Variation of physiological parameters in juvenile treetops of *Eucalyptus tereticornis* from a three-dimensional perspective

Variación de los parámetros fisiológicos en rebrotes juveniles de *Eucalyptus tereticornis* con una perspectiva tridimensional

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Abstract

We analyzed the characterization of physiological variables in treetops of *E. tereticornis*; obtaining An of 28.6-40.6 µmol m^{-2}s^{-1}, E of 8.53-16.90 µmol m^{-2}s^{-1}, Gs of 87.47-335.16 mmol m^{-2}s^{-1}, Pp of 65-320 kPa and SPAD of 20.5-38.40; The analysis showed that external and medium branch in higher treetop obtained physiological behaviors significantly lower than the rest of treetop, because they are growing and leaves have not yet reached their peak. Also, we found that 3D model allowed the developed model that simplified information.

key words

Forest physiology, tree physiology, 3D modeling.

Resumen

Se analizó la caracterización de fisiológicas en la copa de árboles de *E. tereticornis*; obteniendo un An de 28,6-40,6 µmol m^{-2}s^{-1}, E de 8,53-16,90 µmol m^{-2}s^{-1}, Gs de 87,47-335,16 mmol m^{-2}s^{-1}, Pp de 65-320 kPa y SPAD de 2,5-38,40; El análisis de aglomerados mostró que la rama externa y media en las copas de los árboles superiores obtuvo comportamientos fisiológicos significativamente más bajos que el resto de la copa, ya que están creciendo y las hojas aún no han alcanzado su punto máximo. También, se encontró que la implementación del modelo 3D permitió el modelo desarrollado que simplificaba la información.

Palabras clave

Fisiología forestal, fisiología arbórea, modelado 3D.

1. Introduction

Trees are living organisms that are constantly changing and developing (Thomas, 2000), physiological activities such as stomatal conductivity (Pinkard & Beadle, 1998; Shurong, Xuifeng & Yuangang, 1999), transpiration (Utkhao & Yingjajaval, 2015), photosynthesis (Seelig et al., 2009) among others; driving the growth of the individual (Warren & Adams, 2005), which is usually displayed in increasing biomass, diameter growth (Pinkard & Beadle, 1998), leaf area index (van Wijk, Williams, & Shaver, 2007) and root mass index (Wachsman, Sparks, & Benfey, 2015).

The physiological development of an individual is linked to genetic (Davison, Battaglia, & Close, 2004) and environmental factors (Barry & Pinkard, 2013), specifically to precipitation, temperature, relative humidity, photosynthetically active light and wind (Shurong, Xuifeng, & Yuangang, 1999). To explain and predict the relation of environmental factors to individual growth and development, multiple mathematical models have been developed (Utkhao & Yingjajaval, 2015). Two-dimensional (2D) physiological relationships have been commonly performed (Bussiere, Solmon, & Fouere, 2002), in which a physiological parameter is taken and dimensioned in a hasty manner between trees or treatments, maintaining constant aspects as the selection and location patterns of the leaves (Bylesjö et al., 2008).

Therefore, there have been developed multiple modeling techniques modeling of three-dimensional (3D) type that seek to give a physiological comprehensive and detailed interpretation (Paulus, Kuhlmann, & León, 2014); the most implemented models include Bonhomme and Varletgrancher (Bonhomme and Varletgrancher, 1978) implemented in

grasses and based on a geometric description which consists in a first phase where leaves of different ages and plants are dimensioned, organisms are subsequently harvested and through photography in an area with a square template information such as inclination of leaves, inclination of plant and silhouette of leaf distribution is generated, it is a technique that has the advantage of having a high degree of accuracy, but it is very meticulous and destructive. On the other hand, the technique of stereo photography, consists of implementing several cameras at the same time, around the plant and starting points previously located on the floor taking pictures at the same time to produce a precise architectural treetop analysis by using a specialized program (España et al., 1999).

Studies of 3D physiological modeling in agriculture have been implemented in detail with species as *Zea mays* (Paulus, Kuhlmann, & León, 2014), *Arabidopsis thaliana* (Kaminuma et al., 2007), *Trifolium repens* (Gautier, Meïch, Prusinkiewicz, & Varlet-Grancher, 2000), *Hordeum vulgare* (Dornbusch, Wernecke, & Diepenbrock, 2007), among others; with the common feature that these studies have focused on the morphological and anatomical evaluation of plant with a 3D perspective, without evaluating physiological variables. Therefore, the objective of this work was to characterize the variability of physiological parameters in the treetop of in the cup of youth regrowth of *E. tereticornis* through use of a three-dimensional model.

2. Metodology

2.1. Specie and studie site

We used ten regrowths of *E. tereticornis* (from plants that had 30 months old when they were harvested) with an age of six months. Each regrowth was selected four months early, eliminating competing sprouts that affected its development. On average, regrowths had a diameter at 1.3 m above the ground of (19.3±1.91) mm and an average total height of (206.3±25.5) cm. The plants were planted with a sowing density of 10000 tree ha^{-1} and had no kind of silvicultural management applied. The study site was in Cartago, Costa Rica (9°50'57.91" N; 83°54'37.27" W), at an altitude of 1392 m, with an average annual temperature of 24 °C and annual rainfall of 2100 mm, arranged in 7 rainy months (IMN, 2016).

2.2. Segmentation and selection of treetop measuring points

Treetop was defined from the first branch with living leaves to the apical meristem, according to its height divided proportionally into three sections (Figure 1a): higher treetop (HT), intermediate treetop (IT) and lower treetop (LT). In each branch of each section of the cup was measured and divided proportionally in three sections (Figure 1b): internal branch section (IBS), middle branch section (MBS), external branch section (EBS).
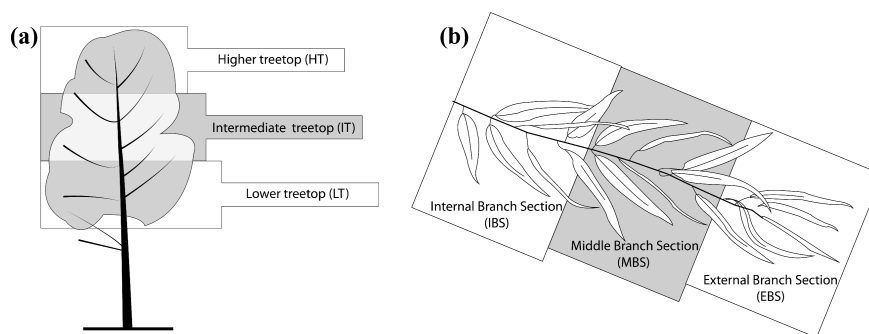
Figure 1. proportional division of treetop (a) and branches (b) in regrowths of *E. tereticornis* with an age of six months.

2.3. Physiological parameters evaluated

Selected leaves were assessed with the following parameters: net photosynthetic rate (An), transpiration (E), relative chlorophyll content (SPAD), stomatal conductivity (Gs) and foliar turgor (Pp). All these parameters were evaluated between 08:00 and 11:00 (GMT-6), with a partly cloudy climate, with a temperature that varied from 23 to 26 °C, relative humidity of 70 to 85 % and speed of wind lower than 10 km/h.

• *Net photosynthetic rate and transpiration (An and E)*: both parameters were evaluated with a CIRAS II gaseous exchange meter (PP Systems ®, USA). The CIRAS-II was implemented with a constant CO_2 flux of 380 ppm.

• *Value of the relative chlorophyll content in the leaves (SPAD)*: Measurements of the potential chlorophyll content were performed with an SPAD-502 (Konica Minolta®).

• *Stomatal conductivity (Gs):* it was evaluated using a Leaf Porometer CS-1 (Degacon Devices, Inc®).

• *Foliar Turgency (Pp):* We implemented with a ZIM-probe unit, connected to a Zim-clamping device (Yara®).

2.4. 3D modelling

Firstly, the total height, number, and length of branches, height of the tree and its angle of inclination of each evaluated tree were measured; additionally, the number of leaves per branch was counted and 25 % of them were collected to determine the specific leaf area (SLA) and leaf distribution; then the normal distributions were made to determine the average SLA of each cluster and define the average form. Subsequently, the prototypical branches (identified average characteristics) were built for HT, IT and LT and with this a prototypical tree was developed, all this was developed in the Solid Works 2016® software (Dassault Systèmes SolidWorks Corp.)

2.5 Analysis of results

The results of each physiological parameter were tested in a two-way analysis of variance (ANOVA) in which was evaluated (1) whether there are significant differences between the three leaves of treetop and (2) differences in three segments of the branches, in addition to

the interaction of both factors. With the parameters that showed differences, a Tuckey test (with a significance of 0.05) was applied. Subsequently, a multivariate cluster analysis (CHAID) was developed to identify the physiological variables that generated the greatest variation in the grouping of treetop sections. This analysis was performed with the program STATISTICA 9.0 and R version 3.2.0. with a significance of 0.05.

After having the ANOVA and general CHAID, results were assembled in the prototypical tree and using the option of Solid Works Simulation, the physiological data obtained were placed with sensors in the prototypical branches to develop analysis of potential variation at the treetop with residual errors less than 20 % and with a significance of 0.05.

3. Results

3.1 Physiological variability within trees

We obtained the tendency of the assimilation was exponential type, obtaining a variation of 28.6 to 40.6 µmol m^{-2}s^{-1} at the point of maximum assimilation of photosynthesis. In assessing whether there were differences in assimilation between the three treetop and branch segments, LT-IBS, HT-MBS and HT-EBS combinations showed significantly lower An compared to the remaining 6 segments of the treetop. In the case of LT-IBS significant differences in the curve were given after 1250 µmol m^{-2}s^{-1}, in which An started to stabilize at 31.6 µmol m^{-2}s^{-1}, while LT-MBS and LT-EBS showed significantly higher assimilation and stabilized at 40.5 µmol m^{-2}s^{-1}. Similarly, HT-MBS and HT-EBS showed significant differences in An compared to HT-IBS after a PPFD of 100 µmol m^{-2}s^{-1} that started stabilizing in 28.6 µmol m^{-2}s^{-1}, whereas HT-IBS continued to increase assimilation up to 40.6 µmol m^{-2}s^{-1}.

The transpiration (E) (Figure 2) varied from 8.53 to 16.90 µmol m^{-2}s^{-1} and its behavior tended to two clusters within the treetop, the first group with significantly lower values (less than 10 µmol m^{-2}s^{-1}) was formed by HT-MBS and HT-EBS, while the second, with higher transpiration levels, was formed by three lower treetop branch sections. While the stomatal conductivity (Gs) showed variations from 87.47 to 335.16 mmol m^{-2}s^{-1} with significant differences within the treetop that tended to form three groups: (i) consisting of segments MBS and EBS in HT that showed a Gs lower than 100 mmol m^{-2}s^{-1}, (ii) the second group with intermediate values of Gs (from 150 to 250 mmol m^{-2}s^{-1}) complied by IBS-HT, the segments of branch in IT and EBS-LT and (iii) with higher conductivity than 300 mmol m^{-2}s^{-1} in sections of IBS and MBS-LT.

SPAD values with variations from 20.50 to 38.40, showed only significantly lower values in the EBS-HT (20.50), the rest of treetop showed no differences, with an average value of 35.23. Finally, Pp showed variations from 65 to 320 kPa with the tendency of three clusters in the treetop, the first formed the three segments of HT, IBS-IT and IBS-LT with average values of 316 kPa, followed by MBS and EBS of IT with average values of 226 kPa and the third group with minor Pp conformed by EBS and EBS in LT with 67.6 kPa.
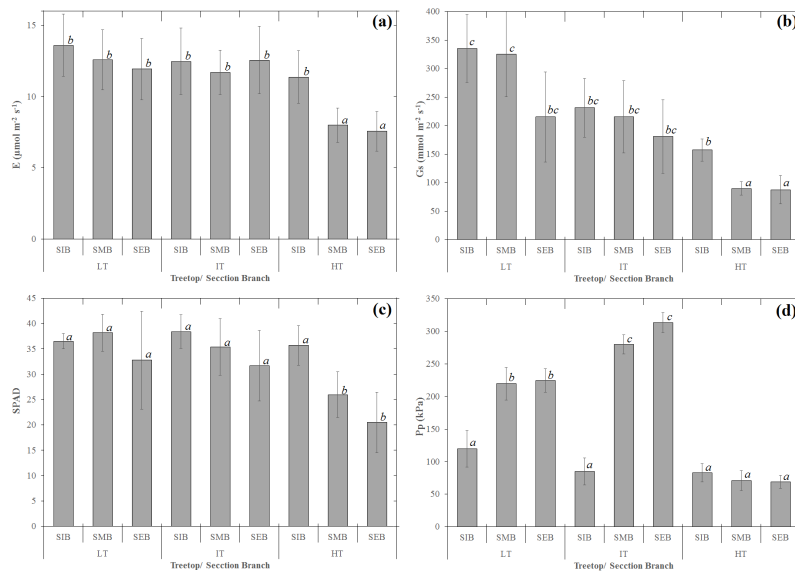
Figure 3. E, GS, SPAD and PP obtained in the three segments of treetop and three sections of branch in regrowths of *E. tereticornis* with an age of six months.

3.2 Modeling and 3D physiological differentiation

Physiological differences of the biotypic branches: Figure 3 shows the branches model defined for each section of treetop and results of agglomerates analysis are presented to evaluate the degree of similarity between the physiological parameters evaluated in three segments of the branch. In HT (Figure 3a) branches had an average length of (28,5±10,9) cm with an average number of (6±4) leaves that showed a SLA (16,8±10,9) cm^2; with IT (Figure 4c) the average length of the branches was (42,5±12,9) cm with (45±18) leaves which presented two groupings in their SLA: (15,9±8,1) cm^2 and (28,6±9,9) cm^2 respectively. In the case of LT (Figure 3e) had the greatest lengths of branches of (88,5±10,6) cm, with an average of (61±12) leaves and three groupings of SLA that were: (18,8±6,6) cm^2, (25,7±5,2) cm^2, and (39,9±10,1) cm^2.
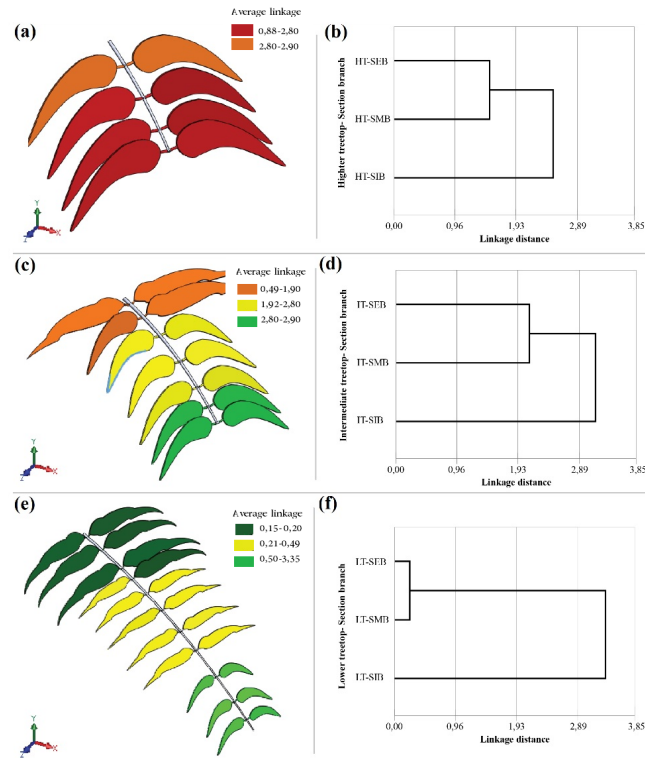
Figure 3. branches models developed for three of treetop

Physiological differentiation of treetop: Variability in physiological behavior is shown in isometric view (Figure 4a) and upper view (Figure 4b and 4c). CHAID analysis showed that the values of Pp and Gs generated the greatest variation between sections of treetop that was conglomerated in five segments (Figure 5d). First cluster consisted of the branch section (MBS and SME) in both IT and LT, it showed groupings with a link distance smaller than 0.56 and after that, they were agglomerated in a secondary line, the third CHAID consisted of the branch section IBS of HT with IT with a link distance of 1.21 and was united with previous conglomerate with a distance of 1.93; the fourth cluster was in HT with EBS and MBS with the closest grouping (0.10), but it moved away to the values present in IT and LT due to the high differentiation in SPAD; finally LT-IBS showed the least closeness to the rest of the parameters of the cup, obtaining a link distance of 3.94.
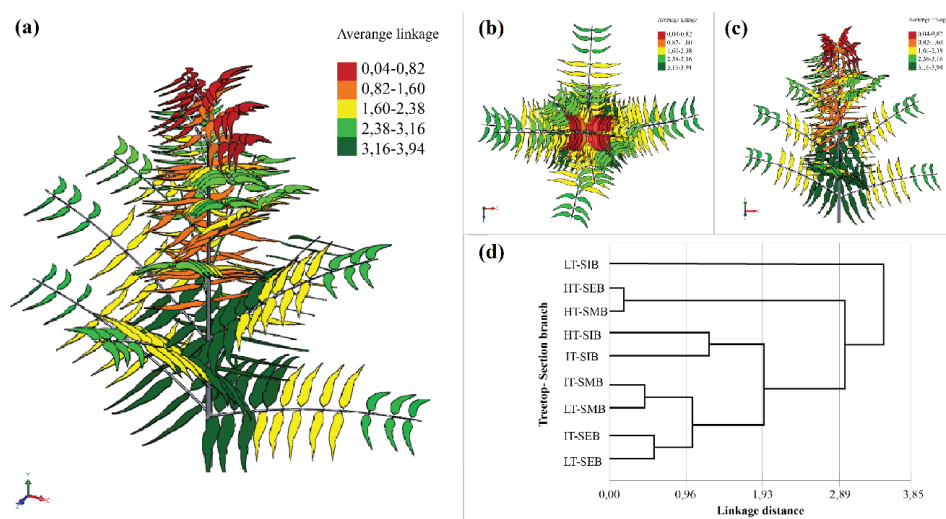
Figure 4. Isometric view (a), upper view (b and c) and results of cluster (d) of the physiological differentiation among the three treetop parts

4. Discussion

4.1 Physiological variability

This study showed that there are differences between physiological values in different segments of treetop in *E. tereticornis*; finding that variables SPAD, E and An showed no distinctions in their values in the majority of treetop (showing that they can be evaluated in almost any part of treetop), but instead, Gs and Pp impacted significantly on groups in treetops. Initially, it was found that MBS and EBS segments in HT obtained physiological values significantly lower in comparison to rest, due that they are areas in which the plant centers its growth (Medhurst, Pinkard, Beadle, & Worledge, 2006), with leaves that have not reached their maximum SLA or optimum stomatal development, causing the production of photosynthesis to be low since leaf does not have enough stomata or chlorophyll a and b (Pinkard & Beadle, 1998); Lisboa et al. (2014) as *E. regnans* mentions, the low capacity of photosynthesizing of upper part is attributed to the low levels of transpiration and stomatal conductivity that prevent leaf from reaching optimum levels of photosynthetic production. At the same time England & Attiwill (2011) found that after 60 days of starting the development of the plant stomatal conductivity, transpiration and photosynthetic assimilation began to stabilize and have values of an adult leaf, being in lower ages inefficient in their physiological productivity.

In the case of LT-IBS section that showed significant physiological variations, due to an An significantly lower the rest of the segments in combination with lower turgidity values. Lisboa et al. (2014) explains that decrease in An is due to the degree of maturity of leaves that causes photosynthetic development to lower product of decrease of chlorophyll b, England and Attiwill (2011) emphasize that apart from reduction of chlorophyll the plant starts to inhibit the leaf by reducing water availability, being shown in Pp values below 90 kPa, which combined with a high Gs (greater than 320 mmol m^{-2}s^{-1}) produce physiological

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distortions in the leaf that will cause the tree to eliminate it, due to the loss of water that generates by little control of stomas closing.

4.2 Potentiality and limitations of 3D modeling

In terms of potential use of 3D differentiation of variables, the distribution and trend of groupings of variables can be more clearly defined. In our case, although numerous variables showed little differentiation, a model would give interconnections showing with simplicity that interactions; however, the modeling presented problems and limitations like those found in other studies.

First, precision is lost with development of morphological perspective modeling, de Moraes Frasson and Krajewski (de Moraes Frasson & Krajewski, 2010), Paulus, Kuhlmann, & León (2014) discussed about angulations of branches and leaves must be standardized, which in some cases affects computational analysis of aspects such as percentage of luminosity to soil or light distribution within the crown, the study had to standardize the angulations of the leaves at 45° and 55° according to branch and angles of 40° to 38°, thereby limiting the actual angulations especially to treetop.

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