Medicinal herbs studied and experimental treatments.

The medicinal herbs studied were *Lippia alba* (Mill.) N.E. Brown (*Verbenaceae*), *Justicia pectoralis* Jacq. (*Acanthaceae*) and *Cymbopogon citratus* (DC) Stapf (*Poaceae*). These species selected because they presented in the ethnobotanical studies the highest IVI (Import Value Index), as well as, they are in the lists of the Essential Medicines and of plants of interest of the SUS (Brazilian health system). These species were cultivated in a greenhouse under the different treatments below and according to figure 1:

- Luminous treatments: 100% light (L1 = 1800 μ mol / m-2 / s-1) and 50% light (L2 = 900 μ mol / m-2 / s-1)
- Water treatments: With Irrigation (A1) and without irrigation (Water stress = A2)
- Soil treatments: Terra Preta (T1) and Soil fertilized (T2)



Fig 1: Medicinal herb experiment (*Lippia alba*, *Justicia pectoralis* and *Cymbopogon citratus*) under different light, water and soil treatments, Santarém, Pará, Brazil.

2.3 Physic-chemical characterization of soils.

The soil T 1 (black soil) showed a trend towards acidity, with pH between 4.51 and 5.26, while soil T2 (fertilizer) showed a tendency to neutrality, with pH of 7.0 to 7.40. As for the phosphorus content (P) and Potassium (K), soil T1 had a low P value (20.69 mg / kg - 24.18 mg / kg) and a lower K content (0.15 cm² / kg and 0.16 cm² / kg). T2 presented high levels of P between 268.07 mg / kg and 343.06 mg / kg and a high K content between 8.66 mg / kg and 8.89 mg / kg.

2.4 Physiological variables studied

A. Stomatal Conductance (gs) - Four completely expanded and asymptomatic leaves of each species selected. The reading of the stomatal conductance done at three different times (08:00 - 09:00, 11:00 - 12:00 and 17:00 - 18:00) with the aid of an AP4 Porometer (Δ T Devices, Cambridge, England).

B. Specific Leaf Area (AFE) - It was performed by calculating the ratio (AFE = AF / MSF) between leaf area (FA) and dry leaf mass (MSF). The leaf area measured by means of a digital analyzer (Δ T Devices) and the dry weight determined in Ohaus - MB35 model scale, Florham Park, USA.

C. Biomass Partitioning - the distribution or partitioning of the dry biomass of the aerial part (PA), the root portion (SR) and the total part (TP) of the medicinal herbs calculated by weighing these biomasses in analytical balance Quimis model QILA2104. All samples previously dried in a Quimis air forced ventilation oven at 40°C until obtaining constant weight. The following ratios were then calculated: SR / TP; PA / TP and PA / SR.

3. Results and Discussions

The results showed that the stomatal conductance between the medicinal herbs at the most critical hour of the day (1:00 p.m.) was significantly different at the 5% probability level ($p < 0.05$) for all combinations of treatments. Cidreira (*Lippia alba*) obtained the highest values of stomatal conductance when compared to Cumaruzinho (*Justicia pectoralis*) and Capim Santo (*Cymbopogon citratus*). These results indicate that the physiological behavior of Cidreira (*Lippia alba*) with respect to gas exchange differs from the other two species, probably due to osmotic adjustment (increase in the concentration of certain organic compounds) as a strategy to survive water stress. However, the herbs cumaruzinho (*J. pectoralis*) and Santo grass (*C. citratus*) shown to be more sensitive to water stress (L2), since they reduced their stomatal conductance in order to protect the turgescence of their leaf tissues. It is important to note that there is a strong correlation between the presence of stromal conductivity and the presence of a high level of stress. In this way, it expected to find species that mainly use stomatal control to avoid water loss, as observed in aroeira plants (SILVA *et al.* 2005) [69].

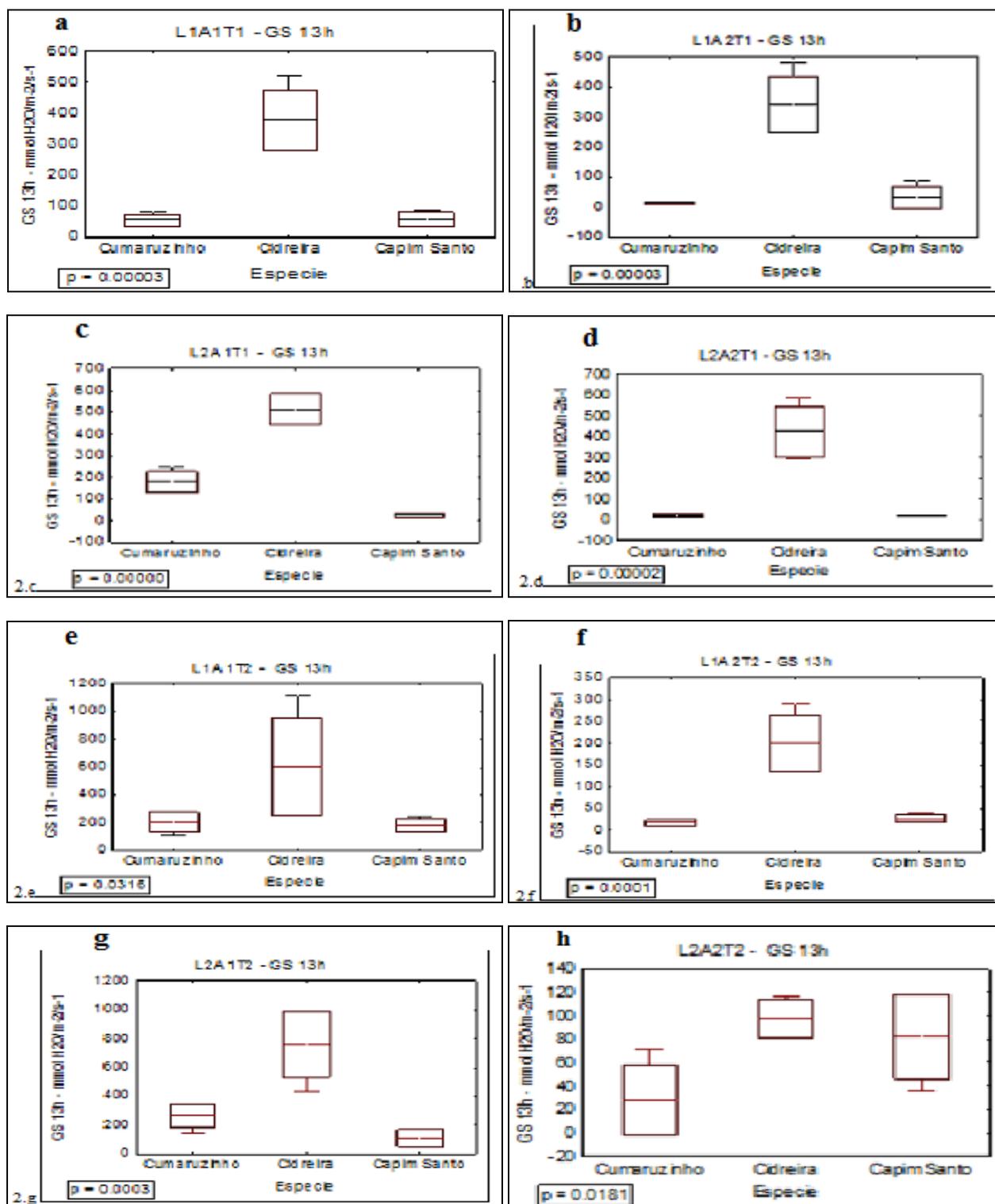


Fig 2: Stomatal conductance (GS = mmol H₂O / m² / s⁻¹) obtained at 13:00 in leaves of Cumaruzinho (*Justicia pectoralis*), Cidreira (*Lippia alba*) and Capim santo (holy grass, *Cymbopogon citratus*) under different experimental treatments: L1 - 100% Light; L2 - 50% light; A1 - no water stress; A2 - with water stress; T1 - Black Earth; T2 - Only fertilized. The centerline of the boxes represents the average, the upper and lower lines of the box represent the standard deviation and the Swiss show the maximum and minimum values.

Specific Foliar Area (AFE)

According to figure 3 it was possible to verify that, in general lines, Cumaruzinho had the highest levels of specific leaf area among the three species in all treatments excluding L2A2T1 (use of sombrite, water stress and black soil) where Cidreira obtained larger values. It was also possible to observe higher values of specific leaf areas in shade treatments (L2). This fact is in line with what is observed in the literature, since as

an adaptive strategy, plants submitted to low irradiance levels expand the leaves to increase light energy uptake and allow greater photosynthetic efficiency and, consequently, greater carbon fixation. In the case of medicinal plants, the most interesting is they have low specific leaf areas, which indicates that the plant incorporates carbon in the leaves, in the formation of organic compounds, with the possibility that these compounds are the secondary metabolites of interest for

pharmacology. In all treatments, Capim Santo obtained the lowest values of specific leaf area, indicating that this species needs small leaf areas for the production of 1 gram of leaf dry

matter, and therefore, this species is more metabolically efficient than the others are.

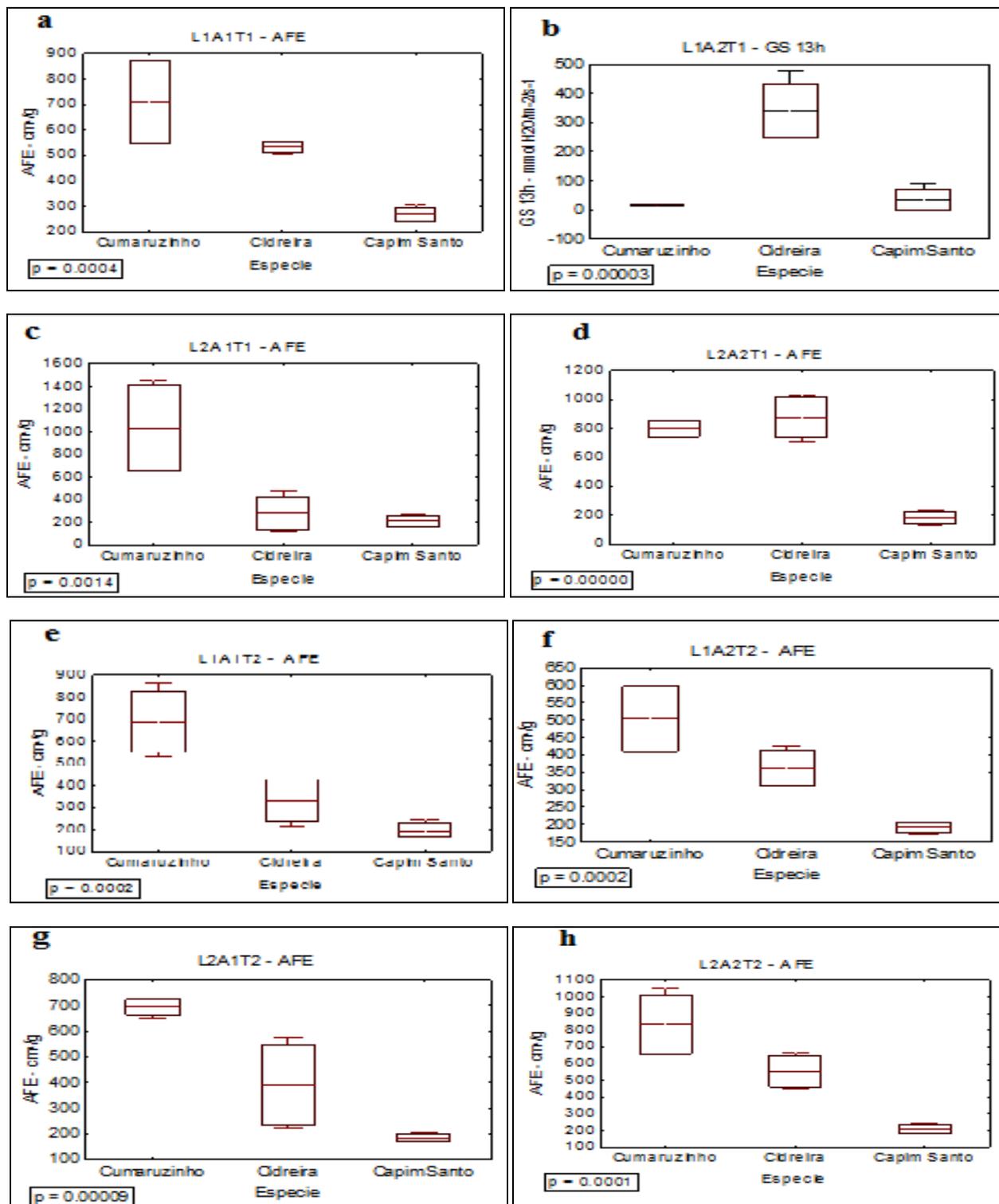


Fig 3: Specific Leaf Area (AFE)(AFE= leaf area/leaf dry matter) Cumaruzinho (*Justicia pectoralis*), Cidreira (*Lippia alba*) and Capim santo (holy grass, *Cymbopogon citratus*) under different experimental treatments: : L1 - 100% Light; L2 - 50% light; A1 - no water stress; A2 - with water stress; T1 - Black Earth; T2 - Only fertilized. The centerline of the boxes represents the average, the upper and lower lines of the box represent the standard deviation and the Swiss show the maximum and minimum values.

Ratio of Aerial and root dry matter (PA / R)

According to figure 4 it is possible to verify that Cumaruzinho presents the highest values in all valid treatments. This indicates that, independently of the environment conditions,

this species is the one that uses the least investment strategy in root growth for survival in water deficit situations. Cidreira maintains the intermediate values throughout the treatments. This information, coupled with the previous result that

indicates that this species is also the one that invests least in closing its stomata among the species studied reinforces the tendency to produce secondary metabolites as a strategy of osmotic adjustment in order to maintain the water balance of the plant. The osmotic adjustment is an increase in the osmotic potential caused by the accumulation of solutes in the cells, which maintains the gradient of water potential and, at the same time, the turgescence necessary for cell growth

(Chaves Filho *et al.* 2001) [16]. This information is in agreement with what is observed in the literature in relation to the plants increasing their levels of active principles in stress situations (FATIMA; FAROQI; SHARMA, 2000) [26]. (GOBBO-NETO; LOPES, 2007) [32]. The smallest reasons found in Capim Santo suggesting that this species leads the partitioning of photo assimilates to the root as a survival strategy to water stress.

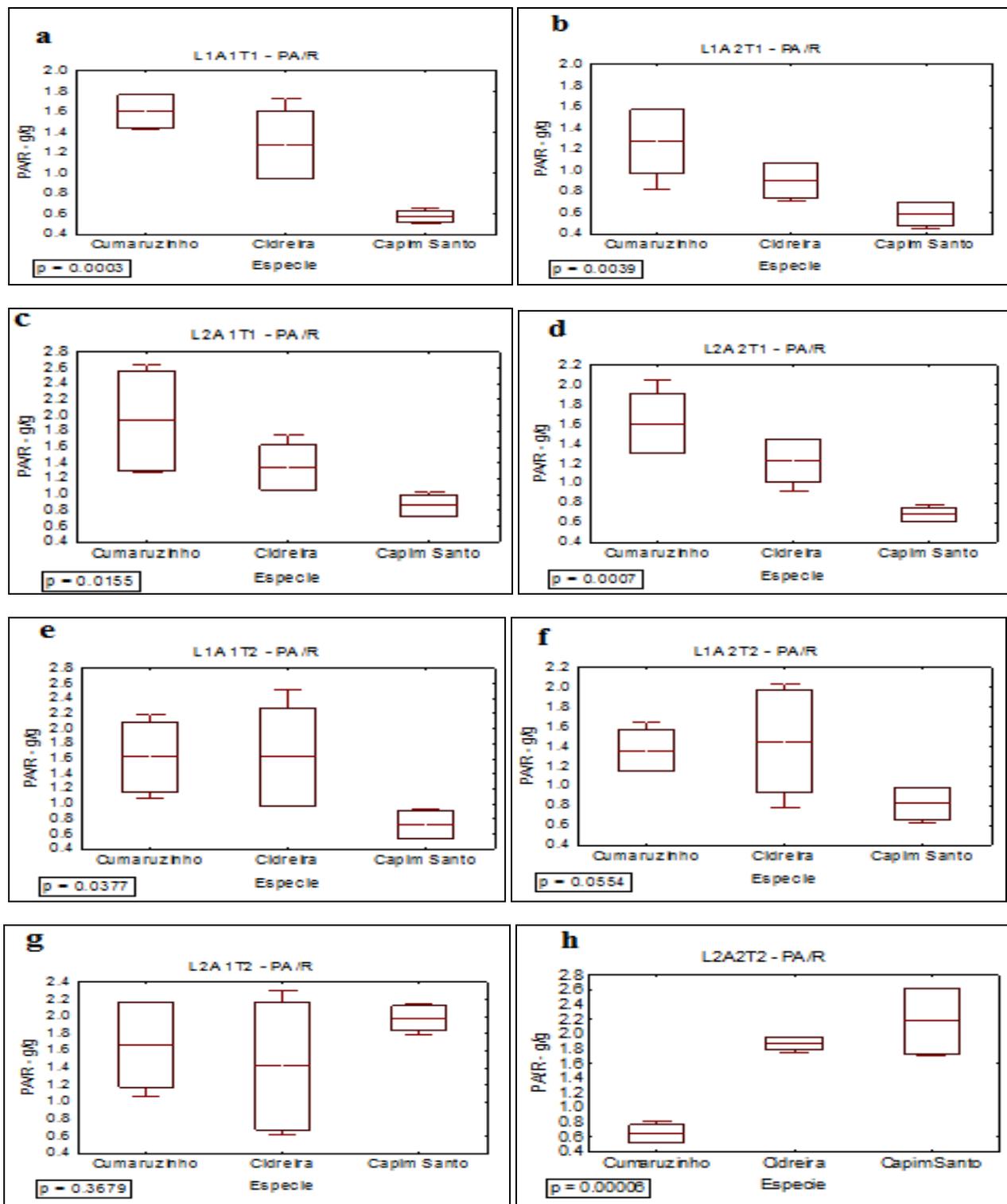


Fig 4: Ratio of leaf dry matter/ root dry matter (PA/R) of Cumaruzinho (*Justicia pectoralis*), Cidreira (*Lippia alba*) and Capim santo (holy grass, *Cymbopogon citratus*) under different experimental treatments: : L1 - 100% Light; L2 - 50% light; A1 - no water stress; A2 - with water stress; T1 - Black Earth; T2 - Only fertilized. The centerline of the boxes represents the average, the upper and lower lines of the box represent the standard deviation and the Swiss show the maximum and minimum values.

Stomatal Conductance

The correlation between the hours of the day and the measures of stomatal opening verified in this experiment demonstrated polynomial behavior in practically all the treatments according to Figure 5. The result reiterates that there is a tendency of the treatments submitted to water stress to present stomatal opening points smaller. Those submitted to the full irrigation regime. This observation in accordance with found in the literature. In a situation of low availability of water in the soil, plants reduce water loss by reducing stomatal conductance (COSTA, MARENCO, 2007) [20].

The experiment demonstrated that among the species studied, the Cidreira was able to maintain a greater stomatal opening

regardless of the time, because it is a species with proven rusticity and a broad phenotypic plasticity with the capacity to develop in poor soil conditions and with Water scarcity (JANANI; SILVIA; BOARO, 2011) [38]. The holy grass is able to regulate the stomatal opening and is very efficient in the use of carbon. The results are not in agreement with those verified in the várzea ecosystem. Since they represented by polynomial equations and present the highest values accompanying the highest radiation occurring in the time of 12-13 hours while in the floodplain are expressed by equations Exponential, logarithmic and geometric values, the highest values of stomatal opening occur during the first hours of the day and are decreasing.

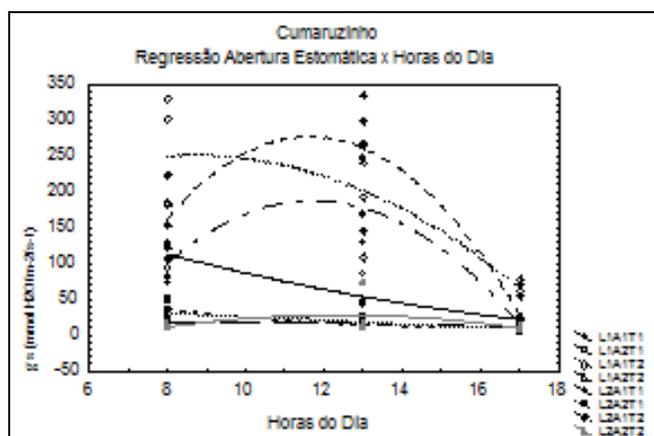


Fig 5: a - Cumaruzinho (*J. pectoralis*)

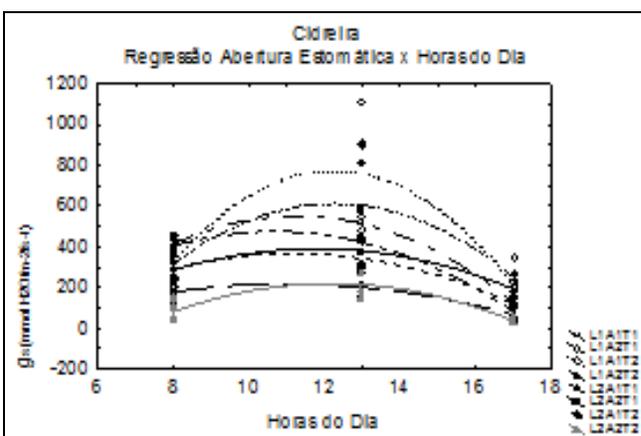


Fig 5:b - Cidreira (*L. alba*)

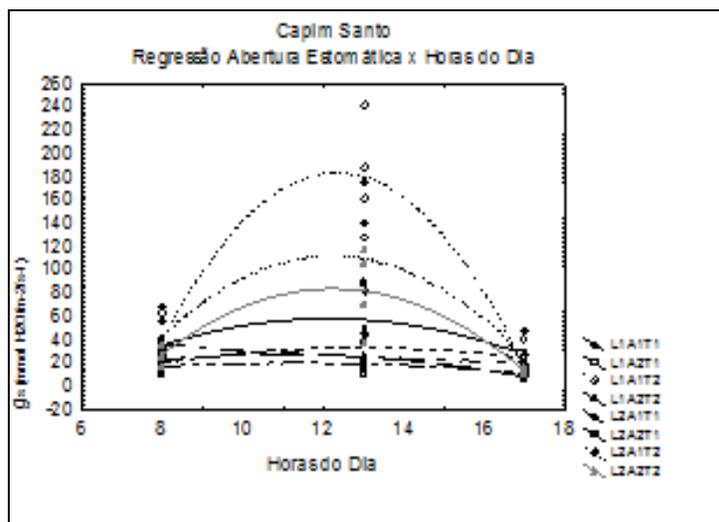


Fig 5: c - Capim Santo (*C. citratus*)

Fig 5: -Regression analysis of stomatal conductance (Gs) (mmol H₂O/m-2/s-1) depending on the timetables (8:00-9:30 h; 12:00-13:30 h; 17:00-18:30). Polynomial models in clockwise from 5a.-Cumaruzinho (*J. pectoralis*); 5. b – Cidreira-Lemon grass (*Lipia alba.*) and 5. c – Capim santo-Holy Grass (*C. Citratus*) in experimental cultivation area, Alter do Chão-Santarém-Pará.

Legends

- L1 – 100% light
- L2 – 50% light
- A1 – full Irrigation
- A2 – water Stress
- T1 – Black soil
- T2 – Fertilized soil

Multivariate Analysis

The Bartlett test demonstrated (Table 1) that species tend to

assume a more similar behavior in relation to the variables stomatal opening, specific leaf area and ratio between the masses of the aerial part and the root part when under water stress, in soil fertilized with Full light (L1A2T2). The lowest likelihood coefficient observed under partial shade, full irrigation and fertilized soil (L2A1T2), where the species assumed different physiological behaviors to cope with the water and light stresses, which could culminate with a greater or lesser production of active principles of interest to herbal medicine.

Table 1 - Multivariate Analysis showing the maximum likelihood coefficients (Phi) in the Bartlett test between the different treatments (L1 = 100% light, L2 = 50% light), Irrigation (A1 = Full Irrigation, A2 = Water Stress) and Soil (T1 = Black Earth; T2 = Black Terra Fertilized) in the species Cumaruzinho, Cidreira and Capim Santo.

Treatments	P	Phi
L1A2T1	< 0.0001	65.3
L1A1T1	< 0.0001	61.8
L2A1T1	< 0.0001	54.8
L2A2T1	< 0.0001	69.4
L2A1T2	< 0.0001	46.2
L1A1T2	< 0.0001	63.7
L1A2T2	< 0.0001	88.3
L2A2T2	< 0.0001	61.9

The multivariate distance from test of Penrose and Mahalanobis (Table 2) showed that in the great majority of treatments the species with the most similar behaviors were Cumaruzinho and Capim Santo, whereas the most physiologically distinct species found in practically all treatments between the Cidreira and Santo grass species. The exceptions found in the L1A2T2 treatments (full sun, water

stress and fertilized soil) that present the longest distance between cumaruzinho and Cidreira and L1A1T2 species in which the smallest distance is also found between cumaruzinho and Cidreira species. Suggesting that under water stress the species tend to adopt patterns of divergent physiological behavior.

Table 2: Multivariate analysis, Penrose and Mahalanobis tests between the different treatments of Radiation (L1 = 100% light, L2 = 50% light); Irrigation (A1 = Full Irrigation, A2 = Water Stress) and Soil (T1 = Black Earth; T2 = Black soil) in the species Cumaruzinho (A), Cidreira (B) and Capim Santo (C).

Treatments	Major Distancie Penrose	Minor Distancie Penrose	Major Distancie Mahalanobis	Minor Distancie Mahalanobis
L1A2T1	B e C 189.2	A e C 13.4	B e C 557.1	A e C 59.6
L1A1T1	B e C 101.4	A e C 16.6	B e C 376.0	A e C 63.4
L2A1T1	B e C 163.0	A e C 16.1	B e C 978.5	A e C 60.1
L2A2T1	B e C 246.9	A e C 5.2	B e C 3003.8	A e C 21.3
L2A1T2	B e C 73.0	A e C 19.6	B e C 472.1	A e C 67.8
L1A1T2	B e C 32.4	A e B 10.8	B e C 217.8	A e B 39.6
L1A2T2	B e C 175.7	A e C 7.8	A e B 1983.7	A e C 23.3
L2A2T2	B e C 44.4	A e C 10.8	B e C 175.4	A e C 30.7

Conclusion

Cidreira (*Lippia alba*) obtained the highest stomatal conductance, Cumaruzinho (*J. pectoralis*) presented the highest levels of specific leaf area and the highest values in all treatments in the PA / R ratio. The lowest PA / R ratios found in Capim Santo (*C. citratus*). In this way, the holy grass presented morphological strategy through the growth of its rhizosphere in front of water stresses. On the other hand, cumaruzinho (*J. pectoralis*) is the least efficient species in the conversion of light to organic leaf compounds, requiring large leaf areas for the production of leaf dry matter grams. Cidreira (*Lippia alba*) showed physiological plasticity, due to its low stomatal conductance in situations of water stress. It is strongly recommended, associated studies of Phytochemistry in conjunction with experiments in plant physiology, where the three species studied here indicate physiological behavior under stress conditions, propitious to the increase of secondary metabolites, which may be some of the active principles of interest to medicine Ayurveda or phytotherapy.

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