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Effects of fertilization, temperature and leaf collection points on chlorophyll content in adult individuals of *Tectona grandis* L.f.

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Abstract:

The aim of the present study was to determine and compare chlorophyll concentrations in adult individuals of Tectona grandis in two plantations in the municipality of Irituia-PA. A completely randomized design was used, with two treatments (T1 = planting without fertilization and T2 = planting with fertilization) where 50 adults individuals of T. grandis were randomly chosen, being 25 trees per planting area. After collection, the chlorophyll content was obtained from 10 different points on the leaf using an atLEAF+ chlorophyll meter,

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in addition to measuring climatic data of temperature and humidity. Finally, the chlorophyll contents of generic units generated by the equipment were converted to mg/cm². The highest measured chlorophyll contents were attributed to the T2 area, which receives constant fertilization, proving the existence of the relationship between the pigment and the disposition of nutrients in the soil. Regarding the levels of chlorophyll by leaf measurement points, the values corresponding to points 1, 2, 3 and 4 were statistically higher than the others, showing that there is greater pigment production in the lower part of the leaf blade. As for the measurement times, a relationship was directly proportional to the chlorophyll contents, in conjunction with temperature. Therefore, based on the rise of chlorophyll levels, there was no photo-oxidation of the pigments during the observed period.

Keywords: Forest plantations, teak, chlorophyll meter, photosynthesis

INTRODUCION

Teak (*Tectona grandis* Lf - Lamiaceae) is a fast-growing tree species, originally from South and Southeast Asia, which has been showing promise in commercial plantations in Brazil, mainly due to the characteristics of adaptation to regional edaphoclimatic conditions and its natural resistance against xylophages (ANGELO et al. 2009; MOTTA et al. 2013). The teak acreage is estimated to occupy 4.4 million hectares, with 83% in Asia, 11% in Africa, and 6% in tropical America (KOLLERT; KLEINE 2017).

The first *T. grandis* plantations in Brazil took place in the 1960s, implemented by the company Cáceres Florestal S.A., in the State of Mato Grasso (CALDEIRA; OLIVEIRA, 2008). Currently, the species has been cultivated with greater prominence in Pará, Acre, Rondônia, Mato Grosso and Roraima states (DIONISIO et al., 2018), achieving in the recent years approximately 94 thousand cultivated hectares (IBA, 2019).

Tree growth is affected by climatic factors such as precipitation, temperature, wind speed and relative humidity (SINHA et al., 2019). However, among the various environmental components, light is crucial for its growth, because in addition to the energy provided for the photosynthetic process, it also regulates the development of the plant by capturing light at different intensities (ATROCH et al., 2001).

Chlorophylls are the main pigments responsible for capturing solar radiation, converting photons of light into chemical energy in the form of ATP and NADPH during the photosynthesis process (TAIZ et al., 2017). These pigments are generally related to the primary productivity of the plant, affecting even the water deficit in cases of loss of chlorophyll, also affecting the photosynthetic rate (BROETTO et al., 2017).

Therefore, carrying out analysis of photosynthetic pigments is an important tool for assessing the internal parts of the cell's health during the photosynthesis process (SILVA et al., 2013). In addition, it also is the basis for making decisions regarding the maintenance of plantations, mainly when it comes to fertilization and irrigation (RIGON et al., 2012). Thus, the objective of the present work was to determine and compare the concentrations of chlorophyll in adult individuals of *T. grandis* in two plantations in the municipality of Iritua-PA.

MATERIAL AND METHODS

Characterization of the study areas

The study was carried out in the city of Irituia - PA, in two areas of *T. grandis* forest plantation, located at coordinates 01°44'17,90" S; 47°27'44,90" W and 01º43'26,92"S; 47°30'03,69"W. The study region has a relative humidity of 85% and an average annual temperature of 25 ºC. Its rainfall regime is generally between 2,250 and 2,500 mm. The rains, although regular, are not distributed regularly, having their highest concentration starting from January to June, which is equivalent to 80% (FAPESPA, 2016).

Two *T. grandis* planting areas were studied in the municipality of Irituia. Area 1 has 18,000 m² and is formed by a 17-year-old plantation, produced from cuttings seedlings, arranged in 4x4 m spacing, aiming to obtain sawn wood as a final product. In this area, soil characterization and pre-plantation treatments were not carried out, with the exception of fertilization and weeding only during the seedling early development period. Area 2 has 30,000 m², but with only a 1 ha of *T. grandis* plantation, arranged in 3x2 m spacing. The planting is approximately 19 years old and was produced from seedlings from both seeds and cuttings. During these years, cultural and silvicultural treatments were carried out, such as thinning, changing the spacing between trees to 4x4m and the application of organic fertilizer from tanned manure.

Data collection and analysis

For the measurement of chlorophyll contents, a completely randomized experimental design was used, with two treatments (T1 = planting without fertilization and T2 = planting with fertilization) with 50 *T. grandis* adult individuals being randomly chosen, using 25 trees by planting area, collecting two leaves from the middle third of each tree with the assistance of ladders and trimmers. After collection, the chlorophyll content was obtained of 10 different points on the leaves (Figure 1), using an atLEAF+ model chlorophyll meter. This research used adult teak individuals and due to the height of the trees the separation the leaves from the mother plant was needed.

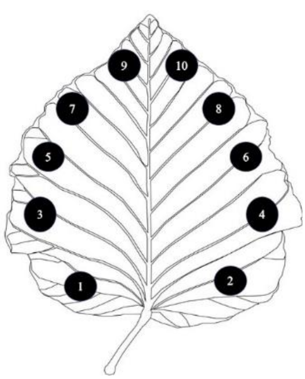

Figure 1. Measuring points of chlorophyll levels in adult individuals of *T. grandis*, Irituia, Pará.

Chlorophyll content readings were taken between 9:00 and 11:00 am, with the operator avoiding the direct incidence of radiation on the sheet to be measured as much as possible, as suggested by Godoy et al. (2008). During the period of collection and reading of the chlorophyll content in the leaves, the climatic data of temperature and humidity were measured. After data collection, the values obtained with the chlorophyll meter were converted to mg/cm² on the atLEAF CHL unit conversion site according to calculations by Zhu et al. (2012).

To verify the assumptions of the analysis of variance (ANOVA), the data were first checked for: a) normality by the Shapiro-Wilk test ($p > 0.05$), b) homoscedasticity by the Bartlett test ($p > 0.05$), and independence between experimental units. Once these assumptions were met, the data were subjected to analysis of variance using the program R version 4.0.2. The comparison between the points collected on the sheet was performed by analysis of variance (ANOVA) and with significant differences between the data, the averages were compared using the Tukey post-hoc test ($p < 0.05$).

RESULTS AND DISCUSSION

Chlorophyll content by area

Table 1 presents that the chlorophyll content in area 2 (with fertilization) was statistically higher ($p < 0.001$) than in area 1 (without fertilization). The difference in chlorophyll content between study areas can be attributed to the fertilization itself, considering that Barros and Filho (2008) and Gross et al. (2006) state that nitrogen in the soil is responsible for part of the formation of chlorophyll molecules, thus increasing pigment production.

Table 1. Average values (± SD) of chlorophyll content in adult individuals of *T. grandis*, Irituia, Pará.

Area	Fertilization	Averages	SD
T1	With	0.023987 b	0.004001
T2	Without	0.025023 a	0.004754

* averages with different letters in the column do not differ by Tukey's test ($p < 0.05$).

This correlation was also observed by Vale and Prado (2009), in which the supply of nitrogen fertilization significantly affected the reading of SPAD (Soil Plant Analysis Development), increasing the concentration of chlorophyll in the leaf blade in the citrus culture. This difference can also be related to the fact that, although the trees are of the same species, they are originally from different populations, and even growing under identical environmental conditions, they can exhibit differences in the specific leaf area (FELSEMBURGH, 2006).

Chlorophyll content by measurement points

Figure 2 presents the average values of chlorophyll content per leaf point in areas with and without fertilization. When compared, there are statistically higher values for points 1 and 2, which correspond to the basal part of the leaf blade. Therefore, the points with the lowest chlorophyll content (3, 4 and 5) corresponded to the middle and upper third. In these points of the leaf, there may be a compensatory physiological measure due to the low irradiance caused by the shading imposed by the upper leaves. There was also an increase in the chlorophyll content as the temperature and time of measurement variables increased, demonstrating that the species has greater resistance to this type of stress.

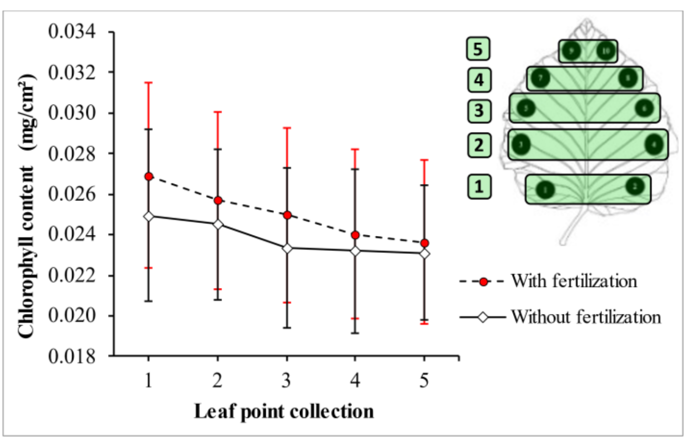

Figure 2. Average content (±SD) of *T. grandis* chlorophyll by measuring points on the leaves.

The number of photosynthetic enzymes and chloroplasts, which increase the photosynthetic capacity per unit area, can explain the difference in chlorophyll contents between specific points and by having a greater amount of biomass in a given area, there is the cost of increasing the absorption capacity irradiance in low light (FELSEMBURGH, 2006). Nakazono et al. (2001) state that a higher photosynthetic rate occurs so that plants in the shaded environment can maximize the capture of light and enhance photosynthesis. Therefore, it is understood that the basal part of the analyzed leaves has higher levels of chlorophyll due to these two factors, the low irradiance submitted by the shading of the upper leaves, and the need for a greater amount of photosynthetic enzymes and chloroplasts.

Chlorophyll content as a function of temperature and time

The chlorophyll content as a function of temperature and measurement times presented a directly proportional relation. The higher the temperature and time of measurement, the higher the chlorophyll contents (Figure 3). Vieira (1996) states that plants are stressed by the incidence of light mainly in tropical environments.

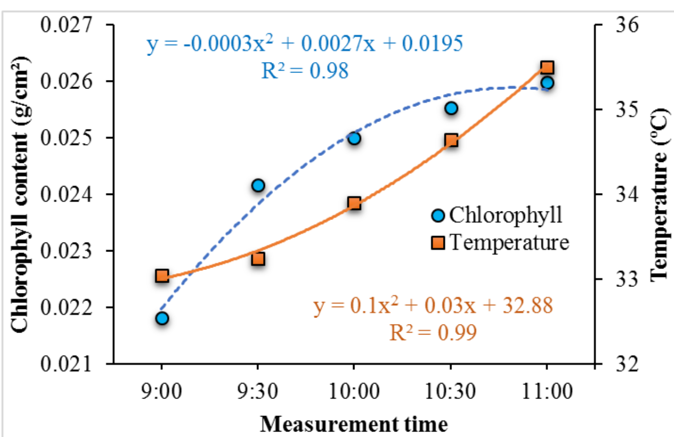

Figure 3. Average chlorophyll content and temperature as a function of measurement times.

Hendry and Price (1993), in addition to reiterating this statement, show that the action of photo-oxidation can be prevented by the presence of carotenoids. Therefore, this relationship between chlorophylls and carotenoids can be used as a potential indicator of photo-oxidative losses caused by strong irradiations. Another biochemical defense mechanism used by plants is the production of the enzyme SOD (superoxide dismutase) that destroys free radicals responsible for the photo-oxidation of pigments (TAIZ et al., 2017).

However, based on the growing increase in chlorophyll content as a function of temperature and measurement times, it is conjectured that there was no degeneration nor photo-oxidation in the present study. Therefore, the amount of carotenoids and SOD enzymes can be abundant, minimizing or neutralizing the impact of photo-oxidation. Another factor with a simpler attribution to this physiological response is the adaptation of the photosynthetic system of plants so that the luminosity is used in the most efficient possible way (ENGEL; POGGIANI, 1991).

Authors such as Wiebel et al. (1994) and Rego and Possamai (2006), when analyzing other tree species, noticed low levels of chlorophyll due to greater irradiance. It is noticeable, therefore, that the species in question is susceptible to photo-oxidation. However, the temperature and time of measurement/irradiance variables were not high enough for the phenomenon to occur, demonstrating greater resistance to this type of stress.

CONCLUSIONS

The highest chlorophyll contents were found in individuals who received constant fertilization.

The atLEAF+ chlorophyll meter was efficient in measuring chlorophyll content in individuals of *T. grandis*, and can be a non-destructive methodological alternative for rapid diagnosis, replacing traditional laboratory methods.

Regarding the part of the leaf as the highest concentration of chlorophyll, the points in the basal third of the leaves were found high levels of chlorophyll.

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