1 ABSTRACT

This study aimed to characterize the effects of environmental variables on the transpiration (E), stomatal conductance (Gs) and photosynthesis (A) behavior of *Eucalyptus grandis* x *Eucalyptus urophylla* clone in the leaf scale over three consecutive days. The measurements were performed in a clonal population of 60 months of age, located in the municipality of Igaratá-SP. Measurements of predawn leaf water potential (Ψpd) by the Scholander pressure chamber and ecophysiological measurements of E, Gs and A were obtained by porometer LcPro + (ADC). Specific climate characteristics to the measurement days were obtained from the automatic weather station Campbell Scientific Inc. installed on the site of study, distant 100 m from the clonal settlement of interest. Environmental variables such as photosynthetically active radiation (Qleaf) and pressure deficit of water vapor (VPD) were chosen to correlate with E, Gs and A. The best associations were observed between E, G, A with Qleaf not being significant for Gs and VPD. The ratio A / E was progressively increased with the decrease of Ψpd. Knowledge of water use efficiency helps to select the best species of *Eucalyptus* to determine certain conditions.

KEYWORDS: plant-water relations; transpiration; water use efficiency

2 RESUMO

Este estudo teve por objetivo caracterizar os efeitos de variáveis ambientais no comportamento da transpiração (E), condutância estomática (Gs) e fotossíntese (A) de um clone de *Eucalyptus grandis* x *Eucalyptus urophylla* na escala foliar ao longo de três dias consecutivos. As aferições foram realizadas em um povoamento clonal com 60 meses de idade, localizado no município de Igaratá-SP. Foram realizadas medições de potencial hídrico foliar antemanhã (Ψpd) pela câmera de pressão de Scholander e as aferições ecofisiológicas de E, Gs e A foram obtidas pelo porômetro LcPro+ (ADC). Características climáticas específicas dos dias de medição foram obtidas da estação meteorológica automática Campbell Scientific Inc. instalada no próprio local de estudo, distante a 100 m do povoamento clonal de interesse. Variáveis ambientais tais como, radiação fotosinteticamente ativa (Qleaf) e déficit de pressão de vapor atmosférico (DPV), foram escolhidas para correlacionar com E, Gs e A. As melhores
associações foram observadas entre E, Gs, A com Qleaf, não sendo significativa para Gs e DPV. A relação A/E foi progressivamente maior com o decréscimo do Ψpd. O conhecimento da eficiência do uso da água ajuda a seleccionar melhores as espécies de eucalipto para determinadas condições.

PALAVRAS-CHAVE: relação planta-água, transpiração, eficiência do uso da água

3 INTRODUCTION

Growth and development of plants is a consequence of several physiological processes controlled by environmental conditions and genetic characteristics of each plant species. Therefore, to better understand the growth, development and hydrological impact of a Eucalyptus plantation, it is necessary to know the factors that control water use. Recently, companies have increased efforts to investigate the contribution of the components of water balance in the productivity of eucalyptus, with the need to integrate the effects of climate and management practices on the production of wood from planted forests of Eucalyptus.

According to Novák et al. (2005) reliable estimates of plant transpiration rates are essential to predict the water flow and crop growth and thus, the rate of transpiration depends on various properties of the continuum soil-plant-atmosphere. Plant ecophysiology is the science that deals with the vital processes and responses of plants according to changes in environmental factors (LARCHER, 2003). Hence, the descriptive study of responses of organisms according to environmental conditions and analysis of the causes of their corresponding physiological mechanisms is involved in different levels of organization. Although many species of Eucalyptus are found in dry conditions, drastic changes in the plant’s water status may influence in its growth and physiology (WHITEHEAD; BEADLE, 2004). The availability of solar radiation is one of the factors that most limits the growth and development of plants. All the energy needed to perform photosynthesis, a process that converts atmospheric CO$_2$ in metabolic energy is derived from solar radiation (TAIZ; ZIEGER, 2004).

There are several studies on the influence of water and environmental conditions over forest productivity and their impacts on water resources of a watershed. Soares & Almeida (2001) developed a model of water use in eucalypt plantations in Aracruz-ES (Eucalyptus grandis W. Hill ex Maiden) from the equation of Penman-Monteith. The model indicated that in years when it rains around the region's historical average (1.350 mm) there is a balance between evapotranspiration and precipitation. The authors also found that eucalyptuses have efficient stomatal control on transpiration during the dry season. Granier et al. (1996) conducted measurements of sap flow in a forest of pine and developed a prediction model of transpiration according to the vapor pressure deficit. Then, estimated the transpiration of the vegetation canopy to a second location using the same model and while comparing the estimated values with those obtained in the field, thy concluded that the model was suitable for estimating canopy transpiration. According to Xavier et al. (2002), computing the water use of Eucalyptus plantations is essential for assessing their environmental impacts and for measuring their sustainability and involvement of growth models. For Soares et al. (1997) and Soares and Almeida (2001), the evapotranspiration of the canopy, when integrated over a given period of time, can be used to express the productivity potential of the crop at a certain place and period.
In an attempt to subsidize the ecophysiological modeling of river basins with planted forests, this study aimed to characterize the effects of environmental variables on the behavior of transpiration, stomatal conductance and photosynthesis of a clone of Eucalyptus sp. in the leaf scale. The work presented here is part of a network of studies involving different scales of observation.

4 MATERIAL AND METHODS

4.1 CHARACTERISTICS OF THE AREA

The study was conducted at Santa Marta Farm, in the municipality of Igaratá-SP at coordinates 23°10'22"S latitude and 46°06'50"W longitude and 745 m altitude. The climate is Cwa according to Köppen classification, being characterized as a high-altitude tropical with dry winters and hot summers, with total rainfall in the driest month less than 30 mm, average temperature in the warmest month above 22°C and the coldest month below 18°C. The average annual temperature is 21.5°C and average annual rainfall of 1.265 mm (SENTELHAS et al., 2007).

According to the geomorphological division of the State of Sao Paulo, the study area is located in the Atlantic plateau, characterized as a highland region, consisting predominantly of Precambrian crystalline rocks, cut by basic intrusive and alkaline Mesozoic-Tertiary rocks. The relief of the basin is called a relief of hills, dominated by local amplitude of 100-300 m and slopes of medium to high - above 15%, with high drainage density, closed to open valleys and restricted inland alluvial plains (IPT, 1981). The soil of the plot of interest is of the type Tb dystrophic Cambisol Oxisols with clay texture.

4.2 ECOPHYSIOLOGICAL MONITORING

For monitoring the ecophysiological behavior, measurements were carried out in a stand of hybrid clonal of Eucalyptus grandis x Eucalyptus urophylla, 60 months after planting at spacing 3 x 2 m.

4.2.1. PREDAWN LEAF WATER POTENTIAL ($\Psi_{pd}$)

The observations of water availability in the soil were performed by measuring predawn leaf water potential ($\Psi_{pd}$) using a Scholander pressure chamber (Scholander et al., 1965), model 3035 (Soil Moisture Equipment Corp., USA) before sunrise in healthy leaves fully expanded. For this, measurements were carried in four individuals per day where we collected, simultaneously, four branches per individual. To avoid water loss through transpiration, the leaves were cut during insertion of the petiole, wrapped in plastic wrap and put under refrigeration. The measurements were carried in the field immediately after collection.

4.2.2. ECOPHYSIOLOGICAL VARIABLES

Transpiration (E), stomatal conductance (Gs) and photosynthesis (A) were obtained with the aid of a mobile elevating work platform disposed in the plantation row, in order to reach the canopy of four trees approximately 18 meters in height. From there, the
physiological measurements were performed by infrared gas analyzer (IRGA) LC-PRO + (ADC bioscientific Ltda., UK). For this end, four healthy random and fully expanded leaves were chosen for each individual. The readings were held at hourly intervals throughout the day in the period from 8 am to 5 pm during the month of August 2008.

4.3 CLIMATE FACTORS

The specific climatic characteristics of the days of measurement were obtained from the automatic weather station Campbell Scientific Inc. installed on the site of study, distant 100 m from the clonal population of interest. Environmental variables such as photosynthetically active radiation (PAR) and vapor pressure deficit of the atmosphere were chosen to correlate with the E, Gs and A.

The PAR on the leaf surface (Qleaf) was determined simultaneously with the measurements of ecophysiological variables, using the sensor coupled to the porometer chamber, always disposed perpendicularly to incident sunlight on the leaf surface throughout each workday.

Environmental variables were chosen to correlate with the E, Gs and A. Thus, additional data on air temperature and relative humidity were collected from this weather station. This information was used to calculate the pressure deficit of water vapor (VPD).

\[ \text{VPD} = \text{es} - \text{ea}, \]  

(1)

The saturation pressure of vapor was calculated using the following equation:

\[ \text{es} = 0.6108 \times 10^{-7.5 \times \text{Tair} / 237.3 + \text{Tair}} \]  

(2)

where \( \text{Tair} \) corresponds to the air temperature in °C and es in kPa.

The partial vapor pressure was obtained by the following equation:

\[ \text{ea} = \text{UR} \times \text{es} / 100, \]  

(3)

where UR is the relative humidity of the place, expressed in%.

4.4 STATISTICAL ANALYSIS

The ecophysiological behavior, depending on \( \Psi_{pd} \), Qleaf and VPD was subjected to analysis of variance and, when significant, the means were compared by Tukey test using Minitab 14.0 software.

5 RESULTS

5.1 MEAN DAILY BEHAVIOR OF ECOPHYSIOLOGICAL VARIABLES OVER THE EVALUATION PERIOD

Figure 1 shows the predawn leaf water potential (\( \Psi_{pd} \)) and the daily mean of gas exchange and environmental variables. Over the three days of measurement, it was observed a continuous decrease of \( \Psi_{pd} \), not necessarily accompanied by a decrease of gas exchange. In general, the behavior of physiological variables E, Gs and A had the same tendencies as the environmental variables and each day of observation was statistically different in all situations. The highest rates of E, Gs and A occurred on 08/12/2008, where individuals not only were under lower water stress, but also with greater availability of energy and VPD (Figure 1e, f), indicating, therefore, a higher consumption of water on this occasion. The maximum changes of E, Gs and A for this day of measurement occurred at 12:00 pm with
11.1 mmol m⁻² s⁻¹, 0.60 mol m⁻² s⁻¹, and 23.12 µmol m⁻² s⁻¹, respectively with Qleafmax also at 12:00 pm, with 1769 µmol m⁻² s⁻¹ and VPDmax between 3 pm and 4 pm with 2.2 kPa. In contrast, on 08/13/2008, characterized by the predominance of many clouds, had the lowest gas exchange rates: E = 3.3 mmol m⁻² s⁻¹, Gs = 0.36 mol m⁻² s⁻¹ and A = 15.1 µmol m⁻² s⁻¹. This event may be related to low incidence of energy and atmospheric demand throughout the period, (Qleafmax at 10 am, 368 µmol m⁻² s⁻¹ and VPDmax at 12 pm, 0.6 kPa) insufficient to boost the ecophysiological activities.

5.2 RELATIONS BETWEEN Ψpd, E, Gs AND A DEPENDING ON Qleaf AND VPD

Interactions between gas exchange and environmental variables Qleaf and VPD are shown in Figure 2. Thus, it is observed that E, Gs and A accompany incrementally the increase of Qleaf (Fig. 2a, 2c, 2d), whose behavior was demonstrated by the correlation coefficients between environmental and ecophysiological variables presented in Table 1. This shows that for these conditions of Ψpd, the behavior of the variables E, Gs and A were better associated with the incident radiation on the leaf. However, lower correlations were found in E x VPD and A x VPD. The only non-significant association was found for Gs x VPD, and as shown in Figure 2d, there is no tendency of interaction between these two variables.
individuals per day. Means followed by same small letter have no statistical difference by Tukey test at 5%. Vertical bars indicate the standard error of the mean.

**Figura 1.** \( \Psi_{pd} \) (A) e média diária da E (B), Gs (C), A (D), Qleaf (E) e DPV (F) em um plantio clonal do clone C041 em Igaratá, SP, Brasil. Agosto 2008. Média de quatro indivíduos por dia. Médias seguidas de mesma letra minúscula não possuem diferença estatística pelo teste de Tukey a 5%. Barras verticais correspondem ao erro padrão da média.

**Figure 2.** Relation between ecophysiological and environmental variables for clone C041. E x Qleaf (A), E x VPD (B), Gs x Qleaf (C), Gs x VPD (D), A x Qleaf (E), A x VPD (F). Santa Marta Farm, Igaratá-SP, Brazil. August 2008.

**Figura 2.** Relação entre variáveis ecofisiológicas e ambientais para o clone C041. E x Qleaf (A), E x DPV (B), Gs x Qleaf (C), Gs x DPV (D), A x Qleaf (E), A x DPV (F). Fazenda Santa Marta, Igaratá-SP, Brasil. Agosto 2008.
Table 1. Simple correlation (r) between the mean hourly values of E, Gs and A with their respective values of Qleaf and VPD of the clone C041 in the period from 12 to 14 of August 2008. Igaratá, SP, Brazil

<table>
<thead>
<tr>
<th>Variables</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>E x Qleaf</td>
<td>0.87 *</td>
</tr>
<tr>
<td>E x VPD</td>
<td>0.56 *</td>
</tr>
<tr>
<td>Gs x Qleaf</td>
<td>0.66 *</td>
</tr>
<tr>
<td>Qleaf</td>
<td>0.07</td>
</tr>
<tr>
<td>Gs x VPD</td>
<td>ns</td>
</tr>
<tr>
<td>A x Qleaf</td>
<td>0.83 *</td>
</tr>
<tr>
<td>A x VPD</td>
<td>0.32 *</td>
</tr>
</tbody>
</table>

* = Correlation significant at 5%, ns = non-significant/ * = correlação significativa ao nível 5% ; ns = não significativo

In order to get a more detailed investigation on how environmental and ecophysiological variables relate, it was established a ratio between the hourly mean of all values observed during the study period of E, Gs and A and their hourly mean of Qleaf and VPD (Figure 3). With this mean ratio, it was attempted to exclude the influence of variation of the concentration gradient of water and Qleaf or VPD. Therefore, aiming to characterize the difference in the behavior of diffusion according only to the structure and physiology of the clones.

The results for all the reasons involving Qleaf were statistically different. On the day 08/13/2008 the ecophysiological variables (Figure 3A, C, E) were more affected by Qleaf, thus, presenting the highest values that can justify the lower gas exchange observed in Figure 1. When comparing the behavior in response to physiological variables according to the environmental variables, it was observed that the limiting factor for gas exchange in the evaluation period was Qleaf. As to VPD, the response tendency of E and A were similar (Figure 3B, F), and were not statistically different for the three days of observation. The ratio Gs / VPD was lower for the day 08/12/2008 (Figure 3D) and statically different from other days, comparing with the already observed in Figure 1F.
3.3. INSTANTANEOUS EFFICIENCY OF WATER USE (WUE) AND INTRINSIC EFFICIENCY OF WATER USE (WUEi)

There were no significant differences in WUE between days in each water regime. However, the ratio A / E was progressively increased with the decrease of Ψpd and the highest values were observed on 8/14/2008, indicating a greater assimilation of biomass in this situation (Figure 4A). In contrast, the ratio A / Gs (Figure 4B) was statistically different between three days and the behavior among them registered the same tendencies observed in Figure 1. The day 08/12/2008 had the highest value of WUEi conditioned by the highest
photosynthetic capacity. The WUEi had decreased over the days, possibly due to the decline in stomatal conductance during water stress, which leads to reduction in efficiency of assimilation by the photosynthetic process, as seen in Figure 1. Some authors suggest that the decline in WUEi, results from a reduction in stomatal conductance, which affects more the photosynthetic rate than the rate of leaf transpiration (AWAL; IKEDA, 2002; LARCHER, 2003).

Figure 4. (A) Mean daily instantaneous efficiency of water use (A / E) and (B) intrinsic efficiency of water use (A / Gs) for clone C041 during the evaluation days. Igaratá, SP, Brazil. August 2008. Mean of four individuals, followed by the same small letter have no statistical difference by Tukey test at 5%. Vertical bars indicate the standard error of the mean.

4 DISCUSSION

The stomata are not only the entry route for gas exchanges for CO$_2$, but also the outflow of water in vapor form, from the inside to the outside of the leaf. In order to absorb CO$_2$ from the outside, the plant inexorably loses water and when this loss decreases, it also restricts the intake of CO$_2$. This interdependence was recognized long ago and numerically expressed by the ratio between total assimilation and water consumption.

According to Lamaud et al. (1996), the efficiency of water use (WUE) represents the ability that vegetation has to absorb carbon while limiting water loss through the stomata. Water exerts influence on various processes such as CO$_2$ assimilation, transpiration, leaf expansion and partitioning of assimilates to different organs of the plant. The reduction in the amount of water available to plants leads to increased respiration, increased activity of hydrolytic enzymes and reduction in the intensity of photosynthesis (KUDREV, 1994). But only the study of the relation available water and physiological activity is not sufficient to explain the behavior of gas exchange and development of plants. By observing the information available in Figures 1a, 1b, 1c, 1d, it can not be concluded that the gas exchange follow the decrease in $\Psi_{pd}$.
In nature, the WUE is influenced not only by water, but also by climatic conditions. Figure 2 showed this relation very well, where one can observe the tendencies of increased gas exchange accompanied by increased Qleaf or VPD and also confirmed by the correlation coefficients (Table 1). Similar situations were reported by several authors in different cultures, including several genera of Eucalyptus sp (GRANIER et al., 2000, WHITEHEAD; BEADLE, 2004; PONI et al., 2005; BOTIA; ROMERO, 2006, MATSUMOTO et al. 2008). Generally, the correlation between Gs and VPD is presented with a high sensitivity of Gs to the increase in VPD (WHITEHEAD; BEADLE, 2004, KUMAGAI et al., 2008). In this study, there was no tendency between these two parameters, perhaps justified by the need for more data or even low levels of VPD during the period of measurements, insufficient to sensitize the stomata. Incidentally, when the ratio of the physiological and environmental variables as verified (Figure 3), only the relationships involving Qleaf were statistically different between the days of observation. An exception was observed only on 08/12/2008 in Gs / VPD (Figure 3d), which is precisely the day with higher atmospheric demand (Figure 1f).

Most studies on efficiency of water use have been made in agriculture, with few studies in forest ecosystems. Recently there has been a large increase in the number of researches on the measurement of CO₂ fluxes and energy between the atmosphere and terrestrial ecosystems, not only to assess the status of these ecosystems, but also to analyze their variations over time.

An important issue in studies on the carbon cycle is related to the responses of vegetation to rising concentrations of atmospheric CO₂. Most studies in this area were conducted under controlled conditions on crops and showed increased rates of photosynthesis and biomass with high content of CO₂ from the air and the consequent increase in the efficiency of water use (LINDROTH; CIENCIALA, 1995). Using techniques of carbon isotopes, Feng (1999) examined tendencies in the efficiency of water use in trees from the last 100-200 years in response to atmospheric CO₂ concentration and concluded that the efficiency of water use of nearly all trees increased with the increased concentration of atmospheric CO₂. According to the same author, this result is due to an increase in the rate of carbon assimilation and/or a decrease in stomatal conductance, indicating an increase in the efficiency of transpiration and a direct connection with changes in plant biomass.

This species, the water use efficiency (WUE) helps the understanding of water relations in plants, representing a relationship between the increase of biomass and volume of water used in the period. Due to the growing concern about the availability of water resources in irrigated agriculture, there is an interest in trying to develop an understanding of how the WUE can be improved and how farming systems can be modified to be more efficient. This, both in the selection of drought tolerant varieties as in the selection of species best suited to each region (Doorenbos & Kassan, 1979; Hatifield et al., 2001).

In order to support what was shown in Figure 4, situations also observed by Schultz (2000), Poni et al. (2005) and Poni et al. (2009) clearly showed that regardless of the stress level in the soil, the intrinsic WUE tends to increase with increasing VPD, while instantaneous WUE usually shows an opposite tendency. In their work, Poni et al. (2005) pointed out the following fact: the results of the effects of WUE on the whole canopy must be reconciled with the conclusion drawn from the assessment of a single leaf. However, one important issue is the sampling of leaves fully exposed to radiation to minimize variability due to different locations of the canopy and interact with the position of the sun. A canopy is comprised of a heterogeneous population of leaves, some of which are located in the shade or form varying angles to the direction of radiation, thus receiving diffuse radiation. The study performed by Intrieri et al. (1998) showed that the leaves that were totally or partially shaded...
usually have low levels of WUE. Indeed, when the efficient use of water is studied in agricultural crops aiming, for example, biomass or reserves per amount of water transpired, there is a consensus that the strategies of water deficit would increase WUE compared to full irrigation (DRY; LOVEYS, 1999).

The values of instantaneous WUE obtained in this study were on average lower than those observed by Tatagiba et al. (2007) in adult trees of Eucalyptus sp. 2.5 to 3 years old in Itauninhas, Bahia - Brazil. This fact can be explained by the decline of ontogenetic development over the years where the plant tends to stability. The WUE can assess the effects of water management, soil and on plant water use and crop production. There is ample evidence that the efficiency of water use by plants varies among species in the same environment, different climatic conditions in the same culture, between sites and seasons (TAYLOR; WILLATT, 1983). The adaptation of species to the effects of climate can help in the management of water in different conditions of moisture, which is why the use of water reflects the complexity of factors involved in the interaction plant/environment. According to Olbrich et al. (1993), the knowledge of the efficiency of water use would help the selection of the best species of Eucalyptus to certain conditions.

5 CONCLUSIONS

Transpiration, stomatal conductance and photosynthesis accompanied the growth of photosynthetically active radiation (PAR) and vapor pressure deficit of the atmosphere. Transpiration and photosynthesis were ecophysiological variables that showed better association with the PAR. There were no good relations observed with the vapor pressure deficit of the atmosphere. The ratio A/E was progressively increased with the decreasing $\Psi_{pd}$ although they showed no statistical difference over the period of work.

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6 REFERENCES


