Changes in leaf physiology for eucalyptus genotypes with increase in integral water stress and its consequence for initial growth in central Chile

Mudanças na fisiologia foliar para genótipos de eucalipto com o aumento da integral do estresse hídrico e sua consequência para o crescimento inicial no centro do Chile

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Abstract

Limited information exists on how small changes in soil water affect the physiological responses of eucalyptus and their relationship with productivity. This experiment evaluated physiological traits during decreased soil water and compared them with growth and water stress integral (WSI) of 22 eucalyptus genotypes with 1.5 years old. The experiment was conducted in sandy soil with low water holding capacity in Chile. Selected genotypes were Eucalyptus globulus, E. nitens, and E. nitens × E. globulus hybrids (E. gloni). Seasonal predawn leaf water potential (Ψpd) was evaluated under well-irrigated conditions and after 40 days without irrigation. Subsequently, two sampling instances were considered for analysis, well-irrigated conditions M0 (470 days after planting) and 40 days without irrigation Mfinal (510 days after planting). During that period, we measured midday leaf water potential (Ψmd), leaf area index (LAI), diurnal changes in photosynthesis (An) and stomatal conductance (gs). The broad range of WSI among genotypes indicated contrasting levels of accumulated water stress during early development. In fact, E. globulus presented 30% more water stress than E. nitens, and genotypes with the highest WSI showed the lowest growth. Between M0 and Mfinal, there was a reduction in An and gs in the morning and midday with small changes in the afternoon, and consequently, values of the intrinsic water use efficiency (iWUE) increased. The Ψmd showed small changes and we observed a large LAI increment for all genotypes. Changes in iWUE were more pronounced for genotypes with lower WSI, which suggested that these genotypes with lower accumulated water stress were more sensitive to changes in soil water, such as E. gloni genotypes allowing them to maintain high productivity.

Keywords: Water stress integral; Photosynthesis; Intrinsic water use efficiency; Forest growth; Leaf water potential.

Resumo

Existem informações limitadas sobre como pequenas mudanças na disponibilidade de água do solo afetam as respostas fisiológicas do eucalipto e sua relação com a produtividade. Este experimento...
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Evaluating the characteristics fisiológicas durante a diminuição da água no solo e as comparou com o crescimento e o estresse hídrico integral (WSI) de 22 genótipos de eucalipto com 1,5 anos de idade. O experimento foi conduzido em solo arenoso, com baixa capacidade de retenção de água no Chile. Os genótipos selecionados foram Eucalyptus globulus, E. nitens e híbridos de E. nitens × E. globulus (E. gloni). O potencial hídrico foliar sazonal (Ψpd), medido antes do amanhecer, foi avaliado em condições bem irrigadas e após 40 dias sem irrigação. Posteriormente, foram consideradas para análise duas instâncias de amostragem, sendo estas em condições bem irrigadas M0 (470 dias após o plantio) e 40 dias sem irrigação Mfinal (510 dias após o plantio). Durante esse período, medimos o potencial hídrico foliar ao meio-dia (Ψmd), o índice de área foliar (LAI), as mudanças diurnas na fotossíntese (A_n) e a condutância estomática (gs). A ampla variação de WSI entre os genótipos indicou níveis contrastantes de estresse hídrico acumulado durante o desenvolvimento inicial. De fato, a espécie E. globulus apresentou 30% mais estresse hídrico do que a espécie E. nitens, e os genótipos com o maior WSI apresentaram o menor crescimento. Entre M0 e Mfinal, houve redução das variáveis A_n e gs pela manhã e ao meio-dia com pequenas variações à tarde e, consequentemente, os valores da eficiência intrínseca do uso da água (iWUE) aumentaram. O Ψmd apresentou pequenas alterações e, ao longo do tempo, observamos um grande incremento do LAI para todos os genótipos. Mudanças no iWUE foram mais pronunciadas para genótipos com menor WSI, o que sugeriu que esses genótipos com menor estresse hídrico acumulado eram mais sensíveis às mudanças na água do solo, como genótipos de E. gloni, permitindo-lhes manter alta produtividade mesmo em condições adversas.

Palavras-chave: Estresse hídrico acumulado; Fotossíntese; Eficiência intrínseca no uso da água; Crescimento florestal; Potencial hídrico foliar.

INTRODUCTION

Water availability is a major factor controlling ecosystem productivity, and drought is the most significant threat for forest plantations (Allen et al., 2010; Lévesque et al., 2013). The severity and temporal scale of water deficit affect the physiology and tree growth (Fontes et al., 2018). The identification of morphological and physiological responses to this deficit is critical for the early detection of potential decrease in tree productivity and their possible adaptation, and these response patterns may determine the tolerance level to lower water availability (Vicente-Serrano et al., 2013; Héroult et al., 2013). Climate change scenarios predict increasing drought duration and intensity in tropical and subtropical areas (Intergovernmental Panel on Climate Change, 2014). Lower water availability in regions with a Mediterranean climate that endure intense summer droughts may severely affect Eucalyptus growth and physiology under water stress conditions, and understanding the genetic response is crucial (Héroult et al., 2013; Correia et al., 2014). In this context, breeding of drought-tolerant trees may be a promising approach (Landsberg & Waring, 2017).

Water deficits have short- and long-term effects on plants. Short term reactions are linked to physiological responses and long-term effects are related to morphology and growth. Thus, the relationship between plant water status (e.g., leaf water potential) and forest yield is necessary to associate the effects of water status on plant development, known as the water stress integral (WSI) (Myers, 1988). The WSI consists of the summation of the leaf water potential at defined intervals over the measurement period. Larger absolute values of WSI represent larger amounts of accumulated water stress (Myers, 1988).

Short-term responses to avoid water stress include the maintenance of higher water potentials, reduction of carbon assimilation, stomatal closure (Chaves et al., 2003; Silva et al., 2016), and reduction of leaf area by shedding or diminishing leaf sizes (Gauthier & Jacobs, 2018). Leaf water potential and stomatal conductance are key factors that influence plant water status (Faroq et al., 2009; Martorell et al., 2014). Higher water-use efficiency is generally linked with stomatal closure for the reduction of transpiration when the soil dries, but it can also be positively correlated with productivity (Hatfield & Dold, 2019).

Water use efficiency has been identified as a key parameter for evaluating eucalyptus adaptation to water deficits and there is a wide variation among eucalyptus genotypes (Navarrete-Campos et al., 2013). However, stomatal closure limits CO2 uptake by leaves, decreasing photosynthetic rates (Myers & Landsberg, 1989; Santos et al., 2019), and thereby reducing plant metabolism. These responses tend to increase during periods of soil water
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decline (Kolb & Stone, 2000). However, eucalyptus genotypes show large variations in physiological responses to water stress (White et al., 2000; Silva et al., 2017). These different physiological responses must be evaluated in experimental plots in the field, since greenhouse seedlings do not necessarily reflect trees phenotype in the field, which currently represents a challenge (Adams et al., 2017). The main objective of this study was to analyze the relationship between early growth and water stress integral on changes in photosynthesis, intrinsic water use efficiency, midday leaf water potential, and leaf area index of different eucalyptus genotypes under a short period of decreased soil water. We tested the hypothesis that all eucalyptus genotypes, evaluated as top-ranking selections for growth by a tree improvement program, subjected to higher absolute accumulated water stress integral would show lower growth during soil water decrease, as a result of decreasing net photosynthesis, intrinsic water use efficiency and leaf area index, and increasing midday water potential.

MATERIAL AND METHODS

Study area

The study site was located 9.6 km east of Yumbel, Bio-Bio region, central South Chile (37°8′0.01″ S, 72°27′34.70″ W, 200 m.a.s.l.) The experiment was established on land previously used for a bioenergy study involving Acacia and Eucalyptus spp., where the stumps were removed mechanically after harvesting, and the remaining residues crushed and incorporated into the soil by harrowing. The soil of the study area is classified as an Entisol (United States Department of Agriculture, 1999), formed by volcanic black sands of andesitic and basaltic origin, with low water-holding capacity. The climate is characterized as Csb (i.e., a warm-summer Mediterranean climate) with winter rains. The average annual precipitation at the site is 1328 ± 333 mm, occurring mainly in winter (600 mm), and the mean annual temperature is 14.2 ± 0.5 °C.

In October 2017, 22 eucalyptus genotypes, evaluated as top-ranking selections by a tree improvement program of the CMPC Forestal Mininco and ARAUCO companies, were planted in a randomized block design with three replicates. Plant genetic material consisted of seven cuttings of E. globulus (EG), three seedlings of E. nitens (EN), and eight cuttings of E. nitens × E. globulus (E. gloni) hybrids (ENG). All genotypes are part of a tree improvement program for the selection of genotypes with desirable fiber and pulp yields and higher growth rates. Experimental plots were established by hand planting 16 plants per 24 m² with four plant internal measurement plots (6 m²) and replanting occurred one month after establishment, and the proportion of replanted individuals was 2%. Details of establishment operations could see in Oliveira et al. (2021). From mid-February 2019 to late March 2019 (approximately 40 days), the irrigation treatment was halted to evaluate plant physiological responses as soil water content decreased.

Growth and water stress integral

Between February 2019 and March 2019, individual tree height (h) and root collar diameter at 0.1 m height (rcd) were measured on four central plants in each measurement plot (6 m²). According to the equation below, the individual tree volume index (vi) was determined from these measurements:

\[ vi = 0.33\pi \cdot \frac{rcd^2}{4} \cdot h, \]

where \( vi \) is the individual tree volume (cm³), \( rcd \) is the root collar diameter measured at a 0.1-m height (cm), and \( h \) is the total tree height (cm).

Volume per hectare estimates (V) were obtained by summing individual tree volumes for each plot and scaling estimates at hectare level. Volume increment (IncVol) was determined as the difference between V in March 2019 and February 2019. Tree survival was not evaluated because there was no mortality during the experiment.
Predawn leaf water potential ($\Psi_{pd}$) was measured at five different times during early growth. Seasonal measurements were taken during the mid-summer (February 2018), early winter (June 2018), mid-winter (August 2018), mid-spring (November 2018), and second mid-summer (February 2019). These dates coincided with different periods of rainfall (PPT) and temperature (T) at the experimental site (Figure 1). Following Myers (1988), we calculated the cumulative water stress integral (WSI) as the cumulative integral of $\Psi_{pd}$ (Galindo et al., 2017) over the entire growth period:

$$WSI = \sum_{i=0}^{n}(\Psi_{i+1} - c). n,$$

where WSI is the cumulative water stress integral (MPa day$^{-1}$), $\Psi_{i+1}$ is the mean of the predawn leaf water potential for interval $i$, $i + 1$ (MPa), $c$ is the maximum value of predawn leaf water potential during the measurement period (MPa), and $n$ is the number of days at interval $i$, $i + 1$.

![Figure 1 - Rainfall (PPT) and Temperature (T) during the first year of growth of Eucalyptus genotypes.](image)

**Volumetric soil water content and sampling instances**

Volumetric soil water content ($\theta_v$) was monitored by installing three soil moisture sensors (CR655, Campbell Scientific, Logan, USA) at each block from February 2019 to March 2019 (40 days). Sensors were inserted to monitor water content at 5 min intervals at 0–20 cm and 20–40 cm soil depths. The sensors were connected to a CR1000 datalogger (Campbell Scientific) to record $\theta_v$ data. From $\theta_v$ measurements, two sampling instances ($M_0$ and $M_{final}$) were determined and used to analyze genotype differences in physiological behavior during the 40 days of $\theta_v$ decrease. $M_0$ represents the final point under irrigation and the starting point under no irrigation (February 18th to February 22nd 2019), and $M_{final}$ represents the final period after approximately 40 days without irrigation (March 21st to March 25th 2019).

**Leaf gas exchanges, midday water potential and leaf area index**

Leaf gas exchange measurements were taken in $M_0$ and $M_{final}$, and the net CO$_2$ assimilation rate ($A_n$ – $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) and stomatal conductance ($g_s$ – $\mu$mol H$_2$O m$^{-2}$ s$^{-1}$) were determined. Measurements were made in one plant per plot (block) to assess mature, fully expanded, and sun-exposed leaves at 9:30 and 10:30 (morning), 12:30 and 13:30 (midday), and 15:00 and 16:00 (afternoon), using a portable infrared gas analyzer LICOR-6400 (Li-Cor, Inc., Lincoln, NE, USA). All measurements were made with reference CO$_2$ of 400 $\mu$mol mol$^{-1}$, light intensity (PAR) in the leaf chamber of 1500 $\mu$mol m$^{-2}$ s$^{-1}$, a constant flow rate of 500 $\mu$mol s$^{-1}$, and leaf temperature (T$_{leaf}$) as close as possible to ambient air temperature (T) for each measurement instance. The intrinsic water-use efficiency (IWUE – $\mu$mol CO$_2$ mol$^{-1}$ H$_2$O) was calculated using the ratio of $A_n$ to $g_s$. Midday leaf water
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potential ($\Psi_{md}$) was determined using a Scholander pressure chamber (PMS Instruments, Corvallis, OR, USA). Measurements were made in mature, fully expanded, and sun-exposed leaves of the upper canopy between 11:30 and 14:00, and occurred in $M_0$ and $M_{final}$. Leaf area index (LAI) was estimated for each plot and occurred in $M_0$ and $M_{final}$ under clear-sky conditions. Estimates were obtained using midday (11:00 to 14:00) photosynthetically active radiation (PAR) interception, by measuring below the canopy ($n = 3$) in the north and east orientation, and outside the experimental area ($n = 1$) using a ceptometer (Li-191R and Li-250; LICOR). For PAR below canopy readings, the sensor was placed in the center of the plot and among the trees in the interrow. To estimate the LAI from PAR measurements, uniform foliar angles ($\theta = 1$) were considered for calculations of the light extinction coefficient ($k$) (Campbell, 1986).

Statistical analyses

A linear model between WSI and IncVol was computed using the ordinary least squares of the function $lm$ in R (R Core Team, 2020). Analysis of variance (ANOVA) was used to evaluate sampling instance ($M_0$ and $M_{final}$), periods (morning, midday, afternoon), genotypes, and interactions for $A_n$, $g_s$, $\Psi_{md}$, and LAI. Sampling instances, periods, and genotypes were considered fixed effects, and the blocks were considered random effects. Analyses were performed using the $lmer$ function in the $lme4$ package (Bates et al., 2015). Fixed effect differences were computed using the function $ls_means$ of the $lsmeans$ package (Lenth, 2016). In addition, a linear model between changes in physiology and LAI during $M_0$ and $M_{final}$ with WSI and IncVol was computed using ordinary least squares of the function $lm$ in R. Models were compared using significance ($p<0.05$), root mean square error (RMSE), adjusted coefficient of determination (Adj-$R^2$), and Akaike information criteria (AIC).

RESULTS AND DISCUSSION

Growth and water stress integral (WSI)

The average $\theta_V$ was $0.12 \pm 0.03 \text{ m}^3 \text{ m}^{-3}$ at $M_0$ and $0.061 \pm 0.02 \text{ m}^3 \text{ m}^{-3}$ at $M_{final}$, with reduction of 50% between sample instances. Across all genotypes, mean IncVol was $18.9 \pm 4.5 \text{ m}^3 \text{ ha}^{-1}$ between $M_0$ and $M_{final}$. At $M_{final}$, average $V$ ranged from 28.6 to 60.9 m$^3$ ha$^{-1}$ for E. globulus, with an increment of 16 m$^3$ ha$^{-1}$ between $M_0$ and $M_{final}$ while for E. nitens the average $V$ ranged from 47.2 to 65.3 m$^3$ ha$^{-1}$, with an increment of 22.7 m$^3$ ha$^{-1}$ in the same period. For E. gloni, $V$ ranged from 29 to 64.2 m$^3$ ha$^{-1}$, with an average increment similar to that of E. nitens (20.8 m$^3$ ha$^{-1}$). As similar percentage increments were obtained for all genotypes, the ranking in wood volume did not present significant changes between $M_0$ and $M_{final}$ (Figure 2).

![Figure 2 - Cumulative volume (v - m³ ha⁻¹) for Eucalyptus genotypes between M₀ and M₉. Percentage represents the variation in average values between two sampling instances. The genotype order represents the largest to the smallest cumulative volume at M₀ for each taxon. Vertical bars correspond to the standard error of the plot means (n = 3).](image-url)
Magnitude seasonal changes in $\Psi_{pd}$ were different for each taxon (Figure 3a, c, and e) during the study period. Genotypes EG17, EG18, EG19, EG21, EG28, ENG3, ENG20, and ENG22 reached $\Psi_{pd}$ values below -0.5 MPa in mid-winter (August 2018), and during the second mid-summer (February 2019) average $\Psi_{pd}$ ranged from -0.2 to -0.4 MPa, with a maximum $\Psi_{pd}$ of -0.05 MPa (c parameter in WSI). The absolute cumulative WSI for the whole period of mensuration (475 days after planting) ranged from 100 to 158 MPa day$^{-1}$ for *E. globulus*, from 67 to 100 MPa day$^{-1}$ for *E. nitens*, and 67 to 161 MPa day$^{-1}$ for *E. gloni* (Figure 3b, d, and f).

The larger WSI indicated genotypes with larger cumulative water stress and *E. globulus* showed the largest absolute average WSI (124 MPa day$^{-1}$) compared to *E. nitens* (-31%) and *E. gloni* (-12%). Before irrigation was stopped, a large range in WSI occurred, indicating that genotypes faced different water stress levels throughout the study period and that could negatively influence growth (Waghorn et al., 2015). However, Myers (1988), who developed the concept of the WSI, observed that the WSI was not only determined by soil water deficits, but also by vapor pressure deficits (VPD). During the study, we observed higher values of temperature in summer, which also increased plant water stress due to a higher VPD (Lim et al., 2020).

![Figure 3](image-url)

**Figure 3** - Eucalyptus genotype changes in predawn water potential and water stress integral for *E. globulus* (a and b), *E. nitens* (c and d), and *E. gloni* (e and f) in the study period.

A significant and negative relationship was observed between IncVol (m$^3$ ha$^{-1}$) and absolute WSI (F = 36.38, p < 0.0001). The slope of the regression model showed a decrease in
of 0.166 m$^3$ ha$^{-1}$ for each 1 MPa day$^{-1}$. Genotypes that accumulated more water stress throughout the experiment were those that showed the least growth between $M_0$ and $M_{\text{final}}$ (Figure 4), apart from ENG3 ($E. gloni$), which revealed the highest absolute WSI and a higher IncVol when compared to the regression model. All $E. nitens$ genotypes (EN12, EN13, and EN14) presented high productivity (above 19 m$^3$ ha$^{-1}$ between $M_0$ and $M_{\text{final}}$) with low water stress (below 90 MPa day$^{-1}$), unlike the majority of $E. globulus$ genotypes (EG18, EG19, EG21, EG28, EG31, and EG34), which displayed the opposite behavior. Interestingly, some $E. gloni$ genotypes were similar to those of $E. nitens$ (ENG2, ENG5, ENG7, and ENG8), while others followed $E. globulus$ (ENG22 and ENG25).

![Figure 4](image)

**Figure 4** - Water stress integral and volume increment relationship between $M_0$ and $M_{\text{final}}$ (40 days) for eucalyptus genotypes. Taxa are differentiated by color.

Despite the different responses of some *Eucalyptus* genotypes, WSI is a useful tool for assessing the effect of water availability on tree growth (Gonzalez-Benecke & Dinger, 2018). Hakamada et al. (2017) highlighted that eucalyptus genotype changes in leaf water potential could be a predictor of plant water status during forest development. In this study, genotypes with lower $\Psi_{pd}$ showed lower WSI, mainly because these genotypes were more stressed during the study. However, water storage in tree tissues such as stem and roots represent an intermediate source of water for leaves to avoid water stress periods, which reflected in no changes $\Psi_{pd}$ values and also small decreased in WSI (Scholz et al., 2011).

### Physiology and leaf area changes during soil water decrease

Gas exchange measurements showed contrasting results, and significant interactions between sampling instances (SI), genotypes (G), and periods (P) were observed for $A_n$ and $g_s$, along with significant interaction between genotypes (G) and sampling instances (SI) for LAI. Only genotypes (G) showed significant effect for $\Psi_{md}$ (Table 1). Across all genotypes and periods, the decrease in soil water between $M_0$ and $M_{\text{final}}$ reduced average physiology performance by 22% for $A_n$, 37% for $g_s$, and 11% for $\Psi_{md}$. Interestingly, LAI was the only variable that increased (by approximately 49%) with soil water decrease. Considering individual taxa, *E. gloni* showed the greatest reduction in gas exchanges measurements, with an average of 25% for $A_n$ and 44% for $g_s$, and *E. nitens* genotypes presented the lowest reduction by average of 13% for $A_n$ and 32% of $g_s$. In addition, *E. nitens* genotypes presented an increase in average $\Psi_{md}$ (4%), although *E. globulus* showed the greatest reduction (16%). All taxa returned an increase in LAI, however *E. gloni* presented the greatest (51%).
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Table 1 - Analyses of variance p-values testing sampling instances (SI – M₀ and Mₚₒ₉ₙ), period (P – morning, midday and afternoon), genotype (G), and interaction effects evaluated for photosynthesis (Aₚ), stomatal conductance (gₛ), midday water potential (Ψₚₙₐ₅), and leaf area index (LAI).

| Effects     | Aₚ  | gₛ  | Ψₚₙₐ₅ | LAI |
|-------------|-----|-----|--------|-----|
| SI          | < 0.0001 | < 0.0001 | 0.2811 | < 0.0001 |
| P           | < 0.0001  | 0.2046 | -      | -      |
| G           | < 0.0001  | < 0.0001 | 0.0485 | 0.3481 |
| SI x P      | < 0.0001  | 0.0230 | -      | -      |
| SI x G      | < 0.0001  | < 0.0001 | 0.2814 | 0.0478 |
| P x G       | < 0.0001  | < 0.0001 | -      | -      |
| SI x P x G  | < 0.0001  | < 0.0001 | -      | -      |
| Mean – M₀   | 19.73 | 0.555 | -1.277 | 3.64  |
| CV (%) – M₀ | 18.59 | 29.65 | 11.24  | 17.58 |
| Mean – Mₚₒ₉ₙ | 15.28 | 0.339 | -1.412 | 5.41  |
| CV (%) - Mₚₒ₉ₙ | 23.72 | 37.13 | 9.15   | 21.72 |

In general, decreased soil water reduced the maximum Aₚ, particularly in the morning and midday. We graphically analyzed changes in the maximum daily value of Aₚ for each taxon and observed larger reductions in maximum Aₚ in the morning for all taxa under decreased soil water, with an average of 19% for E. globulus, 27% for E. nitens, and 23% for E. gloni. During midday, only E. gloni showed a significant decrease (25%). Interestingly, small reductions (10%) in maximum Aₚ were noted in the afternoon, even with an increase in E. globulus (6%) (Figure 5a). In addition, we observed larger differences in Aₚ among E. gloni genotypes with different absolute WSI values before decreasing soil water and IncVol during sampling instances (Figure 5b). Genotype ENG22 (low growth and high-water stress) continually reduced Aₚ along the day with decreased soil water; in contrast, ENG3 (high growth and high-water stress) showed greater reduction in midday Aₚ but stability in the afternoon. Genotype ENG5 (high growth and low water stress) showed the least change, with some reduction in the morning, and stability during midday and afternoon. This indicates that accumulated water stress affects plant physiology responses during decreased soil water conditions. Since we observed a higher reduction in gₛ than Aₚ, iWUE increased between M₀ and Mₚₒ₉ₙ (Figure 5c and d). Average iWUE was 37.43 µmol CO₂ mol⁻¹ H₂O at M₀ and increased to 46.63 µmol CO₂ mol⁻¹ H₂O at Mₚₒ₉ₙ. Among the taxa, E. gloni showed the greatest increase (41%), followed by E. nitens (26%), and E. globulus (12%). The highest increment among genotypes was observed in the morning and afternoon iWUE, and genotypes with low absolute WSI showed a higher increment in iWUE during sampling instances (ENG5).

After 40 days of decreased soil water, there were no specific patterns among eucalyptus genotypes, and there was high variability in the response of Aₚ. In general, average Aₚ decreased for the majority of genotypes in the morning and midday, as expected for eucalyptus with decreased soil water (Mokotedi, 2010). The same author observed that osmotic adjustment helped eucalyptus species maintain photosynthetic capacity during periods of low soil water availability, which makes growth possible when soil water decreases. Interestingly, the genotypes decreased their physiology and increased leaf area under soil water changes, suggesting that a small reduction in Aₚ would compensate for the increased number and/or size of leaves, regardless of accumulated water stress.

Changes in the daily values of leaf gas exchanges in eucalyptus were reported by Battie-Laclau et al. (2016), and were also observed in this study. When irrigation was applied, the average Aₚ was higher in the morning for all taxa, decreasing throughout the day. For E. gloni, higher values of Aₚ were observed in the morning and midday, decreasing in the afternoon. However, Aₚ did not reach maximum values at midday, even in the well-irrigated period (M₀) and declined on average for all taxa with decreasing soil water. Midday depression in Aₚ can arise in relation to the negative association with weather conditions (Borišev et al., 2015) where Aₚ increases during other periods of the day, such as morning or afternoon (Baldocchi, 1997).
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Figure 5 - Maximum $A_n$ and $iWUE$ for each taxon (a and c) and changes in $A_n$ and $iWUE$ for E. gloni genotypes (b and d).

The $g_s$ showed greater reduction than $A_n$ during sampling instances for all genotypes. This results in an increase in the average intrinsic water use efficiency ($iWUE$), which is most pronounced in E. gloni genotypes, revealing a higher response of this taxa to decreased soil water. During the dry season, Battie-Laclau et al. (2016) observed that E. grandis clones responded to a water deficit, increasing their midday values of $iWUE$, mainly because there was a temperature and VPD at this period. In this study, $iWUE$ values were better correlated with $g_s$ than $A_n$, and its absolute correlation increased between $M_0$ and $M_{final}$ (0.47 to 0.54).

These patterns reflected stomatal closure during the day, reducing the uptake of CO$_2$ in the leaves and decreasing intercellular CO$_2$. Previous studies have shown that stomatal closure is the first event that occurs in response to decreasing soil water levels and that it limits the photosynthetic rate (Flexas et al., 2004) as a means to avoid water loss (Mejía de Tafur et al., 2017). Eucalyptus closes their stomata for long periods or at different hours of the day to avoid water loss during drought (Silva et al., 2016; Gonçalves et al., 2017). However, there is a high variation in physiological responses, even in genotypes of the same taxon, as reported in this study. Since a greater reduction in $g_s$ was observed, $iWUE$ increased for all genotypes during sampling instances, indicating that genotypes improved their efficiency in water use at leaf level. Also, other studies showed that $iWUE$ could be used as a tool to select genotypes that are tolerant of water stress (Navarrete-Campos et al., 2013; Saadaoui et al., 2017) and E. gloni genotypes showed the highest average increment in $iWUE$ during sampling instances, from 33.84 to 49.34 µmol CO$_2$ mol H$_2$O, and E. nitens showed the lowest response, from 33.01 to 40.27 µmol CO$_2$ mol H$_2$O. Also, between $M_0$ and $M_{final}$ small changes in $\Psi_{md}$ occurred, indicating that most genotypes were highly conservative in changing their water status, showing an isohydric behavior (stomata closure to maintained values leaf water potential), specially for productivity genotypes, that were more capable to increase or stabilize $\Psi_{md}$ during soil water decrease, maintaining plant water status.

A negative relationship was observed between WSI and changes in $iWUE$, mainly in the midday and afternoon. In addition, IncVol showed a significant relationship with changes in $iWUE$ and $\Psi_{md}$ (Table 2). Genotypes that presented higher IncVol during $M_0$ and $M_{final}$ and lower accumulated WSI had improved $iWUE$, because of stomata closure. In addition, genotypes with higher IncVol during soil water decrease were capable of stabilizing or increasing their $\Psi_{md}$. 
indicating that they were less stressed (Figure 6). In contrast to iWUE, An showed non-significant relationships with IncVol and WSI. The majority of genotypes showed a decreased $A_{\text{net}}$ and a large $A_{\text{net}}$ variation in response to soil water decrease. Non-significant relationships were also observed for LAI, even with larger genotype variations in productivity during sampling instances.

Table 2 - Linear models adjusted for changes in physiology and leaf area index during sampling instances ($M_0$ and $M_{\text{final}}$) with water stress integral (WSI) and volume growth (IncVol). Linear Models are represented by the expression $Y = \beta_0 + \beta_1 X$ and goodness of fit of linear models was evaluated by Adjusted coefficient of determination ($Adj - R^2$), root mean square error (RSME), Akaike information criteria (AIC) and model significance ($p$-value $< 0.05$).

| $\Delta(\%)$ | WSI | $\Delta(\%)$ | IncVol |
|---------------|------|---------------|--------|
| | $\hat{\Delta}_0$ | $\hat{\Delta}_1$ | Adj $- R^2$ | RSME | AIC | $p$-value |
| LAI           | 44.81 ns | -0.141 ns | 0.05 | 15.72 | 189.67 | 0.3002 |
| $\Psi_{\text{md}}$ | 8.458 ns | -0.172 ns | 0.10 | 9.81 | 154.11 | 0.0966 |
| $A_{\text{net}}$ Morning | -20.63 ns | -0.149 ns | 0.01 | 16.20 | 182.57 | 0.3277 |
| $A_{\text{net}}$ Midday | -6.276 ns | -0.135 ns | 0.16 | 16.47 | 174.83 | 0.4425 |
| $A_{\text{net}}$ Afternoon | 1.397 ns | -0.068 ns | 0.01 | 25.31 | 210.61 | 0.7544 |
| Average $A_{\text{net}}$ | -16.66 ns | -0.021 ns | 0.01 | 11.31 | 167.48 | 0.8377 |
| iWUE Morning | 19.58 ns | -0.092 ns | 0.12 | 26.12 | 183.90 | 0.7431 |
| iWUE Midday | 72.27 ** | -0.444 ns | 0.12 | 26.72 | 203.59 | 0.0697 |
| iWUE Afternoon | 133.9 ** | -0.989 ** | 0.46 | 27.58 | 195.45 | 0.0006 |
| Average iWUE | 83.55 ** | -0.558 ** | 0.35 | 18.87 | 188.97 | 0.0028 |

| $\Delta(\%)$ | $\hat{\Delta}_0$ | $\hat{\Delta}_1$ | Adj $- R^2$ | RSME | AIC | $p$-value |
|---------------|------------------|------------------|------------------|-------|-------|---------|
| LAI           | 13.56 ns | 0.881 ns | 0.02 | 15.62 | 189.38 | 0.2491 |
| $\Psi_{\text{md}}$ | -29.77 ** | 1.082 * | 0.15 | 9.54 | 153.00 | 0.0434 |
| $A_{\text{net}}$ Morning | -29.95 ns | -0.176 ns | 0.01 | 15.84 | 181.63 | 0.8247 |
| $A_{\text{net}}$ Midday | -44.77 ** | 1.316 ns | 0.12 | 13.79 | 167.74 | 0.0816 |
| $A_{\text{net}}$ Afternoon | 1.600 ns | -0.402 ns | 0.01 | 22.34 | 187.01 | 0.7357 |
| Average $A_{\text{net}}$ | -12.64 ns | -0.318 ns | 0.01 | 11.23 | 167.17 | 0.5746 |
| iWUE Morning | -10.58 ns | 1.017 ns | 0.01 | 25.80 | 192.77 | 0.4566 |
| iWUE Midday | -16.93 ns | 2.628 ns | 0.12 | 24.91 | 182.09 | 0.0783 |
| iWUE Afternoon | -80.14 ** | 6.095 ** | 0.48 | 27.55 | 185.93 | 0.0006 |
| Average iWUE | -32.83 ns | 3.211 ** | 0.31 | 19.67 | 181.92 | 0.0061 |

ns = not significant; * $p < 0.05$; ** $p < 0.01$.

Greater increments in iWUE were observed for higher productive genotypes and with lower absolute WSI, suggesting that these genotypes were more sensitive to soil water changes, thereby closing their stomata. In addition, less stressful genotypes (lower changes or increment in $\Psi_{\text{md}}$) had the higher growth during sampling instances, showing coordination in plant water status and development. In general, selecting more productive genotypes, those that are less stressed and more efficient in water use, is critical considering the expansion of eucalyptus plantations to regions with a water deficit in South America.

Our results indicate that silviculture and breeding programs must find a balance between productivity and physiology for eucalyptus management, since the most productive eucalyptus genotypes generally show the highest positive changes in water use efficiency, and this must be taken into account in an environment with higher water deficits (White et al., 2009). Further experiments should be conducted involving physiological evaluations during a longer period of water stress by guaranteeing the exclusion of rain to avoid any changes in soil water availability and focus in genotypes differences in tissues water storage which could play in maintained physiological function during stress periods. Differences in responses of iWUE among eucalyptus genotypes still show the possibility of progress in forest breeding and ecophysiology, mainly with $E. \ nitens$ and $E. \ gloni$ genotypes in central Chile. In general, eucalyptus genotypes with high productivity and lower water stress are more efficient in using water than less productive and higher water stress genotypes.
Changes in leaf physiology for eucalyptus genotypes with increase in integral water stress and its consequence for initial growth in central Chile

Figure 6 - Changes in intrinsic water use efficiency (iWUE) and its relationship with water stress integral (WSI) (a) and changes in midday water potential (Ψmd) and its relationship with volume increment (IncVol) (b) between M₀ and Mfinal.

CONCLUSIONS

E. globulus genotypes (EG1, EG18, EG19, EG21, and EG31) and E. gloni genotypes (ENG22 and ENG25) with higher absolute WSI after 1.5 years of development also present the lowest IncVol, indicating that accumulated water stress affected growth performance. Photosynthesis (An), stomatal conductance (gs), and midday water potential (Ψmd) reduce under soil water decrease, resulting in increment of intrinsic water use efficiency (iWUE) being most pronounced by E. gloni genotypes (ENG2, ENG5, and ENG7). Regardless WSI, all eucalyptus genotypes in this study invest in leaf area during small changes in soil water. In general, leaf water potential is a key parameter to connect productivity, physiology and water use in young eucalyptus plantations, and differences responses in water potential among genotypes on the same environment still show the possibility of progress in our understanding drought risks in forest management.

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