Trees Lose Their Leaves Later in Agroforestry Systems
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Abstract— In Brazilian agroforestry systems (AFS), Cordia oncocalyx trees, a native species of Caatinga, lose their leaves late in relation to the trees of the same species occurring in secondary forest. Our hypothesis is that, due to environmental factors, the trees of the AFS maintain better water status. This work aims to present environmental humidity (rainfall, soil moisture and air relative humidity) and trees (photosynthesis, stomatal conductance and transpiration) data to explain the late loss of leaves in an agrosilvopastoral system (AGP) in the Brazilian semi-arid region compared to a secondary forest (SF). Meteorological data were obtained from two weather stations installed in the AGP and SF areas. The physiological traits were measured using an infrared gas analyzer. There was a correlation between physiological processes (transpiration and stomatal conductance) and soil water content in plants of AGP, but not in SF, showing some independence of the plants of this system to variations in soil moisture. This indicates that AGP plants may have developed the physiological and anatomical features that enable to them to keep photosynthesis even when climatic conditions are more severe. Although the most inhospitable environmental conditions in the AGP system, the lower density of plants, and therefore less competition for water, favoring photosynthesis longer, causing the leaves to fall later.

Keywords— Cordia oncocalyx, tree density, gas exchange, semi-arid, secondary forest.

I. INTRODUCTION
The development of plants depends on both intrinsic (inherent to the plants themselves) and extrinsic (environmental) factors. Plant can respond to changes in the environment with both morphological and physiological modifications and adaptations. In high temperature situations, high incidence of solar radiation or water shortage, i.e., may decrease the photosynthesis by closing or reducing the number of stomata [1], modifying hormone levels [2] or modifying the storage of proline, soluble sugars and amino acids [1]. Not only the abiotic factors (extrinsic) from the environment act on the growth and development of plants [3], these are also affected by biotic factors such as population density and intra and interspecific competition, which, in turn, are also affected by extrinsic factors, such as soil conditions [3]. For example, trees in stands with higher densities are thinner and absorb more superficial water, by which they start to compete, in view of the smaller amount of available water in this horizon of the ground. While trees in lower density have thicker stem and the roots can capture deeper water [4].

In agroforestry systems, which are production systems that combine crops and trees, the number of trees is reduced relative to that of the forest. Due to this reduction in density, greater intensity of solar radiation reaches the treetops and the ground. Thus, in semi-arid environments, higher temperatures are expected to be recorded in the soil, higher soil water evaporation and lower relative humidity. While, in relation to plants, a reduction in tree height and higher densities are thinner and absorb more superficial water, by which they start to compete, in view of the smaller amount of available water in this horizon of the ground. While trees in lower density have thicker stem and the roots can capture deeper water [4].

Agroforestry systems have provided an alternative to conventional cropping systems by including larger plants (shrubs and trees). Some studies show the benefits of agroforestry in comparison to monoculture [6, 7], despite the potential for water, light and nutrient competition between cultivated plants and trees [8, 9]. The agrosilvopastoral systems (AGP) is a kind of agroforestry system where both agricultural and animal production are developed together and the trees remain. In this way, another component - animal - emerges as an influencer of tree development.

A more in-depth analysis of water cycling patterns between environmental pools and plants (including transpiration, water use efficiency and water absorption zone) is needed to better understand differential responses in the water absorption patterns of plant species and communities. For example, plants with low water use
efficiency consume more water, depleting the resource in order to maintain stomatal conductance and photosynthesis. Water competition between species through interference or exploration is thus intensified, especially when root distribution patterns overlap. On the other hand, with lower tree density, competition for water resources could be lower.

Authors [5] observed, in a Brazilian agroforestry system, that the trees of Cordia oncocalyx- Allemão, a species native to the Caatinga, lose leaves late in relation to trees of the same species that occur in a forest area. The investigation of this fact started from the hypothesis that the AGP trees maintain a better water state at the beginning of the dry period. This work aims to present environmental and physiological data of the trees to explain the late loss of leaves in an agrosilvopastoral system (AGP) in the Brazilian semiarid compared to a secondary forest (SF).

II. MATERIAL AND METHODS

Study area
The study took place at Crioula farm, which belongs to the National Center for Research on Sheep and Goats of Brazil’s Agricultural Research Corporation (EMBRAPA). The farm is located in the municipality of Sobral (3º41’ S, 40º20’ W) in the State of Ceará. Mean annual temperature and precipitation are respectively 30ºC and 821.6 mm [10]. The dry season lasts for seven to eight months (June to January), and the wet season is shorter (January to May). The climate is semiarid, classified as BSw’h’ according to Köppen: very hot and with most rainfall occurring during the fall. Typical Chromic Orthic Luvisols are the predominant soil type in the study area [11]. The predominant vegetation in the region is a type of woody savannah [12], locally known as Caatinga. It is composed mainly of deciduous species which lose their leaves during the dry season [13].

A long-term experiment was established in 1997 on this farm to evaluate traditional cropping systems (slash-and-burn) and alternative AFS (AGP and silvopastoral). An area was also left under native vegetation (secondary forest) and is used as a control. For this work, we selected two of these experimental areas, and their main characteristics are:

- AGP: area covering 1.6 ha, where rows of Leucaena leucocephala (Lam.) de Wit were established. In the 3-m wide alleys maize (Zea mays L.) and/or sorghum (Sorghum bicolor L. Moench) are grown, with 1 m between plants. Tree density in this plot is approximately 200 trees ha⁻¹, which corresponds to 22% soil cover. No fertilizers are applied and all cropping operations are completed manually. After crop harvest, sheep and goats are allowed to graze the area.
- SF: area covering 1.6 ha under tree-dominated Caatinga, which represents approximately 50 year old secondary vegetation.

The AGP and SF plots contained, respectively, approximately 360 and 2,600 plants with height ≥ 1 m and with a stem diameter ≥ 3 cm at soil level, per hectare. Nine tree species were represented, and the four most common are Cordia oncocalyx-Allemão, Mimosa caesalpinifolia Benth., Poincianella bracteosa (Tul.) L.P. Queiroz and Bauhinia cheilanthes (Bong.) Steud [14].

Meteorological data
Meteorological data was obtained from two weather stations (Campbell Scientific, INC, Utah, USA) installed in the AGP and SF plots. Relative humidity of the air (RH) and soil volumetric moisture content at depths of 30 and 50 cm (Vw30 and Vw50) were measured. Weather stations collected data on these parameters every 30 seconds, and means over 15-minutes increments were stored in dataloggers. Data was collected between 1st May and 30th September 2011. Each day, therefore, 96 readings were recorded.

One rain gauge, connected to the weather stations, was installed in each experimental area, and it was located among the trees in SF and 3 m away from a Cordia oncocalyx tree in AGP. Amounts of rainfall over 15 minutes increments were stored in the dataloggers. Rainfall data shown here represent the total of all rain events over each month, between May and September.

Physiology in Cordia oncocalyx
Cordia oncocalyx is a member of the Boraginaceae. It is abundant in the State of Ceará and dominant in the study area, with a frequency of 49% in SF and 50% in AGP [15, 16]. The SF and AGP plots contain 670 and 80 C. oncocalyx adult individuals per hectare, respectively.

Net photosynthesis (A), transpiration (E) and stomatal conductance (gs) were measured using an infrared gas analyzer (IRGA, LI-6400XT, LI-COR Biosciences, USA). Measurements were made on three sun-exposed leaves on three trees in each of the land-use systems. The trees where measurements were made were selected based on similarities in stem diameter. Scaffolds were erected to a height similar to that of the trees (between 8 and 9 m above the ground). Measurements were taken once a month between noon and 1pm, in May, June, July, August and September. Water use efficiency (WUE) was calculated as the ratio of photosynthesis to transpiration.

Rooting depth in C. oncocalyx
Main lateral root length of *C. oncocalyx* was measured by excavation in July for three trees in AGP and three in SF. Soil was removed from the surface until main lateral roots were found, and these were followed away from base of tree. Rooting depth was determined by cutting trees and completely removing roots from the soil.

Isotope analyzes

*C. oncocalyx* roots samples were collected, along with soil and rainwater to analyze stable oxygen isotopes (δ¹⁸O). Cylinders (5.0 cm high and 0.5 cm diameter) from the stalk of roots were taken. These samples were placed in plastic bottles, capped and sealed with semitransparent, flexible and watertight plastic film. As the stalk cylinders from each of the one tree in each treatment were composited placed in a single sample per treatment for isotopic analysis, no statistical analysis was possible. Soil samples were collected at depth increments of 0-20, 20-40 and 40-60 cm under the crown of trees, and processed similarly to roots. All samples for isotopic analysis were placed on ice to further minimize evaporation during transport to the laboratory where they were stored, refrigerated, until water extraction by vacuum distillation. *C. oncocalyx* roots and soil were collected on 19th May 2011.

Approximately 1 µL of water extracted from root and soil samples, along with rainwater, were used to measure ratios between concentrations of water molecules with different combinations of H and O isotopes (HD¹⁶O, H₂¹⁶O and H₂¹⁸O), using a liquid water isotope analyzer (DLT-100, LGR, CA, USA). The ratios, corrected using a calibration curve and working standards, were expressed as δ in parts per mil (‰) as relative deviations from the V-SMOW international standard, as calculated by equation δ = \(((R_{sample}/R_{standard}) - 1) \times 1000\), where R is the ratio ¹⁸O/¹⁶O of the sample and the standard.

Data analysis

The significance of the differences between means of photosynthesis, water use efficiency and air relative humidity for the two land use systems was assessed using the Student t test, α=0.05. Correlations were made between the trees physiological parameters (stomatal conductance and transpiration) and soil volumetric water. The graphs were constructed using Microsoft Excel and the statistical program MicrocalOrigin™.

III. RESULTS AND DISCUSSION

In the period from May to the end of the first half of July the rainfall was 302.7 mm in the AGP. No rainfall was recorded in the months of August and September. The volumetric soil water content in the depth of 30 cm was greater than 50 cm throughout the study period and declined progressively from July, reaching the lowest values at the end of September, when the average was bit above 0.2 cm³ cm⁻³ (Figure 1). Researches [17] also show a trend of higher soil moisture near the surface and a decrease in depths greater than 30 cm. In the shallower soil (30 cm depth), the oscillations in the volumetric content notably accompanied the rainfall, rather than in deeper layers, a fact also recorded [18].

![Rainfall and soil volumetric water to 30cm (Vw30) and 50cm (Vw50) depth in agrosilvopastoral system (AGP)](image)

In secondary forest (SF), rainfall recorded in the months of May to July was only 187.7 mm. This value, 115 mm lower than that of the AGP, reflects the interception by the crown of the trees, which are in greater density in SF. This difference in recorded rainfall, however, did not lead to differences in soil moisture at 30 cm depth between systems. This is because only a small part of the water intercepted by the trees is absorbed by the leaves, most of it flows down the trunk and reaches the ground. The interception of rainwater by the canopy can vary from 13
to 22% of the precipitated total [19] and for precipitations of less than 11.0 mm the intercepted water does not even reach the soil, due to its rapid evaporation [20].

From the second half of July, when there are no more rainfall, the Vw30 in SF starts to reduce gradually, reaching the lowest values at the end of September (0.24 cm$^3$ cm$^{-3}$) (Figure 2), which are equivalent to the values found at the same depth in the AGP (Figure 3). The soil volumetric water content in SF was higher at depth of 50 cm and peaks were recorded on rainy days, although less notable than at 30 cm. The biggest difference between the systems in the Vw is in the depth of 50 cm. In the AGP, in September, the mean values fluctuated around 0.18 cm$^3$ cm$^{-3}$, while in SF the averages were slightly above 0.3 cm$^3$ cm$^{-3}$. There are biotic and abiotic factors that can justify this difference, such as differences in ground cover and soil characteristics, which may induce variations in soil moisture in depth [21, 22].

In September, the driest of the observed months, AGP and SF presented the same value of Vw30; however, the SF presented higher soil moisture throughout the day in 50 cm depth and lower in the depth of 30 cm and the opposite was recorded in the AGP (Figure 3). As recorded by other authors [23, 24], soil moisture usually increases in depth, as in the case of SF. Among the factors that alter the moisture along the soil profile are evapotranspiration and infiltration. The conditions for the occurrence of higher evaporation can be better recorded for the AGP due to the direct contact of the sun's rays with the soil, whereas in SF, a more closed canopy favors the permanence of water in the soil; this explains, in general, why there is more water in SF soil than in 50 cm depth AGP soil. On the other hand, higher moisture values were recorded at a lower depth (30 cm) than at 50 cm in the AGP. In order to explain the differences in the behavior of soil moisture between systems it is important to evaluate the water absorption pattern of the plant species of the systems. In this sense, it was evaluated, through the isotopic constitution of soil water, rainfall and roots, the preferred source of water absorption. The Figure 4 shows the results.

Soil excavations demonstrated that C. oncocalyx does not have a taproot, but numerous similar roots which reach 20 to 80 cm in depth, which one varies with the size of the plant, in both land use systems. The roots extend laterally up to 3 m in SF and 4 m in AGP. This shows that all water absorbed by the root comes from the soil profile less than 100 cm. In addition, this large lateral extension of the roots allows it to explore more water at the horizon of the soil than at depth.
Isotopic analyzes showed similar values between C. oncocalyx root sap water and the deeper soil (40-60 cm) in the AGP and with the more superficial soil (0-20 cm) in the SF (Figure 4). This point to preferential water absorption at these depths. Such evidence could explain the differences in soil moisture levels recorded throughout the study period for these two areas: lower water content at 30 cm depth in SF and lower at 50 cm depth in AGP. However, the source of water taken up by plants can change depending on the season [25] or according to water availability and rooting depth [26]. When the water requirement of the plant exceeds the supply, it must find other sources of water or use it more conservatively to minimize water stress and maintain metabolic functions. Indeed, plants can take up more superficial water during rainy periods and deeper water during dry periods [27]. Such a capacity to rapidly change the source water from different regions of the soil can give an advantage to plants, when water competition occurs within the ecosystem.

Photosynthesis (A), in general, decreased from the rainy season to the dry season, following reductions in RH, both in AGP and SF (Figure 5). In the months of May to June (rainy season) the photosynthesis was similar between the two systems, ranging from 10 to 12 μmol m^{-2} s^{-1}, when higher RH values were recorded in SF. This could indicate a good acceptability of C. oncocalyx to maintain the rates of photosynthesis under HR of 80 to 88%. However, in spite of the still high levels of RH in July (81% and 86%, for AGP and SF, respectively), the A reduced in the plants of both systems to 7.43 (AGP) and 8.51 μmol m^{-2} s^{-1} (SF). In the dry months (August and September) the photosynthesis was higher in the AGP plants, when RH was similar between the two systems. These observations indicate that other physical factors of the environment, besides RH, are influencing this physiological process in the trees. Among these factors, Larcher [28] points out the air temperature, winds, incidence of solar radiation, soil nutrients and soil moisture. In view of these findings, it is necessary to evaluate the influence of other parameters, which is done by checking the effect of soil moisture on stomatal conductance (gs) and transpiration (E).

![Fig.4: Oxygen isotope ratios (δ^{18}O) of C. oncocalyx xylem root sap (CO) and soil water at the 0-20, 20-40 and 40-60 cm depth increments under an agrosilvopastoral system (AGP) and secondary forest (SF)](image)

![Fig.5: Photosynthesis (Photo) in leaves of Cordia oncocalyx and air relative humidity (RH) in agrosilvopastoral system (AGP) and secondary forest (SF). * Photosynthetic averages are different between the two systems, p <0.05 by the t test (n = 9). # Relative humidity averages are different between the two systems, p <0.05 by the t test. RH values represent the average of the photosynthesis recording day (n = 96)](image)
The variation in transpiration values was higher in SF (from 2.56 to 6.0 mmol m$^{-2}$ s$^{-1}$) than in AGP (from 3.79 to 5.75 mmol m$^{-2}$ s$^{-1}$). Similar to stomatal conductance (0.08 to 0.29 mol m$^{-2}$ s$^{-1}$ in AGP and 0.04 to 0.31 mol m$^{-2}$ s$^{-1}$ in SF). This higher variance in the physiological parameters of the trees in SF indicates a greater variation in the physical parameters of the environment, since the response of the plants is proportional to the variations in the environment [29], to some extent. Solar radiation may be one of the factors that explain these variations. The solar radiation that surpasses the canopy in a forest is quite variable and of inferior quality that arrives at the canopy [28]. This variable amount and quality of the radiation reaching the lower branches and leaves leads to greater oscillations in photosynthetic processes, such as stomatal conductance and transpiration, when compared to plants growing in more open areas whose entire canopy can receive similar levels of radiation, leading to lower oscillations in the rates of these processes.

Statistical analysis showed correlation of soil water content at both 30 cm and 50 cm with transpiration and stomatal conductance in *C. oncocalyx* plants in AGP (Figure 6A and B). Lower values of transpiration and stomatal conductance were observed in lower levels of water in the soil, showing the dependence of this environmental parameter on the trees to keep their stomata open and thus to continue photosynthesizing. However, there was no such correlation for SF trees (Figure 7A and B), pointing to a certain independence of these physiological processes in relation to soil moisture. The physiological processes linked to photosynthesis occur in response not only to an isolated environmental factor, but to the whole of them, one influencing the sensitivity of the plant in relation to another factor. Shen et al. [30] observed that the sensitivity of the canopy conductance process in response to the air vapor pressure deficit decreased when the soil moisture content was reduced. Thus, there are possibly other factors influencing the response of stomatal conductance and transpiration to soil moisture in SF. It is known that physiological processes in plants respond to environmental conditions [29]. On the other hand, it is also known that many species anatomically modify to adapt to stressful environmental conditions or alter biochemical processes to tolerate environmental stresses [31]. Investigations in this sense may help to understand these differences in the physiology of trees between the two different land use systems.

**Fig.6:** Correlation between soil water volumetric content at 30 cm and 50 cm depth (Vw30 and Vw50) and transpiration (A) and stomatal conductance (B) in *Cordia oncocalyx* in agrosilvopastoral system. $E$: $n = 9$; Vw: $n = 96$ (readings along the day of photosynthesis recording). Each point represents the averages of one day per month. (A) Vw30xE: $p = 0.017; R = 0.94$. Vw50xE: $p = 0.004; R = 0.97$. (B) Vw30xgs: $p = 0.01; R = 0.93$. Vw50xgs: $p = 0.001; R = 0.98$. $E$ – transpiration; gs – stomatal conductance.
Fig. 7: Correlation between soil volumetric water content at 30 cm and 50 cm depth (Vw30 and Vw50) and transpiration (A) and stomatal conductance (B) in Cordia oncocalyx in agrosilvopastoral system. E: n = 9; Vw: n = 96 (readings along the day of photosynthesis recording). Each point represents the averages of one day per month. \( A \) Vw30x E: \( p = 0.07, R = 0.83 \); Vw50x E: \( p = 0.10, R = 0.79 \). \( B \) Vw30x gs: \( p = 0.06, R = 0.86 \); Vw50x gs: \( p = 0.08, R = 0.82 \).

There was a trend towards lower water use efficiency (WUE) in the SF trees throughout the study period, although there was an effective difference (\( p < 0.05 \)) only in the months of May and September (Figure 8). As the WUE represents how much carbon is being fixed for each unit of water used, this result points to a higher water expenditure per unit of carbon fixed in SF trees, which is only possible for Caatinga plants if there is a water supply. However, the AGP plants showed lower water expenditure in the photosynthetic process, which becomes fundamental in the period of water scarcity, considering that the soil and air are drier, and, in the absence of physiological or anatomical strategies for survival at climate more arid, the plants may have their development impaired. In addition, low water use efficiency situations indicate the need for more water to make photosynthesis compared to those with high WUE; so when there is water shortage in the soil, tree low WUE are the first to present reductions in photosynthesis.

![Graph showing water use efficiency (WUE) in C. oncocalyx trees in agrosilvopastoral system (AGP) and secondary forest (SF). * WUE means are different between the two systems, \( p < 0.05 \), by t test \((n = 9)\)](image_url)

**IV. CONCLUSION**

Although the most inhospitable environmental conditions in the AGP system, the lower density of plants, and therefore less competition for water, besides greater independence of water variations in soil and greater water use efficiency, favoring photosynthesis longer, causing the leaves to fall later. In view of the above, it’s possible that AGP plants have developed physiological and anatomical features that enable to them to keep photosynthesis even when climatic conditions are more severe.

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