

**University of São Paulo  
“Luiz de Queiroz” College of Agriculture**

**Physiological responses and soil water balance of clonal  
*Eucalyptus* under contrasting spacings and genotypes**

**Rodrigo Eiji Hakamada**

Thesis presented to obtain the degree of Doctor in  
Sciences. Area: Forest Resources. Option in:  
Silviculture and Forest Management

**Piracicaba  
2016**

**Rodrigo Eiji Hakamada**  
**Forestry Engineer**

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under contrasting spacings and genotypes**

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:  
Prof. Dr. **SILVIO FROSINI DE BARROS FERRAZ**  
Coadvisor:  
PhD. **ROBERT MARSH HUBBARD**

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## **DEDICATION**

To my lovely wife Cacilia, my dear parents Cesar e Lourdes, my dear brother Henrique and my uncle Machado *in memorium*



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## SUMMARY

RESUMO.....	11
ABSTRACT .....	13
1 INTRODUCTION.....	15
References .....	17
2 BIOMASS PRODUCTION AND POTENTIAL WATER STRESS INCREASE WITH PLANTING DENSITY IN FOUR HIGHLY PRODUCTIVE CLONAL EUCALYPTUS GENOTYPES.....	20
Abstract .....	20
2.1 Introduction.....	20
2.2 Material and methods .....	22
2.3 Results .....	26
2.4 Discussion .....	29
References .....	31
3 TRANSPIRATION EFFICIENCY USED AS STRATEGY FOR FOREST ADAPTIVE MANAGEMENT IN HIGH PRODUCTIVITY <i>Eucalyptus</i> PLANTATIONS .....	36
Abstract .....	36
3.1 Introduction.....	36
3.2 Materials and Methods .....	38
3.3 Results .....	43
3.4 Discussion .....	47
References .....	51
4 HOW IS THE BEHAVIOR OF HIGH-DENSITY SHORT-ROTATION SYSTEM IN CLONAL <i>Eucalyptus</i> REGARDING SOIL WATER BALANCE? A STUDY CASE IN BRAZIL.....	57
Abstract .....	57
4.1 Introduction.....	57
4.2 Materials and Methods .....	59
4.3 Results .....	66
4.4 Discussion .....	74
References .....	78

5. Hypothesis, conclusions and recommendations ..... 84

## RESUMO

### Respostas fisiológicas e balanço hídrico do solo em *Eucalyptus* clonais sob espaçamentos de plantio e genótipos contrastantes

A densidade de plantio e o genótipo possuem estreita relação com as relações hídricas nas plantas. Sob um cenário de maior ocorrência de eventos climáticos extremos e do avanço dos plantios florestais de eucalipto para regiões de elevado déficit hídrico, elaboramos três perguntas-chave para esse estudo: (1) Qual a relação entre a densidade de plantio e o potencial estresse hídrico? (2) O espaçamento de plantio interfere na eficiência do uso da água (EUA), i.e., na quantidade de biomassa produzida pela quantidade de água transpirada? (3) Pode a densidade de plantio alterar o balanço hídrico do solo (BHS)? Para responder a essas questões, instalou-se um ensaio de campo em Mogi Guacu, SP, em fevereiro de 2012. Foram plantados quatro materiais genéticos (*Eucalyptus grandis* x *E.urophylla*1 {Urograndis1}, *Eucalyptus grandis* x *E.urophylla*2, *E.urophylla* e *E.grandis* x *E.camaldulensis* {Grancam}) com distintos níveis de tolerância à seca e quatro espaçamentos de plantio (3,4, 7,0, 10,5 e 16,9 m<sup>2</sup> planta<sup>-1</sup>, que correspondem às densidades de 2.949, 1.424 e 1.028 e 591 plantas ha<sup>-1</sup>). No capítulo 1, avaliou-se o potencial hídrico foliar (representando o potencial estresse hídrico) dos quatro clones durante 1 ano, entre 1,5 e 2,5 anos. Independentemente do material genético, quanto maior a produtividade madeireira atingida, maior o potencial hídrico foliar. Os plantios mais adensados (2.949 plantas ha<sup>-1</sup>) geraram povoamentos 39% mais produtivos, no entanto, o potencial estresse hídrico chegou a atingir 33% acima do plantio menos adensado (591 plantas ha<sup>-1</sup>). No segundo capítulo, durante o mesmo período, avaliou-se a eficiência do uso da água, que não variou conforme a mudança de densidade de plantio, mas apresentou diferença entre os genótipos, com EUA de 2,3, 2,2 e 1,5 g L<sup>-1</sup> para os clones Urograndis, Urophylla e Gramcam, respectivamente, na densidade de 1.424 plantas ha<sup>-1</sup>. Por fim, o terceiro capítulo avaliou durante dois anos, entre 1,7 e 3,7 anos, a transpiração (T), evaporação do solo (Es) e interceptação de água pela copa (Ei), que somados compunham e evapotranspiração (ET). A subtração da precipitação (P) da ET resultou no balanço hídrico do solo (BHS). O BHS foi positivo ou próximo de zero para os dois clones avaliados (Urograndis1 e Gramcam) quando a densidade de plantio foi inferior ou igual a 1.028 árvores ha<sup>-1</sup>. No plantio mais adensado, o balanço foi de -25%. Estes estudos demonstram que: a maior produtividade madeireira acarreta em maior potencial estresse hídrico, gerando um claro dilema entre a produção e a sobrevivência dos plantios. No entanto, o estudo detalhado dos materiais genéticos releva que há possibilidades de incremento na eficiência do uso da água sem que ocorra o aumento no uso da água, trazendo um maior compartilhamento da água na escala da microbacia. Por fim, plantios acima de 1.028 árvores ha<sup>-1</sup> resultaram em um balanço hídrico do solo negativo médio de -25% no pico do crescimento. Em conjunto, o presente trabalho releva que o espaçamento associado a materiais genéticos específicos, podem servir como ferramenta na busca pelo equilíbrio entre a produção madeireira e a conservação de recursos naturais.

Palavras-chave: Densidade de plantio; Florestas plantadas; Potencial hídrico foliar; Evaporação do solo; Interceptação da copa; Transpiração; Uso de água; Eficiência de uso da água



## ABSTRACT

### Physiological responses and soil water balance of clonal *Eucalyptus* under contrasting spacings and genotypes

Planting density and genotype have close relationship with the water relations in plants. The scenario of increased occurrence of extreme weather events and the change of *Eucalyptus* forest plantations to high water deficit regions, led us to the three key questions of this study: (1) What is the relationship between planting density and the potential water stress? (2) The planting spacing interferes the water use efficiency (WUE), i.e. the amount of biomass produced by the amount of water transpired? (3) Can the planting density change the soil water balance (BHS)? To develop this work, we settled a field trial in Mogi Guacu, SP, in February 2012. We planted four genetic material (*Eucalyptus grandis* x *E.urophylla*1 {Urograndis1}, *Eucalyptus grandis* x *E.urophylla*2, *E.urophylla* and *E.grandis* x *E.camaldulensis* {Grancam}) with different levels of tolerance to drought and four planting spacings (3.4, 7.0, 10.5 and 16.9 m<sup>2</sup> plant<sup>-1</sup>, which correspond to densities of 2,949 , 1,424 and 1,028 and 591 plants ha<sup>-1</sup>). In Chapter 1, we evaluated the leaf water potential (representing potential water stress) of the four clones for 1 year, between 1.5 and 2.5 years. Regardless of the genetic material, the higher the wood productivity, the greater the leaf water potential. The denser planting (2,949 plants ha<sup>-1</sup>) stands generated 39% more wood, however, water stress potential reached up to 33% higher than the least dense planting (591 plants ha<sup>-1</sup>). In the second chapter, during the same period, we evaluated the efficiency of water use, which did not vary according to the change of planting density, but showed differences between genotypes with US 2.3, 2.2 and 1, 5 g L<sup>-1</sup> to Urograndis, Urophylla and Gramcam, respectively, at a density of 1,424 plants ha<sup>-1</sup>. Finally, the third chapter evaluated for two years, between 1.7 and 3.7 years, transpiration (T), soil evaporation (Es) and canopy interception (Ei), which together made up evapotranspiration (ET). Subtraction of precipitation (P) per ET resulted in soil water balance (SWB). The SWB was positive or near zero for the two clones evaluated (Urograndis1 and Gramcam) when planting density was less than or equal to 1,028 trees ha<sup>-1</sup>. In the denser planting, the balance was -25%. These studies show that: a higher wood growth results in a higher potential drought stress, generating a clear trade-off between production and survival of trees. However, the detailed study of genetic materials fall under that there are increasing opportunities in water use efficiency, though without the increase in water use, bringing a greater share of water in the watershed scale. Finally, plantations above 1,028 ha<sup>-1</sup> trees resulted in a negative soil water balance of -25% at the peak of growth. Together, this study reveals that spacing associated with genotypes can serve as tools in the search for balance between timber production and conservation of natural resources.

Keywords: Planting density; Plantation forests; Leaf water potential; Soil evaporation; Canopy interception; Transpiration; Water use; Water use efficiency



## 1 INTRODUCTION

Global demand for wood products is expected to triple by 2050. With the continuous reduction of natural forests, the main source of supply of these resources, and the growing pressure for the conservation of these ecosystems, planted forests start to exercise utmost importance and can supply up to 50% the entire global wood demand by 2050 (World Wildlife Fund, 2012).

In Brazil, planted forests, especially those implanted with *Eucalyptus*, have significant numbers, such as 35% of supply of the country's current demand for wood comprising the same only 1% of the national territory coverage and the contribution of 4% of gross domestic product (GDP) and 20% in exports (Associação Brasileira de Produtores de Florestas Plantadas, 2014).

The main aspect of *Eucalyptus* competitiveness in the country is its high productivity, with an average of  $42 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$ , which can be considered the highest global average (ABRAF, 2014). This is due to the adaptation of the genre to climatic conditions, the significant advance in the genetic improvement of the main species planted in the country and improvement of silvicultural practices (GONÇALVES et al., 2013).

Among the main silvicultural practices, the choice of planting spacing is one of the critical steps in the planning process and the formation of a forest stand. The decision of the proper spacing for an area is related to several factors, including the amount of biomass to be produced, the cycle, the management system, the design of machines, labor and inputs, the susceptibility of plants to water deficit and the use and final value of the timber (NYLAND, 2002).

The first report on a *Eucalyptus* tree in spacing trial in Brazil is 1961, published by Navarro de Andrade, considered the father of the culture in the country (LEO, 2000). Since then, it has been studied intensively around the world. The focus has been given to the impact of spacing on aspects related to timber production (BALLONI; SIMÕES 1980; SCHONAU; COETZEE, 1989; BERNARDO et al., 1998; NEILSEN; GERRAND, 1999), the assortment and development branches (ALCORN et al., 2007, FORRESTER et al., 2013), nutrition (HARRISON et al., 2000) and the light use efficiency (SILVA, 2006; STAPE; BINKLEY, 2010).

Some recent trends create new gaps in knowledge of plant spacing interaction with different genotypes, such as: i) the increased occurrence of extreme

weather events (ALLEN et al., 2010; BOOTH, 2013) exposing the crops to high level stress, especially to water stress; ii) the increased demand for crops in areas subject to high water stress (FAO, 2012), with emphasis on Brazil to the north and northeast; iii) the emergence of new pests and diseases, which related to water stress, may interact strongly in the survival of plants (GONCALVES et al., 2013).; iv) the need for adaptation of forestry practices that relieve the water consumption of forest plantations due to high pressure from society by incorporation of conservation practices (FERRAZ et al., 2013) and finally, v) implantation of genetic material highly productive, which can interfere with the genetic interaction x planting density (FORRESTER et al., 2013).

This scenario is directly related to water stress in which trees can be submitted. One way to alleviate the plant drought stress is through proper choice of spacing, either through initial density or by thinning. White et al. (2009) evaluated the effect of different densities in *Eucalyptus globulus* in Australia and concluded that the reduction from 1200 to 600 h<sup>-1</sup> plants by grinding did not change the yield and reduced mortality after 8 years of about 20% to 5%. Medham et al. (2011) showed the close relationship between planting density and water consumption. The authors evaluated three planting densities (300, 600 and 1200 stems per hectare) in *Eucalyptus globulus* planted in southwest Australia and its impact on the water content in the soil 2 to 8 years old. They concluded that the soil water deficit at the beginning of rotation (up to about four years) was more pronounced the higher the density.

For that seek a better understanding of the interaction genotype x spacing x water relations, it is crucial to the understanding of physiological variables such as transpiration, leaf area index, conductance canopy, leaf water potential and the use of efficiency water (ALBAUGH et al., 2013).

Faced with gaps of knowledge mentioned above, it defined the three chapters of this thesis with the general objective of understanding the response of *Eucalyptus* clonal plantation to manipulation of spacing and genetic on physiological traits and soil water balance. In the first chapter, we discuss the relationship of planting density and different genotypes to water stress, giving a physiological character to the discussion. The second chapter will address the efficiency of water use, a key issue for physiological ecology, but also of practical interest in the forest hydrology and forestry. Finally, the third chapter will deal with the forest hydrology

purest way, evaluating the effect of planting density on soil water balance. The chapters are:

- 1) Biomass production and potential water stress increase with planting density in four highly productive clonal *Eucalyptus* genotypes;
- 2) Water use efficiency used as strategy for forest adaptive management in high productivity *Eucalyptus* plantations;
- 3) How sustainable is the high-density short-rotation system in clonal *Eucalyptus* regarding soil water balance? A study case in Brazil.

With these three chapters we expect to partially fulfill the gaps of knowledge regarding planting density. On the other hand, we also hope they will open more research issues for future works.

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## 2 BIOMASS PRODUCTION AND POTENTIAL WATER STRESS INCREASE WITH PLANTING DENSITY IN FOUR HIGHLY PRODUCTIVE CLONAL EUCALYPTUS GENOTYPES

### Abstract

The choice of planting density and tree genotype are basic decisions when establishing a forest stand. Understanding the interaction between planting density and genotype, and their relationship with biomass production and potential water stress, is crucial as forest managers are faced with a changing climate. However, few studies have investigated this relationship, especially in areas with highly productive forests. This study aimed to determine the interaction between biomass production and leaf water potential, as a surrogate of potential water stress, in different clonal *Eucalyptus* genotypes across a range of planting densities. Four clones (two clones of *E. urophylla* x *E. grandis*, one clone of *E. urophylla*, and one clone of *E. grandis* x *E. camaldulensis*) and four planting densities (ranging from 591 to 2949 trees ha<sup>-1</sup>) were evaluated in an experimental stand in southeast Brazil. Biomass production was estimated 2.5 years after planting and predawn ( $\psi_{pd}$ ) and midday ( $\psi_{md}$ ) leaf water potential were measured 2 and 2.5 years after planting, in February (wet season) and August (dry season) 2014. For all clones, biomass production increased and leaf water potential decreased with planting density, and their interaction was significant. Thus, wood biomass at tighter spacings were higher but exhibited lower leaf water potentials, resulting in a trade-off between productivity and potential water stress. In light of the increased frequency of extreme climate events, silvicultural practices that are tailored to the potential productivity of each region and that result in low potential water stress should be considered.

Keywords: Leaf water potential; Brazil; Plantation; Wood productivity

### 2.1 Introduction

Global demand for wood products could triple by 2050 (World Wildlife Foundation, 2012). The reduction of natural forests, which are the main source of these resources, and the growing pressure for the conservation of these ecosystems underscore the importance of planted forests, which could supply up to 50% of the global demand for wood by 2050 (World Wildlife Foundation, 2012).

Planted forests, especially those established with trees of the *Eucalyptus* genus, comprise a relatively large share of the world economy. In Brazil, *Eucalyptus* plantations supply 35% of the current demand for wood, despite taking up only 1% of the land area, and contribute approximately 4% of gross domestic product (GDP) and 20% of exports (ABRAF, 2014).

The high productivity of eucalyptus is the main aspect associated with its competitiveness (STAPE et al., 2010). This high productivity is a result of the

adaptation of the genus to different climate conditions, significant advances in breeding technology, and improvement of silvicultural practices (GONÇALVES et al., 2013).

The choice of planting density is one of the critical steps in the process of planning and implementing plantation forest operations. The choice of planting density among trees is based on several factors, including the amount of biomass produced, rotation length, management system, the amount of machinery, labor, inputs, susceptibility of plants to water stress, and the use and final value of timber (NYLAND, 2002).

The first planting density experiment with eucalyptus in Brazil was conducted by Andrade (1961), who found that maximum profitability and wood growth were achieved at planting densities between 1000 and 1500 stems ha<sup>-1</sup>. Since then, several spacing trials have been planted around the world. These studies have focused primarily on the effects of planting density on timber production (BALLONI; SIMÕES, 1980; SCHONAU; COETZEE, 1989; BERNARDO et al. 1998; NEILSEN; GERRAND, 1999), branch size and development (ALCORN et al., 2007; FORRESTER et al., 2013), nutrition (HARRISON et al., 2000), and light-use efficiency (SILVA, 2006; STAPE; BINKLEY, 2010).

However, substantial gaps in knowledge of the interaction between plant genotypes and planting density remain as a result of recent trends such as: i) increased frequency of extreme climate events (ALLEN et al., 2010; BOOTH, 2013), exposing plantations to elevated stress levels, especially water stress; ii) increased demand for planted forests in areas under high water stress (FAO, 2013), particularly in northern and northeastern Brazil; iii) the emergence of new pests and diseases, which may interact strongly with water stress and affect plant survival (GONÇALVES et al., 2013); iv) the need for adaptation of silvicultural practices that reduce water use by planted forests in light of increased pressure for adoption of more sustainable practices (FERRAZ et al., 2013), and lastly, v) the use of highly productive genotypes, which may affect the genotype x planting density interaction (FORRESTER et al., 2013).

Experiments that investigate the interaction between genotypes, planting density, and plant water stress are crucial to gain insights into these knowledge gaps. This study aimed to determine the interaction between biomass production and leaf water potential (as a surrogate for potential water stress (SCHOLANDER et al.,

1965)) in different clonal *Eucalyptus* genotypes across a range of planting densities. We tested the hypothesis that individual trees growing on stands with higher biomass production, regardless of tree genotype and stocking, are subjected to lower leaf water potential, resulting in a trade-off between plant productivity and potential water stress.

## **2.2 Material and methods**

### **2.2.1 Site description**

The field experiment was set up on February 2014 in Mogi Guaçu (SP), state of São Paulo, Brazil (22°20'58" S and 46°58'16" W, 664 m asl). The experiment is part of the Clonal *Eucalyptus* Tolerance to Water and Thermal Stress network (TECHS – <http://www.ipef.br/techs/>), which is an international collaborative research project involving 42 institutions (companies, universities, and research centers) coordinated by the Brazilian Forestry Science and Research Institute (IPEF). The local climate is humid mesothermal (Cwa) according to the Köppen classification, with an average annual temperature of 22 °C and an average annual precipitation of 1200 mm, 81% of which is concentrated in the summer (October to March). Water deficit in the region is 50 mm according to the Thornthwaite and Matter water balance model, based on a 150 mm soil water storage capacity (DEMATTE, 2000). Precipitation for the 2.5 years of tree growth was 2643 mm, 18% below the historical average, whereas the accumulated water deficit for the period was 279 mm, 109% greater than the historical average.

The stand where the experiment was established has been cultivated with eucalyptus for over 50 years and at the end of the previous rotation (age 7) had a mean annual increment of 55 m<sup>3</sup> ha<sup>-1</sup> year<sup>-1</sup>, which is a high-productivity value for eucalyptus plantations on a worldwide level (GONÇALVES et al., 2013).

### **2.2.2 Experimental design**

We used a systematic design with continuous and sequential increases in tree spacing (Fig. 1). This design is effective to evaluate different genotypes across a wide range of planting densities in a small area (STAPE and BINKLEY, 2010). Row spacing was 3.0 m and tree spacing varied according to the desired planting density. In this study, we selected four planting densities with 14 trees per plot (eight trees per measurement plot) for a spacing of 3.4, 7.0, 9.7, and 16.9 m<sup>2</sup> plant<sup>-1</sup> (planting

densities of 2949, 1424, 1028, and 591 plants  $\text{ha}^{-1}$ ) (Fig. 1). The selected range corresponds to the main planting densities used in the log sawmilling industry (591 stems  $\text{ha}^{-1}$ ), planted forests in Brazil (1000–1500 stems  $\text{ha}^{-1}$ ), and for the use of wood biomass for energy production (2949 stems  $\text{ha}^{-1}$ ) (BALLONI; SIMÕES, 1980). Neighboring trees are not spatially independent, but we think they provide a fair estimate of tree physiology because any interaction between trees is competitive, leading to increased (rather than decreased) variation in physiology, and also encompassing realistic competitive interactions that happen in operational plantations.

Four clonal genotypes with different origins from different *Eucalyptus* species/hybrids were investigated (Table 1). Water deficit in the origin region were estimated by using Thornthwaite e Matter (1955) method. In short, we evaluated four clones x eight trees per clone x four planting densities, totaling 128 trees. The four clones occupied a total area of 7560  $\text{m}^2$ . The site is very uniform, but slight differences in soils might have some influence on tree physiology. However, the 4-fold range in spacing is likely a much stronger factor than any minor variation in soils.

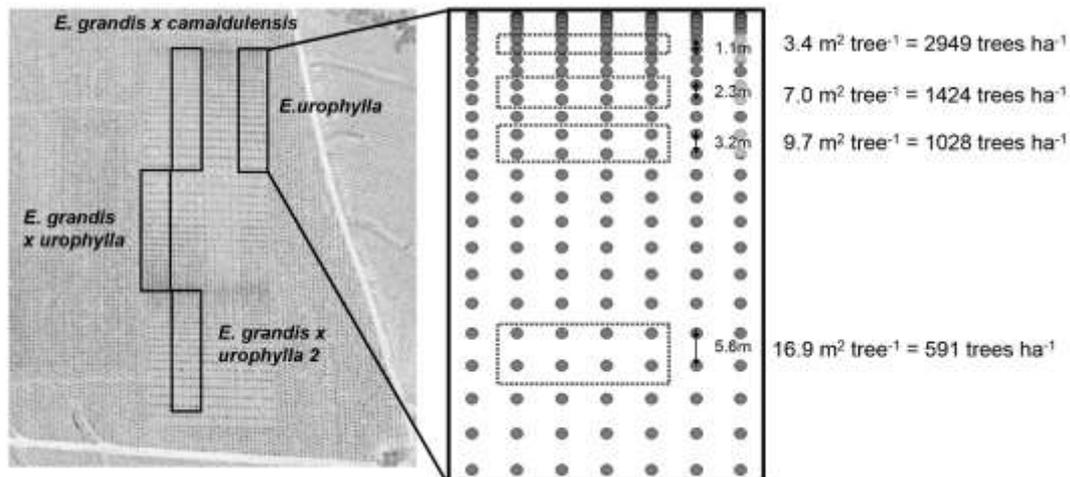


Figure 1 - Satellite image of the study site showing the distribution of the four *Eucalyptus* clones (left) and graphical representation of plant spacing and corresponding planting densities (right). Dotted rectangles represent planting densities of 591, 1028, 1424, and 2949 stems  $\text{ha}^{-1}$ . Google Earth<sup>®</sup> image 12 months after planting

Table 1 - Species, clone, site of origin, climate characteristics for the sites, and water deficit for the four *Eucalyptus* clones evaluated in the study

Species	Clone	Origin (state)	Mean annual temperature (°C)	Mean annual rainfall (mm)*	Mean annual water deficit (mm)*
<i>E. urophylla</i> x <i>E. grandis</i>	Urograndis	Minas Gerais	22	1200	0
<i>E. urophylla</i>	Urophylla	São Paulo	21	1300	59
<i>E. urophylla</i> x <i>E. grandis</i> 2	Urograndis 2	Minas Gerais	20	1400	70
<i>E. grandis</i> x <i>E. camaldulensis</i>	Grancam	Bahia	24	1450	165

\*Source: Sentelhas et al. (1999)

Soil preparation consisted of subsoiling to 60 cm depth every 3 m of row spacing. Rates of fertilization were tailored to remove nutritional limitations (GONÇALVES et al., 2013). Fertilizer applications consisted of 70, 110, and 160 kg ha<sup>-1</sup> of N, P, and K, respectively, applied at the planting date (all P, 33% of N and K) and six and 12 months after planting (33% of N and K in each operation). The study plots were kept completely free of pests and diseases, and weed competition was suppressed since planting. Survival rates were 100% at all planting densities for both experimental and buffer trees and so do not affect the regression analysis (ODA et al., 2008).

### 2.2.3 Tree growth measurements

Growth rates were determined in August 2014, 2.5 years after planting by measuring tree height (H) and diameter at breast height (DBH, 1.3 m above the soil surface). Tree height and circumference at breast height (CBH) were measured using an electronic hypsometer and a tape measure, respectively. CBH was later converted into DBH. Tree volume was estimated using DBH and H data according to the model of Schumacher and Hall (1933) and converted into stem wood biomass based on the basic density of each *Eucalyptus* clone (unpublished data). Next, we used the

individual tree as the unit of observation. So, each one of the eight tree-level biomass values were extrapolated to total biomass per hectare.

#### **2.2.4 Measurements of leaf water potential**

Leaf water potential ( $\Psi$ ) measurements were conducted 2 and 2.5 years after planting in February and August 2014, representing the wet and dry seasons, respectively. Predawn  $\Psi$  ( $\Psi_{pd}$ ) and midday  $\Psi$  ( $\Psi_{md}$ ) measurements were taken between 03:00 and 06:00 h and 11:00 and 14:00 h, respectively, using a PMS1003 (PMS Instrument Co., Corvallis, Oregon, USA) Scholander-type pressure chamber (SCHOLANDER et al., 1965). Predawn measurements were used as a proxy for soil water potential and therefore soil water availability. Leaves from the middle third of the canopy, chosen for  $\Psi_{pd}$  and  $\Psi_{md}$  measurements, were fully expanded and healthy. We collected two leaves per tree in four trees per planting density, totaling eight leaves per planting density in the four clones evaluated (total per sample: 2 leaves x 4 trees x 4 planting densities x 4 clones = 128 leaves). The time between leaf excision and chamber pressurization was < 30 s.

#### **2.2.5 Statistical analysis**

Biomass and leaf water potential data were tested using analysis of variance (ANOVA). The unit of observation was the individual tree for this evaluation of tree physiology response to varying planting density. As a conservative measure, we also analyzed the effect of planting density by pooling all eight trees at each spacing into a single average, giving four observations for testing the effect of planting density. The average trend would be the same in this analysis, but the reduction in degrees of freedom gives a far more conservative test of the effects of planting density than in our basic design that used each tree as a unit of observation.

A logarithm regression was used to determine the correlation between total biomass 2.5 years after planting (dependent variable) and leaf water potential (independent variable). All analyses were performed using SAS/STAT<sup>®</sup> 9.3 software (SAS INSTITUTE, 2011).

## 2.3 Results

### 2.3.1 Biomass production

Individual tree biomass decreased with planting density in the four *Eucalyptus* clones evaluated. Mean individual biomass was 52 kg tree<sup>-1</sup> (range: 47–57 kg tree<sup>-1</sup>) at a planting density of 591 trees ha<sup>-1</sup> and 17 kg tree<sup>-1</sup> (range: 13–24 kg tree<sup>-1</sup>) at 2949 trees ha<sup>-1</sup> (Fig. 2a). Conversely, total stand biomass (kg ha<sup>-1</sup>) increased with planting density for each clone. Biomass accumulation at the highest stocking was 51 t ha<sup>-1</sup> 2.5 years after planting, 39% higher than at the lowest stocking (31 t ha<sup>-1</sup>) (Fig. 2b).

The response of total biomass to planting density differed among the clones. A 127% increase in total biomass was observed in the Urograndis clone from the lowest to the highest planting density, and increases of 52 and 49% were observed in the Urophylla and Urograndis2 clones, respectively. The Grancam clone showed the weakest response to planting density, with only a 34% increase in total biomass between the 591 and 2949 trees ha<sup>-1</sup> densities.

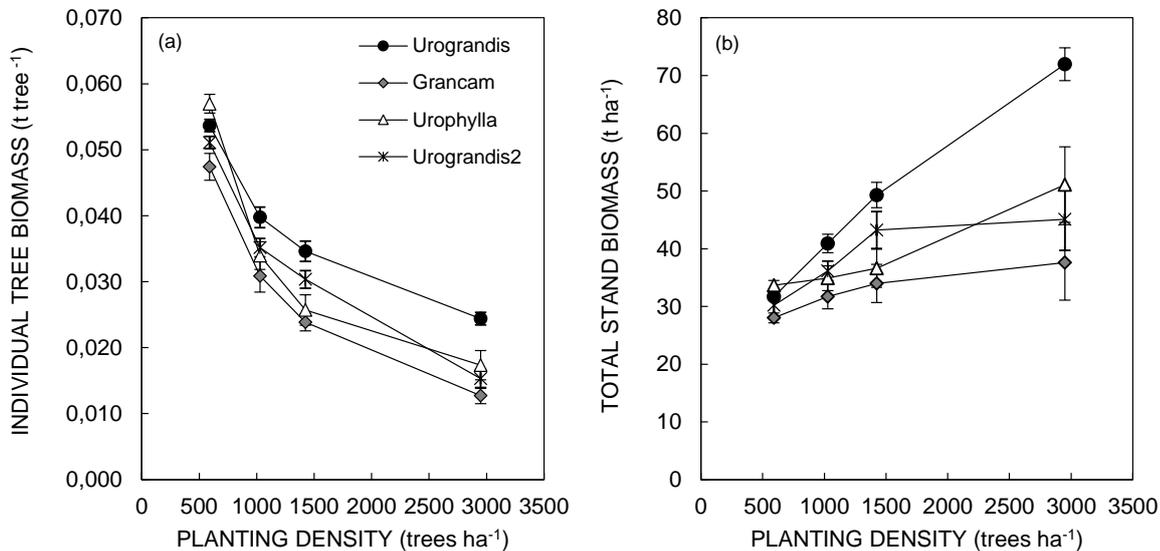


Figure 2 - Relationship between (a) individual and (b) total biomass per clone and planting density (trees ha<sup>-1</sup>). Bars represent the standard error among trees (n = 4). All clones had the logarithm regression adjusted between planting density and individual tree ( $p < 0.01$ ,  $r^2 > 0.79$ ) and total stand biomass ( $p < 0.01$ ,  $r^2 > 0.22$ )

### 2.3.2 Leaf water potential in relation with planting density and genotypes

Leaf water potential was measured in the wet (February) and dry (August) seasons. Wet season predawn leaf water potential ( $\psi_{pd}$ ) was not significantly affected by planting density and did not vary across clones (mean = -0.25 MPa, range: -0.21 – -0.31 MPa; Fig. 3a). However, all clones showed a trend towards  $\psi_{md}$  reduction as planting density decreases (Fig. 3b). Mean leaf  $\psi_{md}$  was -1.7 MPa at a planting density of 2949 trees ha<sup>-1</sup> and -1.2 MPa at 591 trees ha<sup>-1</sup>. Additionally, mean leaf  $\psi_{md}$  differed significantly across clones; the Grancam clone had the lowest mean  $\psi_{md}$  (-2.1 MPa), followed by Urograndis2, Urophylla and Urograndis clones (-1.6, -1.1 and -0.9 MPa, respectively).

Dry season predawn and midday leaf  $\psi$  were negatively affected by planting density and varied across clones. Mean  $\psi_{pd}$  for all clones was -1.7 MPa at the highest planting density, 28% lower than at the lowest planting density (-1.2 MPa) (Fig. 3c), whereas mean  $\psi_{md}$  was -3.2 MPa at the highest planting density, 33% lower than at the lowest planting density (-2.2 MPa) (Fig. 3d). The Urograndis clone had the lowest dry season leaf  $\psi$ , followed by the Urophylla, Urograndis2 and Grancam clones.

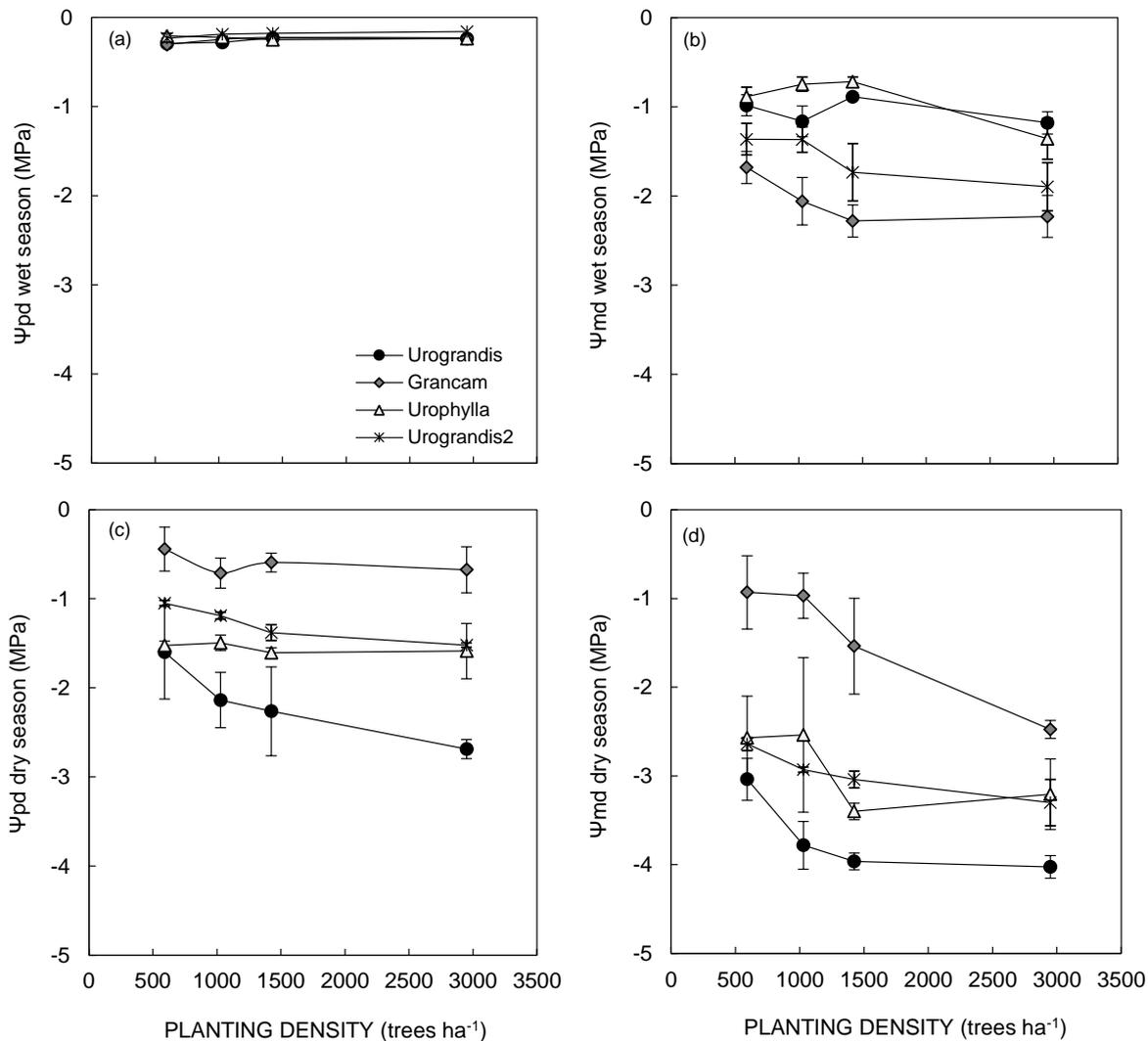


Figure 3 - Relationship between wet (February) and dry (August) season predawn ( $\psi_{pd}$ ) (a and c) and midday ( $\psi_{md}$ ) (b and d) leaf water potential and planting density (trees  $ha^{-1}$ ). Bars represent the standard error among trees ( $n = 8$ ). With the exception of  $\psi_{pd}$  in wet season (non-significant), all clones had the logarithm regression adjusted between planting density and  $\psi_{md}$  in wet season ( $P < 0.05$ ,  $r^2 > 0.15$ ),  $\psi_{pd}$  in dry season ( $P < 0.01$ ,  $r^2 > 0.51$ ) and  $\psi_{md}$  in dry season ( $P < 0.01$ ,  $r^2 > 0.33$ ).

### 2.3.3 Relationship between growth and leaf water potential

Dry season predawn ( $\psi_{pd}$ ) and midday ( $\psi_{md}$ ) leaf water potential correlated negatively with total biomass production 2.5 years after planting ( $R^2 = 0.58$ ,  $p < 0.001$ ; Fig. 4). This result was independent of genetic material, i.e., the higher the biomass in each treatment, the lower the leaf water potential.

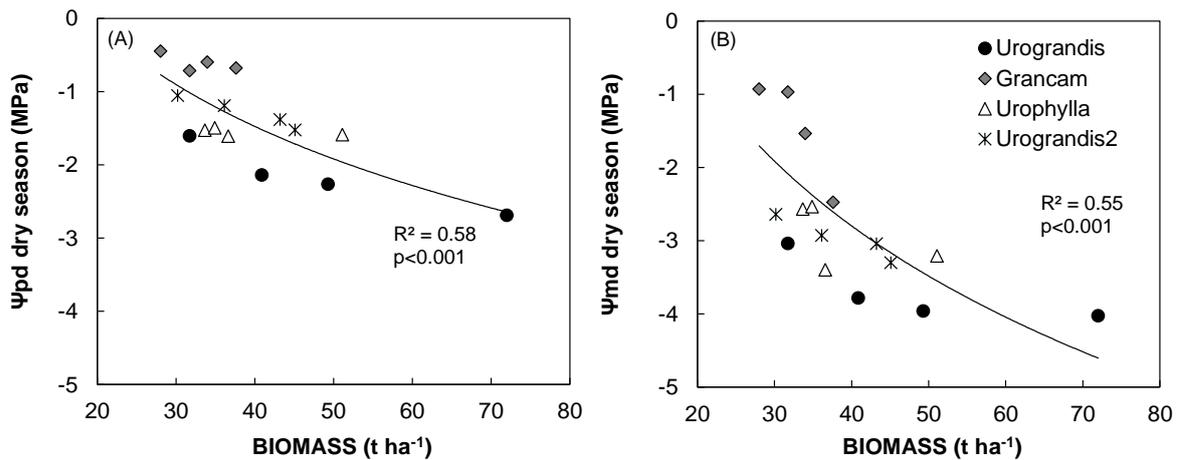


Figure 4 - Relation between dry season (August) predawn ( $\psi_{pd}$ ) (a) and midday ( $\psi_{md}$ ) (b) leaf water potential with accumulated biomass ( $t\ ha^{-1}$ ) 2.5 years after planting

## 2.4 Discussion

In our study, we showed that biomass production and leaf water potential were affected by planting density and tree genotype: total biomass increased with planting density regardless of genotype. This result follows the typical pattern of spacing experiments with various genera such as *Eucalyptus* (STAPE; BINKLEY 2010; FORRESTER et al., 2013), *Pinus* (BLEVINS et al., 2005), and *Populus* (TOILLON et al., 2013). However, in our study, biomass production rates varied among genotypes. Clones of species with high leaf area index (LAI), i.e., *E. urophylla* (XAVIER et al., 2002), showed a greater increase in productivity with increasing planting density. Conversely, the Grancam clone, which is a hybrid of *E. camaldulensis* (JAMES; BELL, 1995) and has a low LAI, showed a small increase in productivity with increasing plant density.

Clones with the greatest increase in biomass also had the lowest leaf  $\Psi_{pd}$  and  $\Psi_{md}$ . Interestingly,  $\Psi$  values followed the water deficit of the region the clones were selected under field conditions (Table 1). The hybrid of *E. camaldulensis* and *E. grandis*, which was selected in a high water deficit region, had the highest  $\Psi_{pd}$  and  $\Psi_{md}$  readings. Conversely, Urograndis, which was selected in a null water deficit area, had the lowest  $\Psi$  value in the dry season. The lowest  $\psi_{md}$  observed in an individual measurement was -4.2 MPa for a Urograndis sample, which is an extremely low value for *Eucalyptus* (WHITEHEAD; BEADLE, 2004). According to

Arndt et al. (2014), rainfall at the site of origin of the different *Eucalyptus* species has a direct effect on traits related to water stress tolerance such as turgor loss point. Thus, the higher the rainfall at the site of origin, the lower the drought tolerance. Because water supply is a key resource determining levels of plantation productivity (STAPE et al., 2010), the study of Arndt et al. (2014) is consistent with the findings of our study, indicating that high-yielding genotypes are likely to deplete water resources faster than low-yielding genotypes, and therefore are more likely to suffer from drought.

Kallarackal and Somen (1997) investigated the effect of planting density on leaf water potential in *Eucalyptus tereticornis* and found that leaf  $\Psi_{pd}$  was twice as low at a stocking of  $\approx 1000$  trees  $ha^{-1}$  than at 1800 trees  $ha^{-1}$ . Other studies regarding different species also detected a reduction in leaf  $\Psi$  with increasing stocking, like in *Eucalyptus globulus* (WHITE et al., 2009; DONOSO; RUIZ, 2001), Douglas-fir (AUSSENAC; GRANIER, 1988), *Pinus contorta* (DONNER; RUNNING, 1986) and *Quercus petraea* (BREDA et al., 1995). However, in these previous studies, productivity was not greater than 30  $m^3 ha^{-1} year^{-1}$ . To our knowledge, this is the first study on the relationship between leaf water potential and timber production in high-productivity eucalyptus stands. Our results show that highly productive stands are associated with an increased risk of water stress, especially in light of the current scenario of extreme climate events. Thus, we propose that forest managers should not follow silvicultural practices (genotypes + planting density) aimed at achieving maximum productivity in areas prone to drought. Ideally, silvicultural practices should be tailored to the potential productivity of each region and tree spacing optimized to achieve timber production goals while minimizing potential water stress.

In order to maximize carbon gain in the face of the near constant trade-off between photosynthesis and water loss via transpiration, many woody plant species regulate stomatal conductance to maintain leaf and plant water potential near the point of catastrophic xylem failure (SPERRY; TYREE, 1988). Consequently, although leaf water potential can be a good indicator of plant water status (SCHOLANDER, 1965) low leaf water potential is not necessarily a precursor to tree mortality (MCDOWELL et al., 2008).

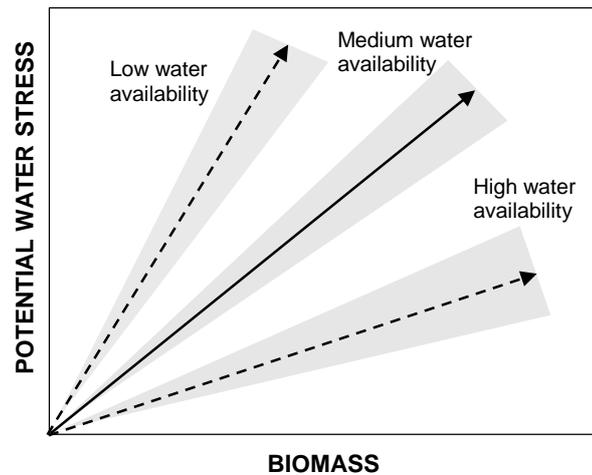


Figure 5 - Diagram of possible patterns of potential water stress response to biomass increase in environments with low, medium (current study condition) and high water availability. Grey buffers represent weather variation in a determined region

An experiment at one site over the course of a single year does not provide statistically based insights into other years and locations. However, the patterns of growth and leaf water potential in relation to planting density were so clear that we expect trees in other years and locations would show similar trends.

Based on these results, we propose a diagram of possible patterns of potential water stress in response to biomass increase with changes in water availability (Figure 5). The current study is located in a medium water deficit region (ALVARES et al., 2013). However, we would expect that at lower water availability regions potential water stress increment will be higher with biomass productivity increase, and the opposite would be true in areas with higher water availability. All curves are subject to natural variation, but then broad pattern should be considered in risk analysis for management decisions. Further studies aimed at identifying specific drought tolerance mechanisms and strategies of *Eucalyptus* clones and genotypes carried out at different water availability regions are needed so that silvicultural planning can be optimized for specific conditions in face of more frequent and prolonged drought periods.

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### 3 TRANSPIRATION EFFICIENCY USED AS STRATEGY FOR FOREST ADAPTIVE MANAGEMENT IN HIGH PRODUCTIVITY *Eucalyptus* PLANTATIONS

#### Abstract

Adaptive forest management objectives to adjust the structure and the consequent functioning of the forest ecosystem to resist harmful impacts of climate change. Improvement of transpiration efficiency for stem biomass production, i.e., the amount of wood biomass produced per amount of water transpired, has been related as one of the main goals in breeding programs and silvicultural management to tackle climatic issues. Several studies have examined these effects on water relations in genetically diverse plantations grown from seed. However, to our knowledge, no study has investigated the effects of planting spacing on transpiration (T) and transpiration efficiency (TE) in high productivity genetically identical clonal *Eucalyptus* plantations. We studied three widely planted clonal *Eucalyptus* genotypes that differ in drought tolerance and productivity (*E. urophylla*, *E. grandis* × *E. urophylla* and *E. grandis* × *E. camaldulensis*) and four individual planting spacing (3.4, 7.0, 9.7 and 16.9 m<sup>2</sup> tree<sup>-1</sup>) at a site in southeastern Brazil. Over a 1-year study period (1.5–2.5 years after planting), individual biomass increment increased significantly with decreasing planting density, ranging from 12 to 18 t ha<sup>-1</sup> at spacing of 591 and 2949 ha<sup>-1</sup>, respectively. Further, sapflow density measurements indicated that transpiration decreased with planting spacing. Mean transpiration was 29% lower at the highest planting spacing (622 mm yr<sup>-1</sup>) than in the lower spacing treatment (879 mm). The increase in transpiration was partially explained by the increase in leaf area index that accompanied increased tree density. Water use efficiency, however, was unaffected by planting density but did vary between *Eucalyptus* clones. In average of all spacings, the most efficient clone was Urograndis, followed by Urophylla and Grancam (2.3, 2.2, and 1.5 g biomass L<sup>-1</sup> transpired H<sub>2</sub>O, respectively). We propose that for an adaptive plantation management point of view, researchers and managers should not only choose a silvicultural treatment with higher biomass and WUE, but the ones which prioritize increased biomass production and water use efficiency while minimizing or maintaining water use. These are preliminary findings and still need to be supported by more experimental evidence and repetitions.

Keywords: Transpiration; Water use efficiency; Planting density; Stocking; Clonal plantations

#### 3.1 Introduction

Plantations of fast-growing tree species with locally adapted genotypes and site-specific silvicultural management will play a key role in the global wood supply in the coming decades, mainly due to their high productivity. In Brazil, *Eucalyptus* forests have an average productivity of 41 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> (ABRAF, 2013), which is 10 times greater than the growth of the cerrado biome, the original vegetation of most

plantations of that genus in Brazil (OLIVEIRA et al., 2002; SCOLFORO et al., 2000).

Among the factors that control productivity, water is the main growth-limiting factor (RYAN et al., 2010; STAPE et al., 2010). Many studies have focused on the influence of superior genotypes and silvicultural practices on increased productivity and water use efficiency, measured by the amount of biomass produced by the amount of water transpired (MYERS et al., 1996; LI, 2000; ALBAUGH et al., 2013; OTTO et al., 2014).

Practices that increase transpiration efficiency usually result in increased productivity (HUBBARD et al., 2010; BATTIE-LACLAU et al., 2016). This is due to the higher level of plant hydration, which results in greater leaf area index at stand scale, stomatal conductance, gas exchange, cell division, and consequently, timber production (WHITEHEAD; BEADLE, 2004).

From a physiological point of view, increased water use efficiency implies improved conversion of solar energy, carbon, nutrients and water into biomass (LARCHER, 2003). However, when we consider hydrological issues on a local scale, increased water use efficiency that leads to an increase in water consumption may not be beneficial to the different parties involved in a watershed (FALKENMARK; ROCKSTROM, 2006).

Currently, there are two main issues that must be investigated when identifying ways of increasing productivity and water use efficiency without significantly increase in water consumption: i) extreme weather events, which have prolonged the dry season in some regions (BOOTH, 2013) and ii) practices that reduce the water consumption of forest plantations without causing losses in timber production (VANCLAY, 2009; FERRAZ et al., 2013). The combination of these factors can cause drought stress of the population due to water shortages (WHITE et al., 2009, HAKAMADA et al., in press) and/or reduce the flow of the stream due to the plants' high evapotranspiration demand (BOSCH; HEWLETT, 1982).

The aim of this study was to evaluate the effect of planting density of three clonal *Eucalyptus* genotypes on biomass increment and water use efficiency. To this end, we tested the hypothesis that water use efficiency will not be affected by planting density. Increment in leaf area index because of higher planting density will lead to largest increase in water use and a proportional increase in timber

production. However, different genotypes will have different levels of transpiration and water use efficiency.

From a practical point of view, we hope this work can identify interactions between genotypes and plant densities that may result in a reduction of water use without altering timber production. Finally, we propose a model that takes into account water shortages and local environments and warns of the need to concomitantly evaluate timber production, water use efficiency and total water use.

## 3.2 Materials and Methods

### 3.2.1 Site description

The field experiment was planted in February 2012 in the city of Mogi Guacu (22°20'58"S, 46°58'16"W) in the northeast region of the state of São Paulo, Brazil. The site is part of the TECHS experimental network (Tolerance of Clonal *Eucalyptus* to Hydric and Thermal Stress; <http://www.ipef.br/techs>).

The local climate is humid mesothermal (Cwa) according to the Koeppen classification. Elevation at the site is 664 m, average mean annual temperature is 22°C, and average mean annual precipitation is 1200 mm, with 81% of rain falling in the summer (October to March). The region has an average water deficit of 50 mm according to the Thornthwaite-Mather water balance model, based on a 150-mm water storage capacity (DEMATTÊ, 2000). The soil in the experimental area is homogeneous and is an Oxisol, with a depth greater than 5 m (DEMATTÊ, 2000) and 38% clay in the 0–30 cm layer. The area has been planted with *Eucalyptus* for about 50 years, and previous 7-year rotation harvested in July 2011 had an average productivity of 55 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup>.

Data was collected between 1.5-2.5 years after planting, during which the mean temperature was 22°C, rainfall was 883 mm (26% below average), with a water deficit of 224 mm. From the time of planting to the end of our study (2.5-year) total rainfall was 2643 mm, 18% below the historical average (Fig. 1). The cumulative water deficit for this period was 279 mm, 109% higher than the historical average.

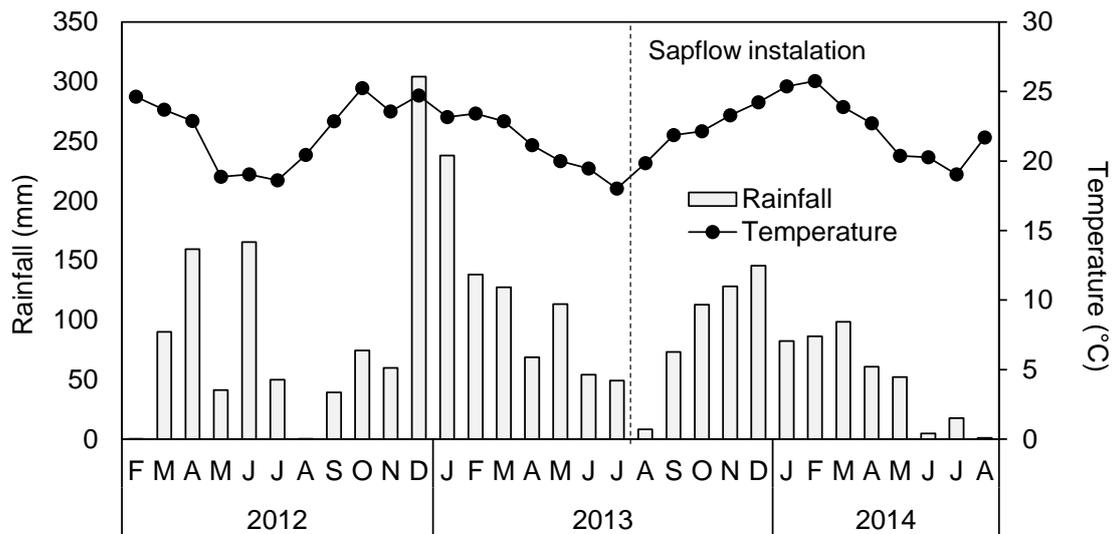


Figure 1 - Rainfall (mm) (bars) and average temperature (°C) (lines) of the *Eucalyptus* plantation site during the 2.5 years of tree growth. The vertical dotted line indicates the beginning of sapflow measurements

### 3.2.2 Experimental design

We examined three clonal genotypes differing in drought tolerance and that are widely planted in Brazil: *E. grandis* × *urophylla* (Urograndis), which has been reported to have low drought tolerance; *E. urophylla* (Urophylla), which is a moderately drought tolerant species; and *E. grandis* × *E. camaldulensis* (Grancam), which is commonly planted in dry areas (ELDRIDGE et al., 1993; GONÇALVES et al., 2013).

We used a systematic plot design similar to Nelder's proposal (NELDER, 1962) in which tree spacing increased sequentially (Fig. 2). The row spacing was 3.0 m, the spacing between plants varied depending on the chosen planting density. In the present study, we selected four planting densities, with spacings of 1.1, 2.3, 3.2, and 5.6 m between plants, representing planting densities of 2949, 1424, 1028 and 591 plants ha<sup>-1</sup>, respectively (Fig. 2). The selected range corresponds to the most commonly used planting densities for *Eucalyptus* plantations in Brazil as identified by Gonçalves et al. (2013). Timber production for saw timber typically employs lower densities (<600 plants ha<sup>-1</sup>). The most planted densities varies from 1000 to 1500 plants ha<sup>-1</sup>, while trees for biomass production are usually planted more densely (>2000 plants ha<sup>-1</sup>).

The unit of observation was one tree, and eight trees were evaluated for each clone  $\times$  density treatment combination, making a total of 96 trees evaluated (3 clones  $\times$  8 trees  $\times$  4 planting densities). One plot was planted per clone and the area occupied by the three genotypes was 5670 m<sup>2</sup>.

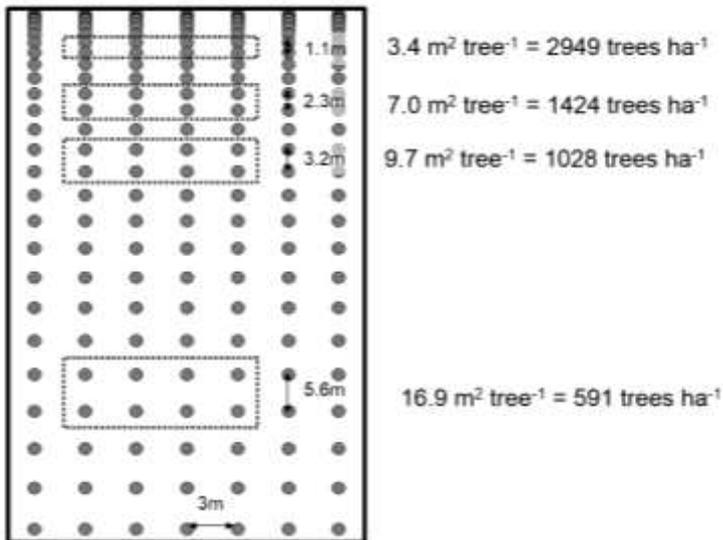


Figure 2 - Schematic sketch of one clone density plot with seven rows and 27 plants per row. In each spacing evaluated (3.4, 7.0, 9.7 and 16.9 m<sup>2</sup> tree<sup>-1</sup>), we measured eight trees

The soil was prepared using a minimum cultivation system (GONÇALVES et al., 2013), with 60-cm deep subsoiling every three meters along the rows. Nutritional limitations were eliminated by supplementing with 70, 110, 160, and 430 kg of N, P, K, and Ca per hectare, respectively. Micronutrients were applied as fritted trace elements. The area was kept completely free of pests, diseases (visually), and weed competition from the time the seedlings were planted. Ants were treated with ant bait made of 0.3% sulfluramide. Weeds were completely controlled with glyphosate herbicide at a dose of 2.88 kg a.i. ha<sup>-1</sup>. We guaranteed survival of 100% of the plantation by replanting less than 1% of the seedlings up to 30 days after the initial planting, in order to avoid artifacts in the regression analyses (ODA et al., 2008).

### 3.2.3 Measurements

#### 3.2.3.1 Growth rate

We quantified stem growth rate with quarterly measurements of height and diameter at breast height (DBH) measured 1.3 m above ground level. We measured eight trees per planting density per clone. Based on the DBH and total height values, we estimated the individual volume of each tree using the Schumacher and Hall (1933) model. Individual biomass was calculated by multiplying the volume by the basic density obtained in an adjacent site planted with an identical design to allow destructive samplings in eight trees per clone (unpublished data). The tree-level biomass estimates ( $\text{t tree}^{-1}$ ) were used to estimate total stand biomass per hectare ( $\text{t ha}^{-1}$ ).

#### 3.2.3.2 Transpiration and water use efficiency

Transpiration was measured using 2-cm thermal dissipation sapflow probes (GRANIER, 1987). Sapflow density was calculated using the equation for *Eucalyptus* (eq. (3)) in Hubbard et al. (2010) for our measurement trees. Sapwood area for our measurement trees was estimated using allometric equations based on diameter at breast height. 18 trees were harvested from the adjacent site with identical planting densities. Thin (1–1.5 cm) disks were cut at breast height and conducting sapwood area was estimated visually by measuring four equally opposed radii of the obvious translucent portion of the disk and calculating area as an ellipse. Sapwood area in relation to DBH was estimated by power models for each clone ( $\text{sapwood} = a + b^{\text{DBH}}$ ,  $R^2 > 0.78$ ). Individual transpiration ( $\text{L tree}^{-1}$ ) was estimated as the product of sapflow density and sapwood area. Data were collected every 15 seconds, and the average was recorded every 15 minutes via a multiplexer coupled to a data logger (CR1000 and AM 16/32, Campbell Scientific, Inc., Logan, UT). To minimize the interference of direct radiation, we positioned probes at each cardinal position (north, south, east, and west), and the sensors were moved 90° clockwise every three months. In this way, differences in flow caused by cardinal positioning were accounted for over the time span of the experiment (GRIME; SINCLAIR, 1999). Styrofoam and foil backed insulation were used to minimize thermal gradients, and plastic bags were placed around the insulation to protect probes against moisture and stemflow. Total stand transpiration ( $\text{mm ha}^{-1}$ ) was estimated from the eight individual transpiration values

obtained per plot. Seasonal water use efficiency (dry period from April to September - 20% of yearly rainfall - and wet period from October to March – 80% of yearly rainfall) (WUE, g biomass L<sup>-1</sup> transpired H<sub>2</sub>O) was calculated as the ratio between the increases in wood biomass and transpiration.

### **3.2.3.3 Leaf area index**

Using the LP-80 ceptometer (Decagon Devices Inc., Pullman, WA, USA), we estimated the interception of photosynthetically active radiation (PAR) for each density treatment by trees 1.5, 1.8, 2.1, and 2.5 years old. We conducted two transects per spacing, with 16 measurement locations per transect. For each plot, we also took a measurement outside the canopy because its value was changing during the measurement period. Measurements were taken from 11:30 to 13:00 on cloudless days with no wind interference that could cause the movement of canopies, thereby minimizing estimation errors (BREDA, 2003). PAR readings inside and outside the canopy were averaged, and Beer's law was used to estimate LAI, assuming a coefficient of light extinction (K) of 0.5, which has been largely used in tropical eucalyptus plantation (ALMEIDA et al., 2004). Stape and Binkley (2010) tested if a different K would change LAI in a large range of spacing but no significant difference were found.

### **3.2.4 Statistical Analysis**

Data were analyzed using linear and/or nonlinear regressions, with plant density as the independent variable and individual and stand biomass, transpiration, LAI, and efficiency of water use as the dependent variables. We also used non-linear regression to calculate sapwood area relative to the diameter at breast height (DBH) for each clone. Significance was assessed at  $\alpha = 0.05$ . We performed homogeneity of variance and normality tests for all regressions to verify that the data had equal variance and were normally distributed. Analysis of variance followed by Tukey's test was performed to compare stand biomass increment, transpiration, and water use efficiency between the three clones at the 1424 trees ha<sup>-1</sup> planting density. Data were analyzed using Sigma Plot software (Systat Software, San Jose, CA, USA).

### 3.3 Results

#### 3.3.1 Leaf area index

Leaf area index (LAI) varied among clones but increased with planting density for all clones (Fig. 3). The Grancam clone had the lowest LAI, which is characteristic of the *E. camaldulensis* species. Urograndis and Urophylla had higher values, ranging from about 2.5 in the lowest planting density to 4.5 in the highest.

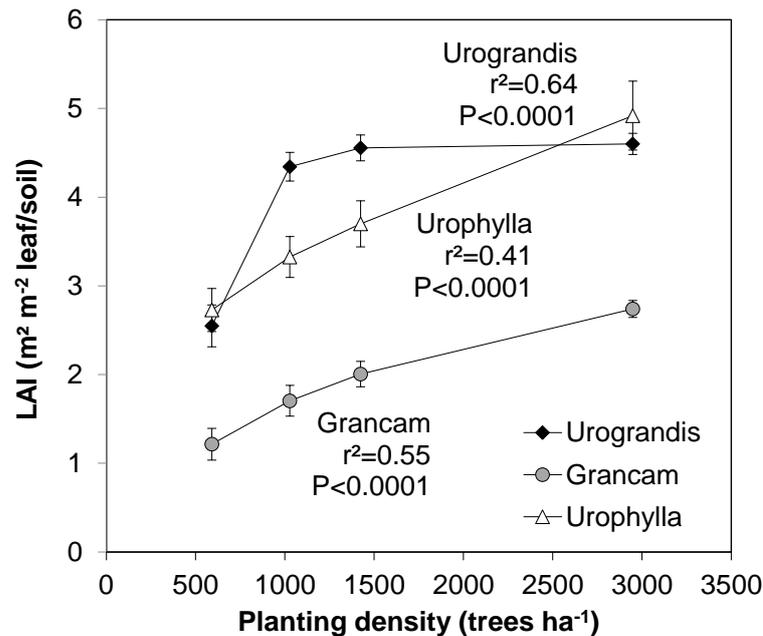


Figure 3 - Leaf area index as affected by planting density as the average of four measurements taken over the 1-year evaluation period (1.5–2.5 years after planting). Error bars represent standard error of the mean of four measurements within the period

#### 3.3.2 Individual Biomass and Transpiration

Tree biomass increased during the 12 months of evaluation and was inversely correlated with planting density (Fig. 4A) for all three clones. The 591 trees ha<sup>-1</sup> treatment averaged an increase of 21 kg tree<sup>-1</sup>, 242% higher than the average biomass increase at a density of 2949 trees ha<sup>-1</sup> (6 kg tree<sup>-1</sup>). Individual transpiration followed the same trend, with the highest values per tree occurring with the smallest planting density (Fig. 4B). While transpiration averaged 29 L tree<sup>-1</sup> day<sup>-1</sup> in the 591 trees ha<sup>-1</sup> treatment, individual trees in the 2949 trees ha<sup>-1</sup> treatment transpired only 8 L tree<sup>-1</sup> day<sup>-1</sup>.

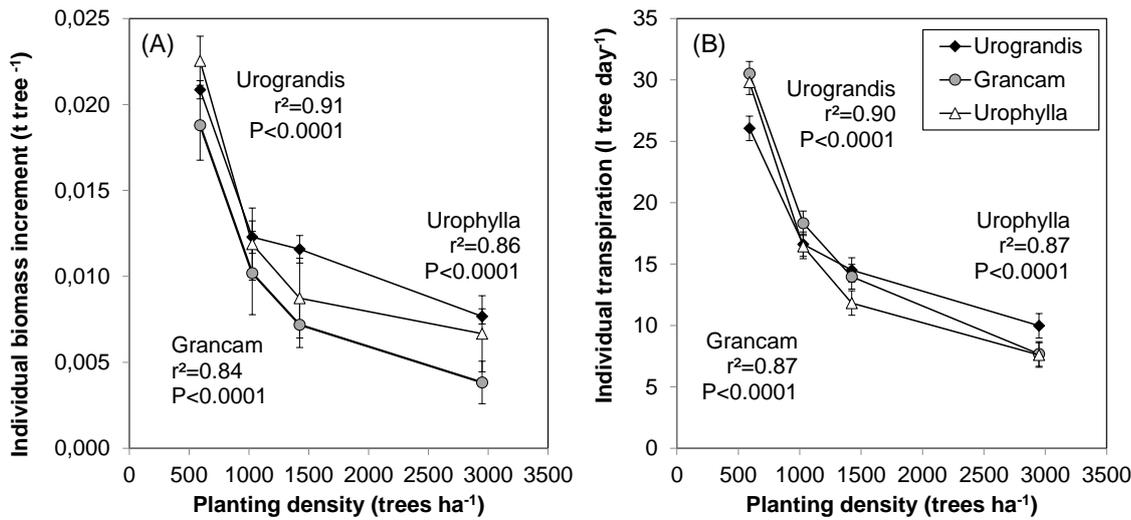


Figure 4 - (A) Individual biomass increment (t tree<sup>-1</sup> yr<sup>-1</sup>) over 12 months (1.5–2.5 years after planting) and (B) average individual transpiration (L tree<sup>-1</sup> day<sup>-1</sup>) as affected by plant density for the Urograndis, Grancam, and Urophylla clones. Error bars represent standard error of the mean of eight trees

### 3.3.3 Stand biomass increment, transpiration, and water use efficiency during the dry and rainy seasons

Planting density affected both growth and transpiration and had mixed effects across clones and seasons. Stand biomass (scaled from individual tree measurements) increased with increasing planting density for the Urograndis and Urophylla clones in both the dry and wet seasons as well as for the full year of evaluation (Tab. 2). In contrast, the biomass of Grancam clone did not show a significant increase during the study period (Fig. 5A, B, C). Stand transpiration remained stable for all planting densities and clones (Fig. 5D) in the dry season. However, during the rainy season and over the entire year, transpiration increased with increasing plant density (Fig. 5E, F). Mean transpiration in 591 plants ha<sup>-1</sup> treatment (averaged over all three clones) was 622 mm, which was 40% lower than that exhibited by the 2949 trees ha<sup>-1</sup> density treatment (879 mm). The greatest difference between clones occurred in the densest treatment: the Urophylla clone had a transpiration rate of 785 mm, 25% lower than that of Urograndis (1047 mm) (Fig. 5F). Planting density did not affect the WUE of Urophylla or Urograndis (Fig. 5G-I), whereas the Grancam clone had a slight reduction in WUE at high planting densities, albeit only during the rainy season.

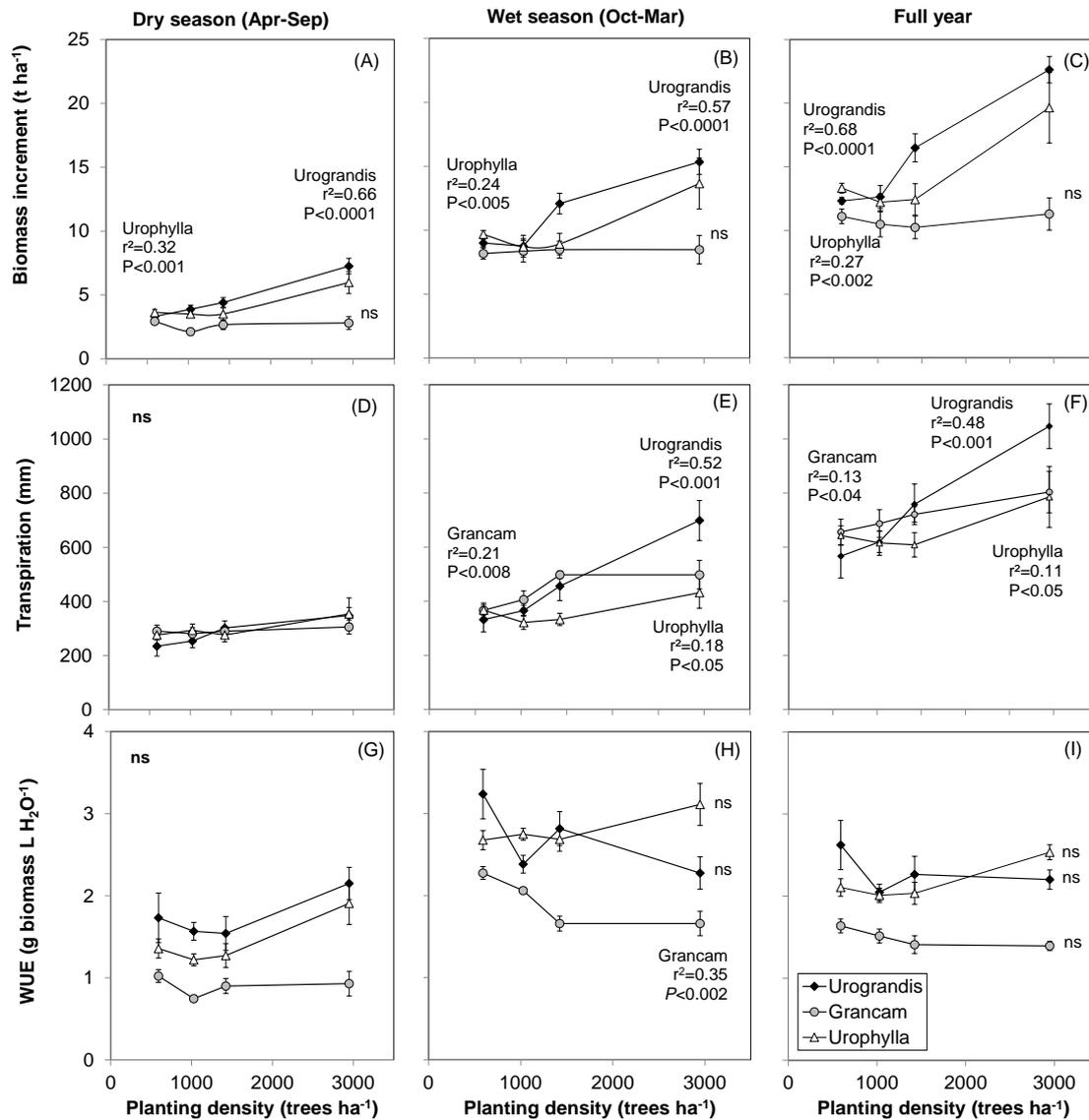


Figure 5 - Increased planting density resulted in increased biomass (a, b, c) and transpiration (d, e, f); however, it did not affect water use efficiency (g, h, i), regardless of whether the evaluation period was the full year (1.5–2.5 years after planting) or the dry or wet seasons. Error bars represent standard error of the mean. ns: not significant

Table 2 - Models correlating wood growth (B), transpiration (T) and water use efficiency (WUE) and planting spacing for hybrid clones of *E. grandis* x *E. urophylla* (Urograndis), *E. grandis* x *E. camaldulensis* (Grancam) and *E. urophylla*

Genotype	Variable	Season	Model	R <sup>2</sup>	E	AIC
Urograndis	B	Dry	$274.72 \times 27.25 \frac{1}{Spacing}$	0.67	110.3	301.1
		Wet	$808.1 \div (1 - 79.3 * e^{-0.15*Spacing})$	0.58	230.9	349.5
		Total	$1,127.5 \div (1 - 87.5 * e^{-0.16*Spacing})$	0.69	289.5	364.0
	T	Dry	-	-	-	-
		Wet	$306.2 \div (1 - 98.4 * e^{-0.16*Spacing})$	0.52	145.1	319.8
		Total	$519.9 \div (1 - 83.8 * e^{-0.149*Spacing})$	0.49	200.3	340.4
Grancam	WUE	Dry	-	-	-	-
		Wet	-	-	-	-
		Total	-	-	-	-
	B	Dry	-	-	-	-
		Wet	-	-	-	-
		Total	-	-	-	-
T	Dry	-	-	-	-	
	Wet	$594.4 - 82.3 \times \ln(spacing)$	0.21	94.3	291.0	
	Total	$810.8 - 10.13 \times spacing$	0.11	148.4	320.0	
Urophylla	WUE	Dry	-	-	-	-
		Wet	$1.49 + 0.0477 \times spacing$	0.34	0.34	-68.5
		Total	-	-	-	-
	B	Dry	$275.28 \times 13.02 \frac{1}{Spacing}$	0.33	145.0	319.0
		Wet	$759.93 \times 6.87 \frac{1}{Spacing}$	0.25	323.3	369.9
		Total	$1,028.5 \times 8.32 \frac{1}{Spacing}$	0.29	460.8	392.0
T	Dry	-	-	-	-	
	Wet	$287.7 - 0.057 \times spacing$	0.18	80.2	280.7	
	Total	$519.5 - 0.11 \times spacing$	0.11	97.1	292.9	
WUE	Dry	-	-	-	-	
	Wet	-	-	-	-	
	Total	-	-	-	-	

E: Standard Error. AIC: Akaike Information Criterion. For all equations, degree of freedom = 30.

PD: plant density; SF: stemflow;  $E_i$ : canopy interception; TF: throughfall;  $T_{stand}$ : transpiration;  $E_s$ : soil evaporation, ET: evapotranspiration;  $Q \pm \Delta S$ : water yield + variation in soil water content.

### 3.4 Discussion

In general, our results supported our hypotheses. Stand biomass and transpiration increased proportionally with planting density for all three clones. Water use efficiency did differ between clones but for each clone, there was no difference in WUE between the planting densities analyzed.

There was a decrease in individual biomass increment and an increase in stand biomass increment with increasing planting density, a trend previously reported by several studies (SCHONAU; COETZEE, 1989; STAPE; BINKLEY, 2010; FORRESTER et al., 2013). The increment in light capture resulting from increased LAI partially explains the greater increase in stand biomass increment that occurred in denser treatments (LANDSBERG; WARING, 1997). As expected, with an increase in LAI, there would be a rise in stand transpiration. Interestingly, the transpiration rate of the Grancam clone was 8% higher than that of the Urophylla clone on average, although its LAI was 78% lower. Nogueira (2013) analyzed the anatomical characteristics of 16 genotypes of *Eucalyptus* and found that the Grancam clone had the highest stomatal area, with stomata occurring on both adaxial and abaxial surfaces. This characteristic may explain Grancam's higher transpiration rate and lower LAI.

Planted forests with a focus on stand wood biomass have been managed in rotations of 5–10 years without thinning, especially in regions of high production potential (GONÇALVES et al., 2008). However, most studies examining the effects of density on transpiration have focused on density control through thinning, a commonly used practice for the production of large diameter timber (STONEMAN et al., 1996; LESCH; SCOTT, 1997; FORRESTER et al., 2012). Importantly, controlling planting density by thinning may have a different effect than when density is defined during planting (WHITEHEAD et al., 1984).

In a study evaluating the effect of planting density on water consumption in seed origin *Eucalyptus tereticornis*, Kallarackral and Somen (1997) obtained transpiration results similar to ours, with a 45% decrease in transpiration observed when planting density was reduced from 1800 to 1090 plants ha<sup>-1</sup>. To the best of our knowledge, the present study is the first report on the influence of planting density associated with different genetic materials in highly managed *Eucalyptus* plantations with high yield potential (> 50 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup>).

Planting density had no effect on WUE within each genotype but there were significant differences between the three clones. WHITE et al. (2014) showed similar results where WUE did not change when a *E. globulus* plantation grown from seed was thinned from 1200 to 300 stems ha<sup>-1</sup>. Using the Grancam clone at 1424 plants ha<sup>-1</sup> as a reference (a density that is widely used in commercial *Eucalyptus* plantations; GONÇALVES et al., 2013), there are significant differences between clones regarding biomass increment, transpiration, and WUE (Fig. 6). Relative to Grancam, the Urograndis clone had 45% more biomass increment and 61% higher water use efficiency (Fig. 6A, C) but no increase in transpiration (Fig. 6B). On the other hand, the Urophylla clone did not show an increment in biomass (Fig. 6D) but had a 16% lower transpiration rate (Fig. 6E), thereby increasing WUE by 44% (Fig. 6F).

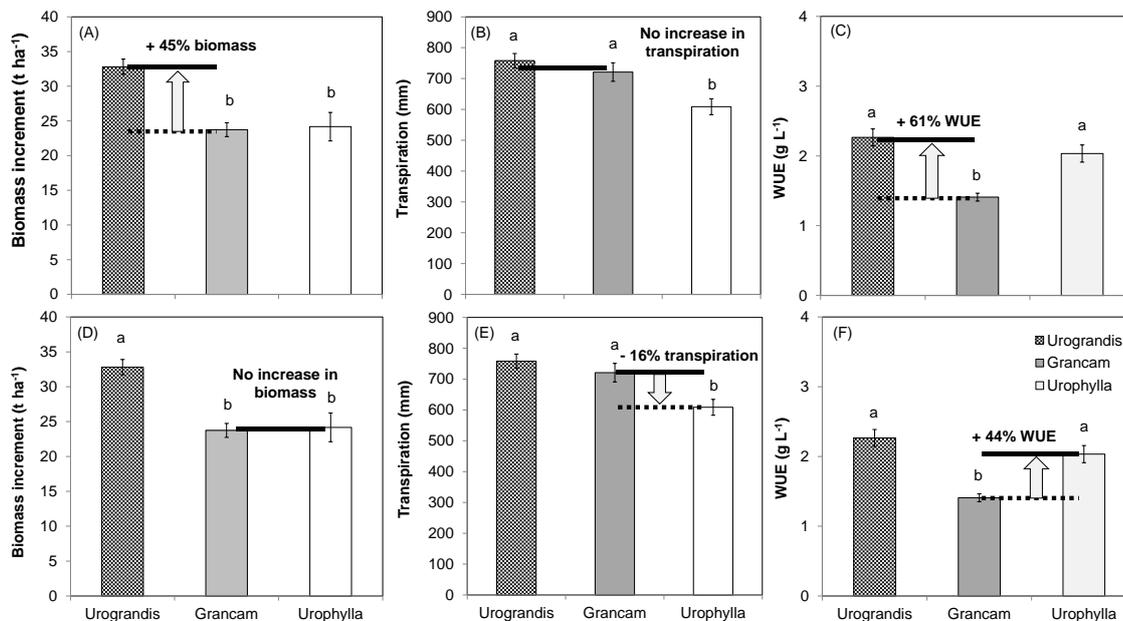


Figure 6 - Biomass increment, transpiration, and water use efficiency (WUE) at a density of 1424 trees ha<sup>-1</sup>, showing changes, relative to the Grancam clone, in the clones Urograndis (A, B, C) and Urophylla (D, E, F). Error bars represent standard error of the mean

Differences in WUE at the leaf scale in different *Eucalyptus* species were reported by Oldrich et al. (1993), Osório and Pereira (1994), and Leroux et al. (1996). At the tree scale, irrigation increased WUE by 52% in an adult *Eucalyptus urophylla* x *E.grandis* plantation (STAPE et al., 2008). Battie-Laclau et al. (2016)

reported a 63% higher WUE in trees supplied with potassium in comparison with a control treatment.

Although we report results for only one site and one year of assessment, the patterns of physiological responses of the three clones followed similar trends. Therefore, we believe that trees planted at different times and in different locations would exhibit trends similar to those observed in the present study. The experimental site is uniform, but small differences in the soil could have influenced the physiology of the trees. However, we expected that a fourfold difference in planting density would have a greater impact on tree physiology than small variations in soil properties.

Based on our results, we propose a diagram that point some results of water use efficiency. prioritizes increased biomass production and WUE while minimizing water use (Fig. 7, directions 2, 3 and 4), which is especially desirable in areas subjected to water conflicts and prolonged dry periods. It is unquestionable that improved plant efficiency in converting transpired water into biomass can greatly reduce the amount of water used to produce a given amount of wood (STAPE et al., 2004; WHITE et al., 2014). However, in the current scenario of extreme climatic events, where periods of drought are more frequent (BOOTH, 2013), and taking into account society's growing demand for hydrosolidarity (FALKENMARK; FOLKE, 2002; LIMA et al., 2011), it is essential to quantify how transpiration rates are affected by silvicultural management strategies.

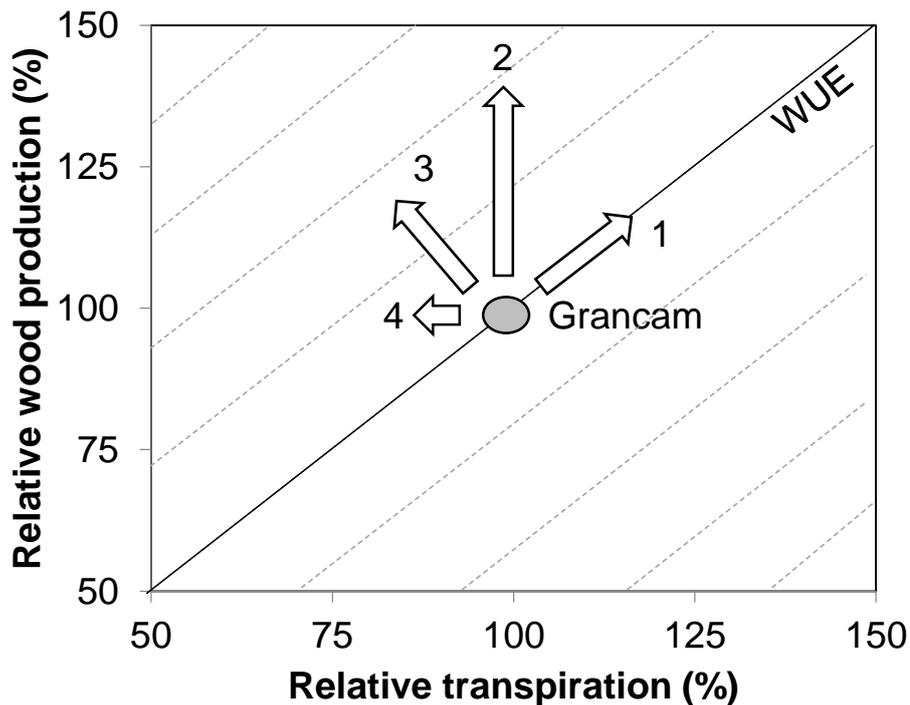


Figure 7 - Diagram of relative transpiration and biomass production for a planting density of 1424 trees ha<sup>-1</sup>. Diagonal lines represent water use efficiency (i.e. WUE increases above the 1:1 line and decreases below). Directions 1, 2, 3, and 4 point to different results of a silvicultural change in timber production, transpiration, and water use efficiency (WUE)

According to the diagram, silvicultural alternatives that lead to a shift toward direction 1 would maintain the same WUE while increasing productivity and water consumption (Fig. 7). Hubbard et al. (2010) reported similar results, where increased productivity through irrigation led to an increase in transpiration, but with equal WUE. This silvicultural alternative is focused only on productivity, since it is concerned with maintaining efficiency but does not take into account the effects of increased water consumption on the watershed. However, in sites where evapotranspiration demand is lower than precipitation, the direction 1 strategy might not be an issue (WHITE et al., 2014).

Our data from the three clones we measured, illustrate potential clonal selection and silvicultural strategies to achieve hydrosolidarity. For example, choosing Urograndis in relation to the Grancam clone, i.e., a shift in direction 2, would increase not only productivity, as did direction 1, but also WUE, while

transpiration remains unchanged. This strategy could be indicated for areas without conflicts over water use. In areas with water use conflicts, directions 3 and 4 would be more suitable. Direction 3 would provide increased WUE and productivity but reduced transpiration. In relation to the Grancam clone, the choice of Urophylla utilizes the direction 4 strategy, i.e., a genotype change can decrease transpiration but maintain productivity by increasing WUE.

In addition to the optimization of water use, our conceptual framework may lead to a lower risk of disturbance of the planted area, due to the lower risk of water stress caused by reducing or maintaining water use. According to the model proposed by Turner et al. (1993), the greater the environmental disturbance caused in an area (either by environmental or biotic factors), the less stable the landscape. This would increase the probability of water stress and/or the appearance of pests and diseases.

To increase timber production is undoubtedly the main objective of a forest plantation. However, there is a growing societal demand for investments that, in practice, encompass adaptive management. This study shows forest managers and researchers that there are alternatives to increase biomass increment that can occur concomitantly with water conservation values. We suggest that future studies address planting density and water use in regions with prolonged periods of drought in order to better understand the effect of such conditions on water relations.

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## 4 HOW IS THE BEHAVIOR OF HIGH-DENSITY SHORT-ROTATION SYSTEM IN CLONAL *Eucalyptus* REGARDING SOIL WATER BALANCE? A STUDY CASE IN BRAZIL

### Abstract

High-density short rotation has been proposed to provide faster and higher amount of wood for energy purposes. However, to the best of our knowledge, there is no study regarding the impact of planting density and different genotypes on soil water balance in high productive clonal plantations ( $>50 \text{ m}^3 \text{ ha}^{-1} \text{ ano}^{-1}$ ). We aimed to evaluate the responses of two hybrid clones that differ in drought tolerance and productivity (*E.grandis* x *E.urophylla* and *E.grandis* x *E.camaldulensis*) planted in densities ranging from 591 to 2,949 tree  $\text{ha}^{-1}$  on components of water balance (transpiration, canopy interception, soil evaporation, stemflow). Independently of genetics, soil water balance measured from 1.7 to 3.7 years-old plantation was influenced by planting density. Scaling from trees to a stand scale, in average of the two genotypes, transpiration (T) increased with density from 53 to 82% of precipitation, varying from 1,078-1,108 mm in lower density to 1,314-2,078 mm in higher density. Canopy interception (Ei) also was higher in higher density, representing 32% of precipitation against 23% in lower density. In an opposite direction, soil evaporation (Es) decrease with the increasing density. *E. grandis* x *E. urophylla*, the less drought tolerant genotype, had a higher sensibility to changes in density, with an increase in evapotranspiration (sum of T, Ei and Es) from 591 to 2,949 tree  $\text{ha}^{-1}$  of 49%. In *E.grandis* x *E.camaldulensis* this difference where of 11%. Soil water balance (SWB), i.e., the difference between precipitation and evapotranspiration, was negative for densities higher than 1,028 tree  $\text{ha}^{-1}$  for both clones, revealing that, in the sense of climate change with extreme drought events, densities higher than this value are not recommended in high productive sites. Results show both genetics and planting density shall be used as silviculture tools to manage sustainably the short rotation plantation. However, because we studied only two genotypes in one site, we suggested more research to expand the extrapolation of our results.

Keywords: Water use, planted forests, tree spacing

### 4.1 Introduction

In the last 25 years, planted forest area in the tropics grew at an average rate of 2.5% per year, reaching approximately 56 MM ha (PAYN et al., 2015) owing to improved forestry and genetic practices that increase plant adaptability to the soil and climatic conditions of the tropics. In fact, eucalypt productivity in these regions often surpasses  $50 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$  (STAPE et al., 2010; GONÇALVES et al., 2013). High productivity *Eucalyptus* forests often yield as much as a 10-fold larger volume than that obtained from temperate climate forests (PAYN et al., 2015), and correspond to

approximately 10-fold faster growth than observed among wild native species under similar conditions (OLIVEIRA et al., 2002; SCOLFORO et al., 2000).

A recent trend in the tropics is the high-density short-rotation system, e.g., more than 3,000 tree ha<sup>-1</sup> with harvesting scheduled each 3-4 years (GUERRA et al., 2014). Some researchers and managers argued this system could be a profitable model to achieve a higher production of biomass to be converted to energy, increasing the representativeness of clean source of energy in the tropics (GUERRA et al., 2014; EUFRADE JUNIOR et al., 2016). However, few studies have investigated the effects of this system on soil water balance, especially in very productive sites.

High productivity forests may accumulate biomass very quickly but will also transpire large amounts of water (WHITEHEAD; BEADLE, 2004). Furthermore, *Eucalyptus* plantations have relatively high leaf area indexes (LAI), often above five (STAPE et al., 2010; GIUNTI NETO et al., 2015), which elevates transpiration rates and increases the amount of rain intercepted by the canopy (BENYON; DOODY, 2015; BIALKOWSKI et al., 2015). Consequently, high productivity eucalypt forests may be more vulnerable to water stress (WHITE et al., 2009, HAKAMADA et al., in press), which could increase tree mortality and have consequences for local and regional carbon balances (MCDOWELL et al., 2008; ALLEN et al. 2015). While eucalypt plantations in Brazil are expanding into warmer and drier regions (GONÇALVES et al, 2013), climate projections for the future indicate much of South America will experience an increase in extreme climatic events and drought conditions (ALLEN et al., 2015). Consequently, silvicultural practices that seek the balance between social, economic and environmental needs are required.

In one point of view, reducing plant density, by either planting fewer saplings per unit area or by thinning, has been proposed as a means of reducing forest water use (BRÉDA et al., 1995; FORRESTER et al., 2012; SOHN et al., 2013; DEL CAMPO et al., 2014). However, the high demand for clean energy (HABERL et al., 2011) and the possibility of having faster and higher profits (GUERRA et al., 2014) has increased the interest for shorter rotation systems controlled by lower spacing plantation. Forest density affects plant morphologic characteristics, such as leaf area, as well as microclimate variables including solar radiation reaching the soil and vapor pressure deficit, all of which affect water balance (WHITEHEAD et al., 1984; BRÉDA et al., 1995).

The use of different species in forest plantations can also change stand level water use by trees. Murunga et al. (2015) reported a daily transpiration of 3.18 and 5.02 mm per day in areas with predominance of *Eucalyptus microcorys* and *E. tereticornis*, respectively. Differences were also reported by WHITE et al. (2002), where maximum transpiration rates were of 1.9 and 2.9 mm per day for *Eucalyptus camaldulensis* and *E. saligna*, likely because of higher maximum stomatal conductance of the second species.

Although planting spacing and genetics both influence soil water balance, there are no studies to our knowledge on the effects of these two variables in high-productivity ( $>50 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$ ) clonal *Eucalyptus* stands. The aim of this work was to quantify how planting density impacted soil water balance components for two high-productivity *Eucalyptus* clones. We selected hybrids with distinct water stress tolerances; the first, a drought sensitive hybrid of *Eucalyptus grandis* and *E.urophylla* (Urograndis), normally planted in areas without water deficit; and the second, a hybrid of *E.grandis* x *E.camaldulensis* (Grancam), which is known for its tolerance to dry and hot conditions (GONÇALVES et al., 2013). Key components of the evapotranspiration (ET), i.e., transpiration (T), canopy interception (Ei) and soil evaporation (Es) were evaluated during two hydrological years in the period of peak of tree growth, between the ages of 1.7 and 3.7 years (RYAN et al., 2010). We hypothesize that i) increasing planting density will increase evapotranspiration for both clones, explained by the increase in leaf area index (LAI) in higher densities (WHITE et al., 2009; FORRESTER et al., 2012); ii) The effect will be greater for the drought sensitive clone Urograndis, once its stomatal conductance is less sensitive than Grancam to the water deficit in the system, in our case, controlled by planting density (GENTIL, 2015).

## 4.2 Materials and Methods

### 4.2.1 Site description

The study was conducted in an experimental plantation that was planted on 02/21/2012 in Mogi Guacu, state of São Paulo, Brazil (22°20'58''S and 46°58'16''W), at an altitude of 664 m. Local climate is considered humid mesothermal according to Koppen's classification. The average annual temperature is 22°C, the average precipitation is 1,200 mm, with 120 rainy days, mostly (82%)

concentrated in summer (October to March). Historically, the potential evapotranspiration (PET) is approximately 1,165 mm, similar to the PET observed during the two hydrological year studied (1,140 and 1,113 mm). The average aridity index ( $AI = PET/P$ ; BUDYKO, 1974), in the region is 0.81. However, during our study it reached 1.48 and 0.91 during the first and second year, respectively. The region typically experiences a water deficit of 50 mm, according to water balance of Thornthwaite & Mather, and considering a water storage capacity of 150 mm (DEMATTE, 2000). The soil in the experimental area is characterized as red oxisol (DEMATTE, 2000), with a clay content of 39%, pH 4.3, and sum of the bases of 15.5  $\text{mm}_c \text{ dm}^{-3}$ . Effective depth is greater than 5 meters, and probably greater than 20 meters (DEMATTE, personal communication).

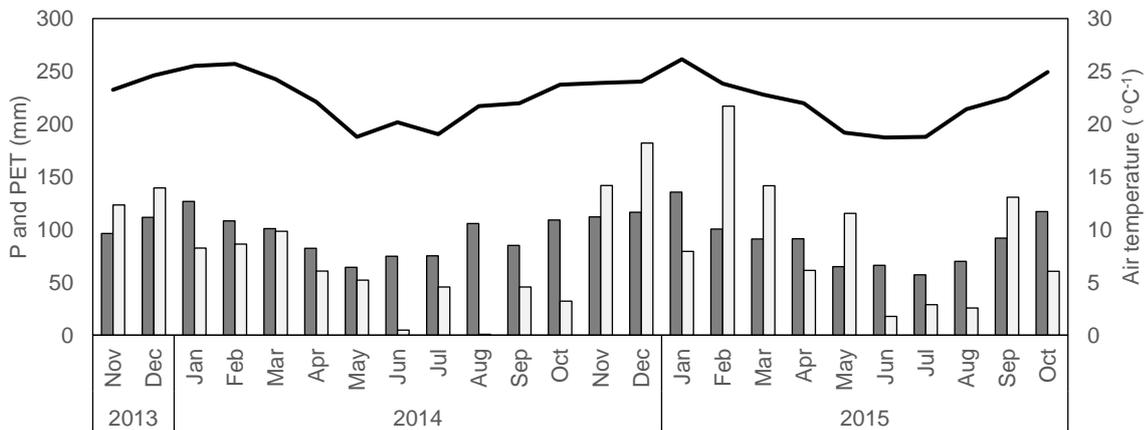


Figure 1 - Precipitation (P) (light bar), potential evapotranspiration (PET) (dark bar) determined by the Penman-Monteith method and air temperature (line) during the 2-year period from November 2013 to October 2015

#### 4.2.2 Experimental design

Trees were planted with increasing spacing between them within each row, whereas spacing between rows was kept at 3 m (Fig. 2). This design allowed us to have a wide range of densities and different genotypes in a relatively small area (STAPE; BINKLEY, 2010). The evaluation of the water balance components was conducted in four different tree spacing with 14 trees per plot, with a useful sample of eight trees per plot. The four densities were 2,949, 1,424, 1,028 and 591 plants  $\text{ha}^{-1}$  (Figure 2). The total area occupied by the two clones corresponded to 3,780  $\text{m}^2$ .

Our unit of observation is the individual tree for this evaluation of water balance components response to varying densities. Even though neighboring trees

are not spatially independent, we think they provide a fair estimate of water balance components because any interaction between trees is competitive, leading to increased (rather than decreased) variation in physiology, and also encompassing realistic competitive interactions that happen in operational plantations. Our experimental site is uniform, but slight differences in soils might have some influence on water balance components; however, the 4-fold range in spacing is likely a much stronger factor in water balance components than any minor variation in soils.

Two genotypes with different tolerance levels to water stress were evaluated. The Urograndis clone is a hybrid of *E.grandis* x *E.urophylla* and the Grancam clone results from the cross *E.grandis* x *E.camaldulensis*. *E.camaldulensis* usually has been planted in regions of elevated water deficit (ELDRIDGE et al., 1993; LE ROUX et al., 1996), whereas *E. grandis* x *urophylla* is known for its low tolerance to high water stress environments (GONÇALVES et al., 2013).

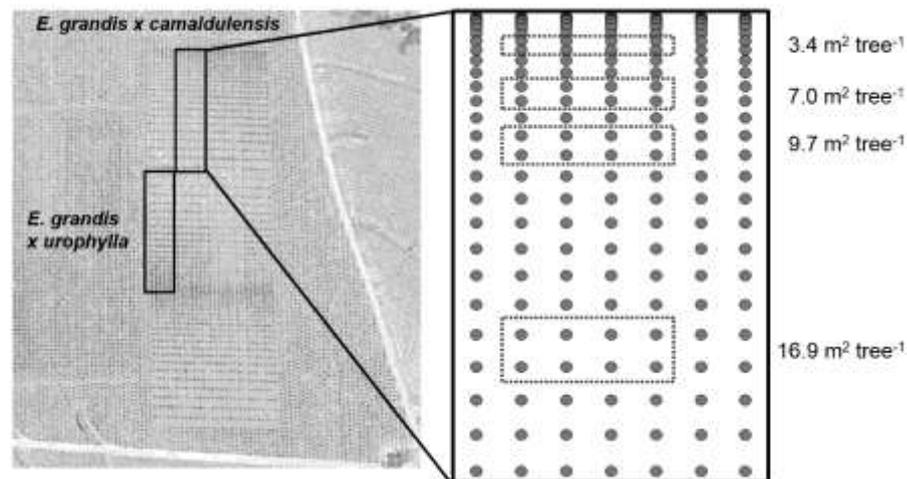


Figure 2 - **Test site.** Left: Photographic image obtained from Google Earth® when trees were 12 months old, solid squares show the location of the two genotypes used in the study. Right: diagram showing the increasing spacing between plants and the plots with different spacings per plant (591, 1,028, 1,424 and 2,949 m² plant<sup>-1</sup>)

The site was prepared by subsoiling at a depth of 60 cm with 3 m between rows. The level of fertilization was defined to eliminate any nutritional limitation to growth with the use of 70, 110 and 160 kg of N, P and K, respectively. Ant control was performed prior to planting and every year afterwards with 0.3% sulfluramid.

Weeds were 100% controlled with the use of Glyphosate at 2.88 kg a.i. ha<sup>-1</sup>. The entire test site was maintained pest and disease free through visual inspection since saplings were planted. Sapling survival was 100%.

#### 4.2.3 Individual ( $T_{tree}$ ) and stand ( $T_{stand}$ ) transpiration measurements

Individual transpiration ( $T_{tree}$ ) was measured for 24 month, from November 2013 to October 2015, when trees were between 1.7 and 3.7 years old. Eight trees were measured per density per clone. Altogether, we evaluated eight trees x four spacings x two clones, for a total of 64 observation units. To measure  $T_{tree}$  during the 2-year period, thermal dissipation sapflow probes were used (GRANIER, 1987) on the same trees that were selected for biomass measurements. Sapflow density was calculated using the equation for *Eucalyptus* (eq. (3)) in Hubbard et al. (2010). We estimated sapwood area for the measurement trees using allometric equations based on diameter at breast height. For these equations, 15 trees were harvested from the adjacent site with identical planting densities. Thin (1–1.5 cm) disks were cut at breast height and conducting sapwood area was estimated visually by measuring four equally opposed radii of the obvious translucent portion of the disk and calculating area as an ellipse (details see OTTO et al., 2014). Sapwood area in relation to DBH was estimated by power models for each genotype (sapwood = a + bDBH,  $R^2 > 0.78$ ).  $T_{tree}$  (L tree<sup>-1</sup>) was estimated as the product of sapflow density and sapwood area. Data were collected every 15 seconds, and the average was recorded every 15 minutes via a multiplexer coupled to a data logger (CR1000 and AM 16/32, Campbell Scientific, Inc., Logan, UT). To minimize the interference of direct radiation on the probes, two probes per treatment plots were positioned at each cardinal position (north, south, east, and west), and the sensors were moved 90° clockwise every three months. In this way, differences in flow caused by cardinal positioning were accounted for over the time span of the experiment (GRIME and SINCLAIR, 1999). To insulate the probes from thermal gradients, styrofoam and foil backed insulation were used. We placed transparent plastic bags around the insulation to protect probes against moisture and stemflow. Stand transpiration ( $T_{stand}$ ) (mm ha<sup>-1</sup>) was estimated from the eight individual transpiration values obtained per plot.

#### 4.2.4 Throughfall (TF), soil evaporation ( $E_s$ ) leaf area index (LAI), stem flow (SF)

Throughfall (the difference between the precipitation outside and inside the canopy) was obtained with the use of funnel collectors placed below the canopy (Fig. 3). We used 12 collectors with an area of  $0.026 \text{ m}^2$  (18.3 cm in diameter) for each tested density and for each clone, totaling  $0.31 \text{ m}^2$  in collection area. In each plot the water collected by the 12 collectors was directed to a reservoir tank. Water from each precipitation event that surpassed 2mm was weighed on a balance in the morning following the event and converted to volume using a density of  $1 \text{ g cm}^{-3}$ . Collectors were distributed across representative areas below the canopies, including within and between rows. An additional group of 12 collectors was installed in an open area 50 m distant from the test site to measure gross precipitation (P).

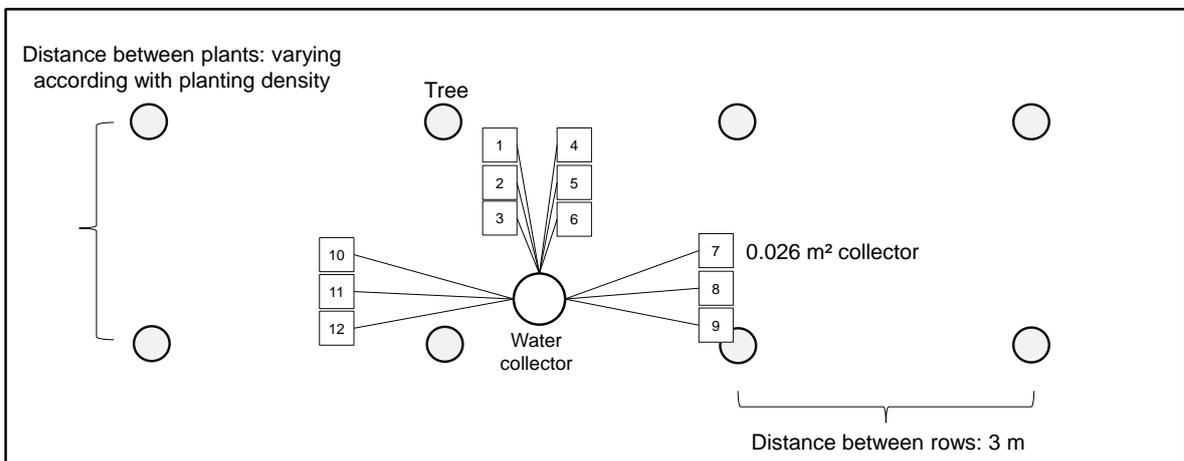


Figure 3 - Lay-out of the 12 collectors distributed in each treatment to estimate gross precipitation (P) and throughfall.

Soil water evaporation ( $E_s$ ) was estimated according to White et al. (2002). From January to October 2014 and from May to October 2015, when PET was above P, we assumed a value for  $E_s$  of  $0.01 \text{ mm h}^{-1}$ , during 10 h per day. This was the lowest value in a study of soil evaporation from dry soil according to a study in similar latitude by Leuning et al. (1994). This study was done to estimate soil evaporation in wheat in Australia. However, the climate conditions is similar and White et al. (2002) already tested its validity for *Eucalyptus globulus* in South Australia. When P was greater than PET estimated according to Penman-Monteith method (ALLEN et al., 1998), i.e., within the periods from November to December of 2013 and from November 2014 to April 2015, the following equation was used:

$$E_s = E_{eq} = \frac{\Delta}{\Delta + \gamma} R_s \quad (5)$$

where:

$E_s$  = soil water evaporation =  $E_{eq}$  = equilibrium evaporation;

$\Delta$  = slope of the relation between saturated vapor pressure and temperature;

$\gamma$  = psychrometric constant;

$R_s$  = liquid radiation below the canopy.

LAI was estimated using Beer's law and the difference between PAR values measured above and below the canopy. In each tested density, PAR was measured nine times between November 2013 and October 2014 each about 3 months with a ceptometer (Decagon Devices Inc., Pullman, WA, USA). Measurements were performed in two transects per plot, with 16 measurements per transect. Measurements were conducted between 11:30 and 13:00 on clear days with no clouds or wind. LAI was estimated based on Beer's law, assuming a light extinction coefficient of 0.5 (ALMEIDA et al., 2007).

To estimate SF, water-collection systems were installed on eight trees per plot. Water from these systems was weighed on balance at the same time as throughfall measurements and converted to volume assuming a density of  $1 \text{ g cm}^{-3}$ . To obtain the value in mm we used the surface area occupied by each tree, which varied according to the planting densities. Total solar radiation, temperature, relative humidity, precipitation and wind velocity data were obtained every 5 minutes from an automated weather station 1,000 m away from the test site. These data were stored every hour in a Campbell CR1000® datalogger.

#### **4.2.5 Estimates of canopy interception (Ei), evapotranspiration (ET), water yield (Q) and soil water balance (SWB)**

Ei was calculated with eq. 6:

$$E_i = P - (TF + SF) \quad (6)$$

ET was given by eq. 7:

$$ET = T + E_i + E_s \quad (7)$$

For each density and clone,  $Q \pm \Delta S$  was calculated as proposed by Lee (1980):

$$\text{SWB} = \text{P} - \text{ET} \quad (8)$$

Surface runoff was negligible, given the relatively flat slope of the plots (<1% slope).

#### 4.2.6 Growth and Biomass measurements

Growth rate during the study period, from 1.7 to 3.7 year of age, was determined from height (H) and DBH using an electronic hypsometer and a metric tape, respectively. Based on DBH and H, we estimated individual volumes using the model developed by Schumacher and Hall (1933). Parameters for the model were estimated from an adjacent site planted with the same design for destructive sampling (unpublished data). To estimate mean annual increment (MAI,  $\text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$ ) at 2.7 years and current annual increment (CAI,  $\text{m}^3 \text{ha}^{-1} \text{yr}^{-1}$ ) at each age, we extrapolated the biomass of each individual as an independent observation unit. Thus, we obtained eight values of total volume ( $\text{m}^3 \text{ha}^{-1}$ ) per treatment for the MAI and CAI estimates. As the results of water balance, biomass results should be viewed as preliminary findings, because of the lack of replication at the plot level. Further studies and replications need to be done to support the tentative conclusions drawn from this paper.

#### 4.2.7 Statistical analyses

The independent variables SF, T, Ei, Es, TF, ET and  $Q \pm \Delta S$  were correlated to the dependent variable, planting spacing, through linear and non-linear models. Model significance was tested with  $P = 0.05$ . We evaluated the normality and homogeneity of variance in all models. To compare whether the models of the two clones were different, we used a test of identity as proposed by Leite and Oliveira (2006). We used each tree as an unit of observation. However, as a conservative measure, we also analyzed the effect of spacing by pooling all eight trees at each spacing into a single average, giving four observations for testing the effect of spacing. The average trend would be the same in this analysis, but the reduction in degrees of freedom give a far more conservative test of the effects of spacing than in our basic design that used each tree as a unit of observation. Our design has 100% of survival across all plots, all neighboring trees with similar size in all plots, and the

site and soil properties were uniform. That is why we think it is not unreasonable to provide an estimated total stand stemwood biomass per hectare for each treatment on a secondary Y axis, in addition to the primary Y axis that shows the biomass per tree which, strictly speaking, was the basic unit of experimentation. All the analyses were conducted in the statistics software Sigma Plot (Systat Software, San Jose, CA, USA) and using an excel spreadsheet for the test of identity.

### 4.3 Results

We evaluated the effects of forest density and genotype on the water balance components comparing highly productive eucalyptus clones during a two years period. Increasing density was accompanied by increases in SF,  $E_i$ ,  $T_{\text{stand}}$  and ET, as well as decreases in  $E_s$  and TF (Table 1). For the Urograndis clone, stemflow declined and ranged from 23 to 53 mm as density increased from 591 to 2949 and SF ranged from 32 to 82 mm in the clone Grancam from the lowest to the highest density (Fig. 4A). Canopy interception followed a similar pattern but increased with density from an average of 481 to 660 mm (Fig. 4B). These values were, on average, 8% greater in the Urograndis clone, because of higher LAI (Fig. 9A). Thus, as expected, TF decreased by 11% with increasing density from 1,596 to 1,417 mm, (Fig. 4C). Soil evaporation also decreased with density in both genotypes, but remained 50% higher in the Grancam clone (Fig. 4D). Higher planting density resulted in greater  $T_{\text{stand}}$  for both clones but Urograndis was 22% greater than the Grancam clone (Fig 4E). The increases in T and density resulted in an increase in ET (Fig. 4F). A model was generated to assess the relation between each component and forest density (Table 2). All of them performed significant correlations (linear models) and high coefficient of correlation, indicating that density is strongly associated with the use of water by eucalyptus forests.

Table 1 - Changes in water balance variables in relation to planting densities of two highly productive eucalyptus clones hybrids of *Eucalyptus grandis* x *E. urophylla* (Urograndis) and *E. grandis* x *E. camaldulensis* (Grancam) during two hydrological years from 17 to 3.7 years old

Clone	Planting density Tree ha <sup>-1</sup>	%								Percentage of P (%)							
		<i>P</i>	<i>SF</i>	<i>Ei</i>	<i>TF</i>	<i>T<sub>stand</sub></i>	<i>E<sub>s</sub></i>	<i>ET</i>	<i>SWB</i>	<i>P</i>	<i>SF</i>	<i>Ei</i>	<i>TF</i>	<i>T<sub>stand</sub></i>	<i>E<sub>s</sub></i>	<i>ET</i>	<i>SWB</i>
Urograndis	591	2077	23	541	1536	1108	307	1956	139	100%	1,1%	26%	75%	53%	14,8%	94%	7%
Urograndis	1028	2077	33	602	1476	1288	188	2078	22	100%	1,6%	29%	73%	62%	9,1%	100%	1%
Urograndis	1424	2077	51	559	1518	1451	166	2177	-66	100%	2,5%	27%	76%	70%	8,0%	105%	-3%
Urograndis	2949	2077	53	654	1423	2078	175	2907	-795	100%	2,5%	31%	71%	100%	8,4%	140%	-38%
Grancam	591	2077	32	442	1636	1078	575	2095	-2	100%	1,5%	21%	80%	52%	27,7%	101%	0%
Grancam	1028	2077	40	488	1589	1192	411	2091	13	100%	1,9%	23%	78%	57%	19,8%	101%	1%
Grancam	1424	2077	47	582	1495	1265	359	2207	-97	100%	2,3%	28%	74%	61%	17,3%	106%	-5%
Grancam	2949	2077	82	666	1411	1314	334	2315	-189	100%	4,0%	32%	72%	63%	16,1%	111%	-9%
Average	591	2077	28	481	1596	1093	441	2015	90	100%	1,3%	23%	78%	53%	21,2%	97%	4%
Average	1028	2077	37	533	1545	1240	300	2072	42	100%	1,8%	26%	76%	60%	14,4%	100%	2%
Average	1424	2077	49	555	1522	1358	263	2176	-50	100%	2,4%	27%	76%	65%	12,6%	105%	-2%
Average	2949	2077	68	660	1417	1696	255	2611	-466	100%	3,3%	32%	71%	82%	12,3%	126%	-22%

*P*: gross rainfall; *SF*: stemflow; *E<sub>i</sub>*: canopy interception; *TF*: throughfall; *T<sub>stand</sub>*: transpiration; *E<sub>s</sub>*: soil evaporation, *ET*: evapotranspiration; *SWB*: soil water balance

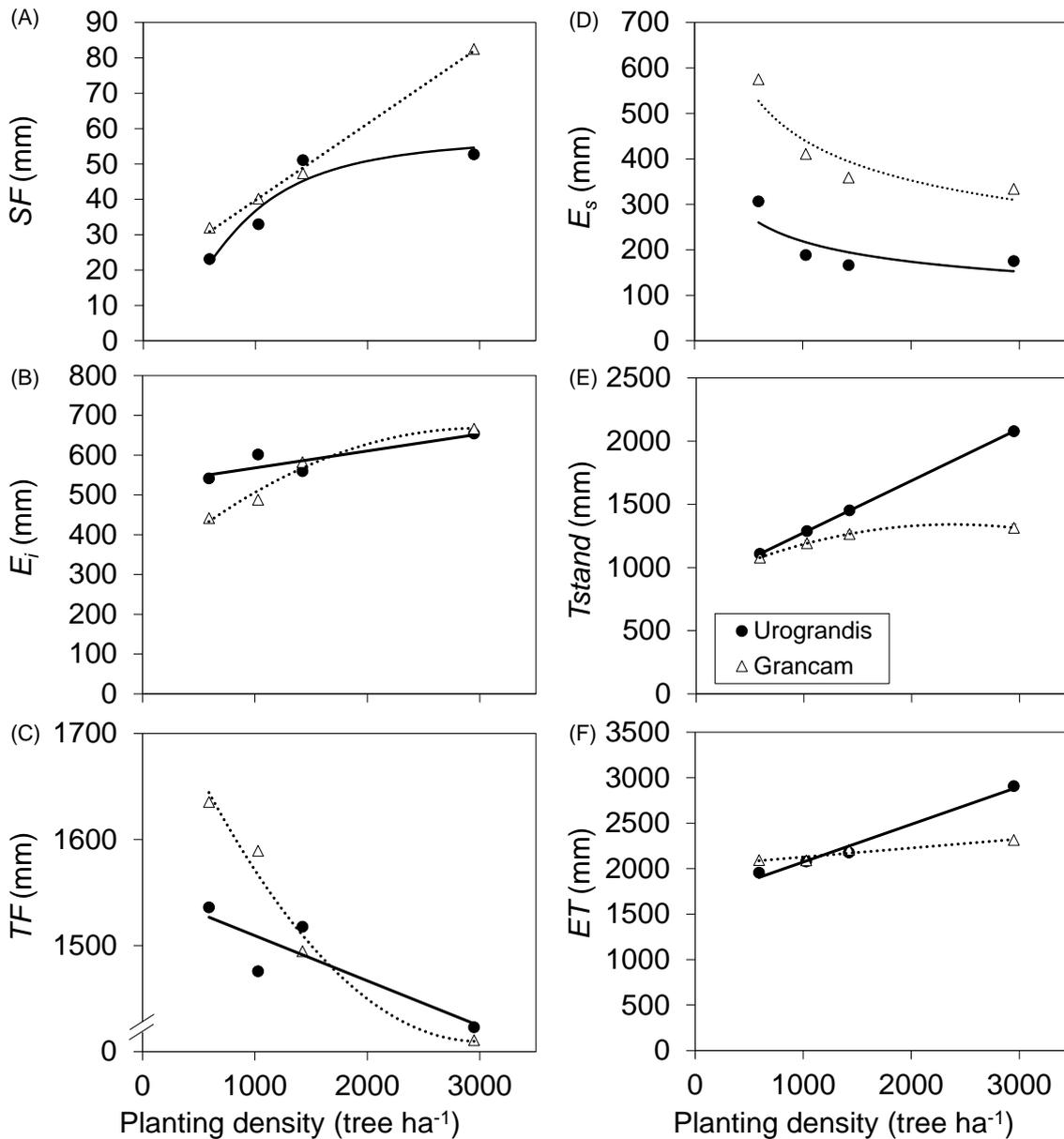


Figure 4 - (A) Stemflow ( $SF$ ), (B) canopy interception ( $E_i$ ), (C) throughfall ( $TF$ ), (D) soil evaporation ( $E_s$ ), (E) transpiration ( $T_{stand}$ ) and (F) evapotranspiration ( $ET$ ) observed in Urograndis and Grancam clones at densities varying from 591 to 2949 plants ha<sup>-1</sup>. Standard error bars are shown for T, other variables had no repetitions

Table 2 - Models correlating water balance variables and plant density for hybrid clones of *E. grandis* x *E. urophylla* (Urograndis) and *E. grandis* x *E. camaldulensis* (Grancam)

Genotype	Component	Model	R <sup>2</sup>	P	AIC
Urograndis	SF	$54.41 - 35.09 * \exp(-3.46 * 10^{-11} * PD^3.41)$	0.98	n.a.	10.4
	E <sub>i</sub>	$0.04247 PD + 525.38$	0.75	<0.001	28.7
	TF	$-0.04261 PD + 1,552.3$	0.76	<0.001	28.7
	T <sub>stand</sub>	$0.4113 PD + 865.09$	0.99	<0.001	-
	E <sub>s</sub>	$2,148.7 PD^{-0.331}$	0.62	n.a.	31.9
	ET	$0.4141 PD + 1,659.2$	0.98	<0.007	34.7
Grancam	SF	$0.0216 PD + 18.061$	0.99	<0.001	3.93
	E <sub>i</sub>	$-0.00004 PD^2 + 0.2418 PD + 304.7$	0.98	n.a.	37.2
	TF	$0.00004 PD^2 - 0.2444 PD + 1774$	0.97	n.a.	37.1
	T <sub>stand</sub>	$-0.00008 PD^2 + 0,3905 PD + 876.2$	0.99	n.a.	13.2
	E <sub>s</sub>	$4333.6 PD^{-0.33}$	0.85	n.a.	31.3
	ET	$0.09917 PD + 2,028.4$	0.91	<0.04	30.5

n.a. = non-applicable. AIC: Akaike Information Criterion

PD: plant density; SF: stemflow; E<sub>i</sub>: canopy interception; TF: throughfall; T<sub>stand</sub>: transpiration; E<sub>s</sub>: soil evaporation, ET: evapotranspiration; Q ± ΔS: water yield + variation in soil water content.

The mean percentage of PAR absorbed by canopy for the planting densities of 591, 1028, 1424 and 2949 trees per hectare varied according to the planting density, ranging between 71 and 82% for Urograndis and between 40 and 66% for the Grancam clone (Fig. 5A). The average LAI from nine assessments was greater at higher densities for both genotypes (Fig. 5B), varying from 3.2 to 4.2 for Urograndis and from 1.6 to 2.8 for Grancam.

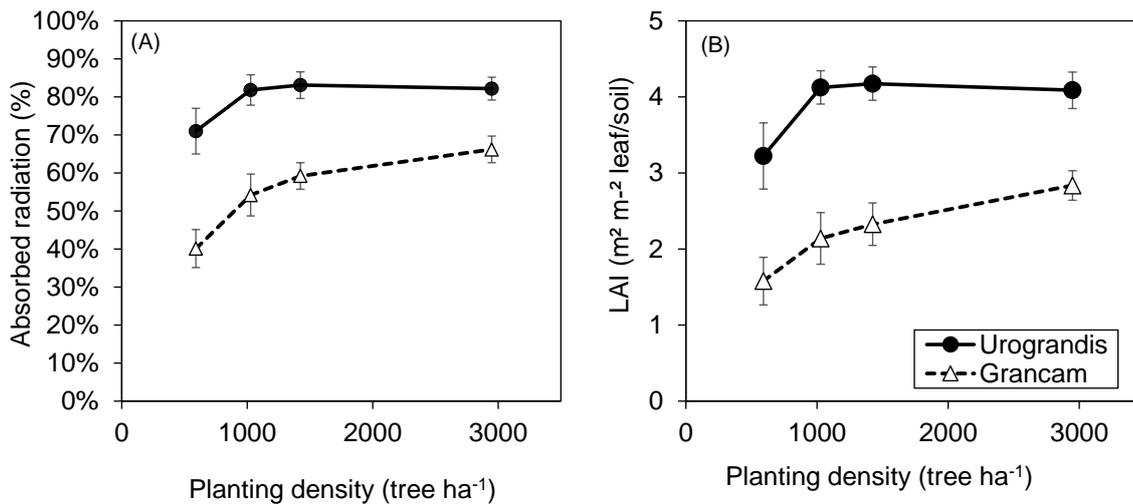


Figure 5 - (A) Percentage of absorbed radiation and (B) leaf area index (LAI) estimated by Beer's law for hybrid clones of *E. grandis* x *E. urophylla* (Urograndis) and *E. grandis* x *E. camaldulensis* (Grancam) in planting densities ranging from 591 to 2.949 trees ha<sup>-1</sup>. Values are an average of nine measurements during the 2-year period of evaluation. Bars represents standard error bar among measurements

Figure 6 compares the values obtained for each variable with the widest and the narrowest spacing. Although a small component of the overall water balance, SF displayed the largest relative difference with an average increase of 243% in the highest compared to the lowest density. The highest density obtained 49% and 11% greater ET in comparison to the lowest density for Urograndis and Grancam, respectively.

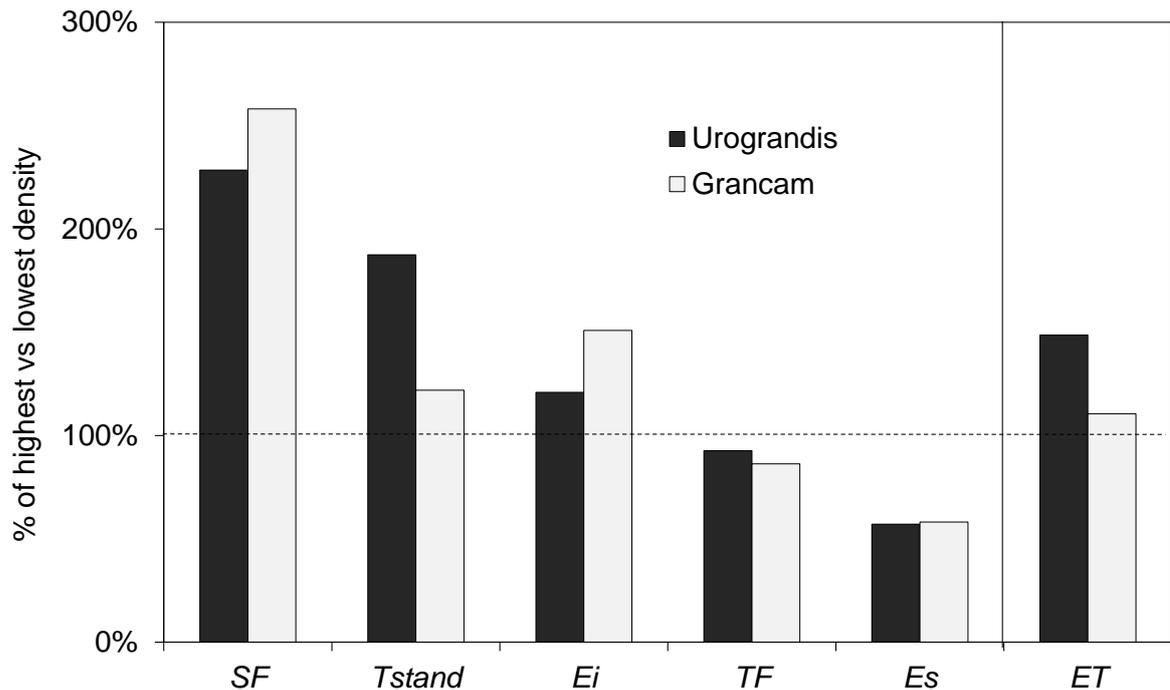


Figure 6 - Percentage of stemflow ( $SF$ ), transpiration ( $T_{stand}$ ), canopy interception ( $E_i$ ), throughfall ( $TF$ ), soil evaporation ( $E_s$ ) and the resulting evapotranspiration ( $ET$ ) at a density of 2.949 stem  $ha^{-1}$  in comparison to a density 591 of stems  $ha^{-1}$  ( represented by the dashed line) during the 2-year period of evaluation

There was a significant increase with density in the sum of the three  $ET$  components ( $T_{stand}$ ,  $E_i$  and  $E_s$ ) for each clone (Fig. 7). Clearly, the component that controls  $ET$  is  $T_{stand}$ , because the sum of  $E_s$  and  $E_i$  for the two clones was similar regardless of plant density. The two hydrological years showed similar trends for both genotypes, with a higher

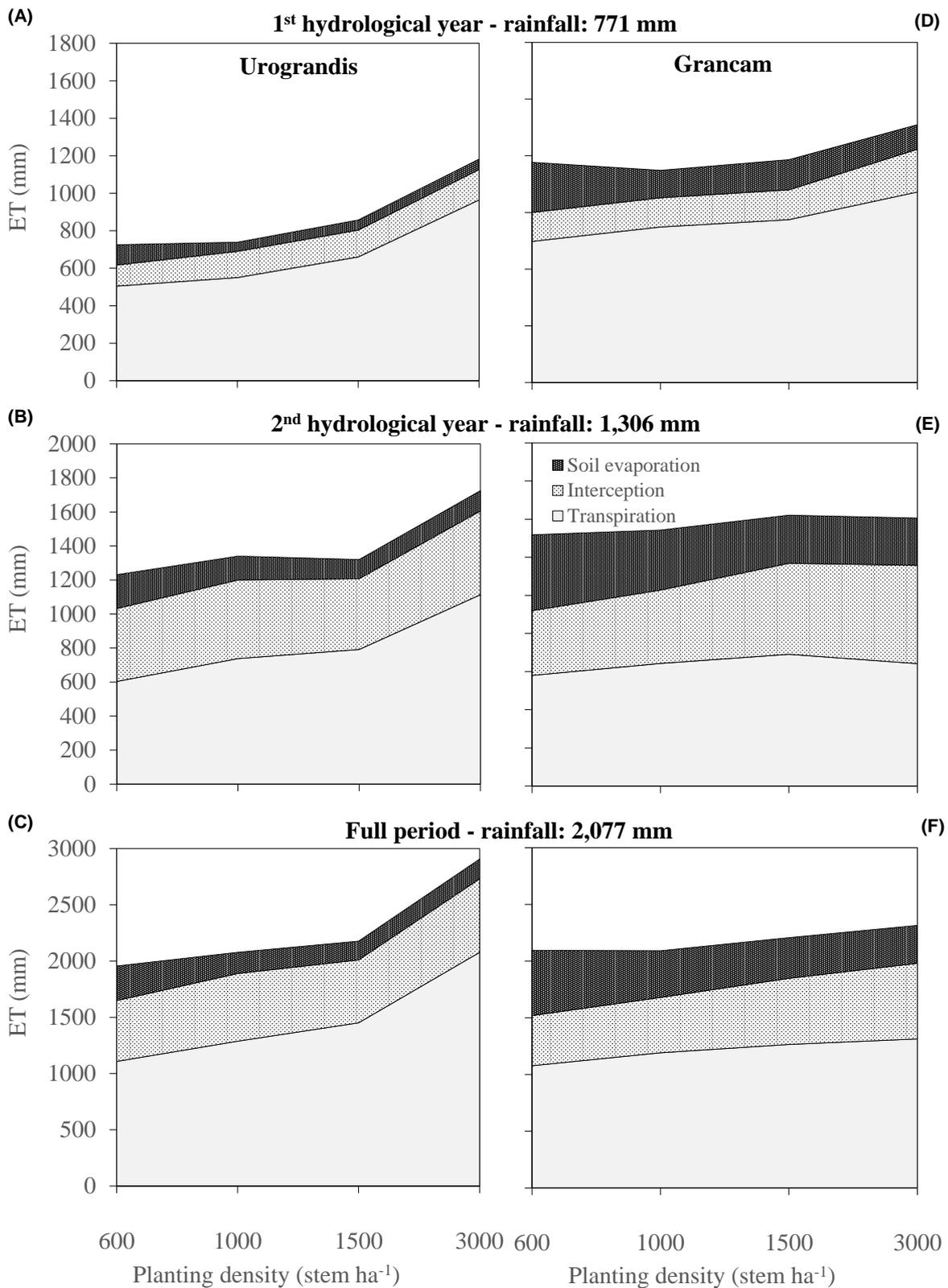


Figure 7 - Sum of water fluxes during the 1<sup>st</sup> and 2<sup>nd</sup> hydrological years and the full period year in forests of (A, B, C) Urograndis and (D, E, F) Grancam with varying densities

SWB was negative at densities higher than 1028 trees ha<sup>-1</sup> (Fig. 8) for both clones. The negative soil water balance in denser stands, i.e., ET > P, reveals a potential problem for the production of blue water, which could affect the equitable use of water at the catchment scale (LIMA et al., 2010; FERRAZ et al., 2013), and plant survival (WHITE et al., 2009; HAKAMADA et al., in press).

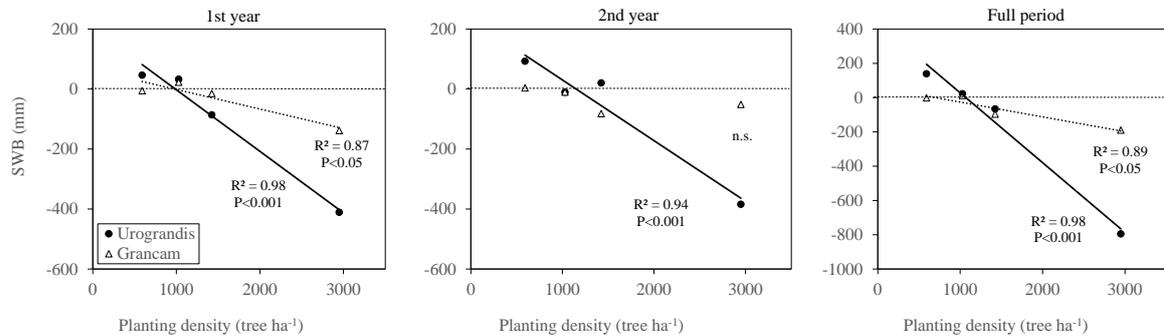


Figure 8 - Relationship between soil water balance (SWB) and planting density during the first and second hydrological year and the full period for two hybrid clones *E.grandis* x *E. urophylla* (Urograndis) and *E. grandis* x *E.camaldulensis* (Grancam). n.s.: not significant

MAI at age 1.7 varied between 31 and 83 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> for the Urograndis clone and between 28 and 42 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> for the Grancam clone, in both cases, MAI increased with density (Fig. 9A). At age 3.7, MAI continued to be higher at denser stands for Urograndis, with values 83 and 42 m<sup>3</sup> ha<sup>-1</sup> yr<sup>-1</sup> for 2,949 and 591 stem ha<sup>-1</sup>. For Grancam, the growth was similar for all densities. During the measurement period, the total volume increment for Urograndis were 65% greater in higher planting density (Fig. 9B) for the Urograndis clone, whereas no differences in growth among densities were observed for the Grancam clone.

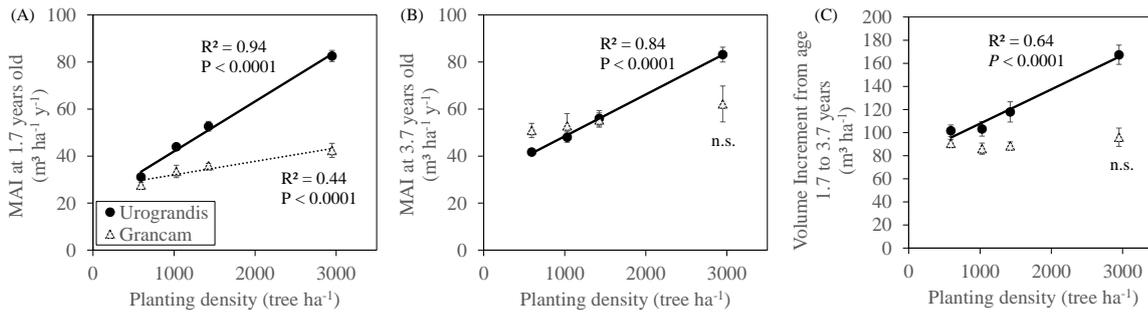


Figure 9 - Mean annual increment (MAI) at age 1.7 (A) and 3.7 (B) and current annual increment (CAI) (C) from age 1.7 to 3.7 for two hybrid clones *E.grandis* x *E. urophylla* (Urograndis) and *E. grandis* x *E.camaldulensis* (Grancam) in planting densities ranging from 591 to 2.949 trees ha<sup>-1</sup>. Error bars represent standard error from the mean. n.s.: not significant

#### 4.4 Discussion

Both spacing and genotype directly affected water balance components. In general, higher densities resulted in more water use and the effect was greater in the Urograndis clone supporting our hypothesis. Average  $E_i$  values for both clones increased at higher densities from 20% of P to approximately 29% of P. However, this pattern was compensated by the inverse pattern of  $E_s$ . Thus, for both genotypes, the component that drove changes to total water use was  $T_{stand}$ . Benyon and Doody (2015) reported similar results using *Eucalyptus globulus* and *Pinus radiata*, where a larger LAI among *Pinus* specimens resulted in greater  $E_i$ , which was compensated by lower  $E_s$  resulting in a similar balance between the two species.

$E_i$  is directly related to leaf angle (CROCKFORD; RICHARDSON, 2000) and branch architecture (BIALKOWSKI, 2015), which could partly explain an average difference of 11% between the two genotypes. In addition Urograndis LAI was on average twofold larger than observed with Grancam at different densities (Fig. 9B). Similar results were reported in previous work, where thinning of 50% of the basal area reduced  $E_i$  from 14% to 8% in *Criptomeria Japonica* (SHINOHARA et al., 2015). In *E.globulus*, the  $E_i$  reached 19% for plants at different ages (BENYON; DOODY, 2015), and varied between 17% and 25% for *E.regnans* trees in their natural habitat, ranging in age from 30 to 200 years (HAYDON et al., 1997). In a region near the current study site, the native vegetation displayed an  $E_i$  of 8% (CABRAL et al., 2015), indicating how planted eucalyptus forests might further impact site water balance.

$T_{\text{stand}}$  was the component that most contributed to the distribution of rainwater, with a general average of 65%. This finding agrees with those reported in previous studies in different ecosystems where  $T_{\text{stand}}$  varied between 59% and 80% of P (DIRMEYER, 2006, JASECHKO et al., 2013, SCHLESINGER; JASECHKO, 2014). However, significant differences occurred between clones and among densities. On average, Urograndis trees had a  $T_{\text{stand}}$  of 741 mm  $y^{-1}$ , 22% above what was observed for Grancam trees. Moreover, there was a significant increase in  $T_{\text{stand}}$ , from 53% to 82% of P, with increasing density. The lower impact of increasing density for Grancam trees (Fig. 5) might be a contribution of *E.camaldulensis* genes present in the clone. This species responds with a smaller reduction of xylem flow when submitted to induce water deficit, in this case, by density (BOURNE et al., 2015).

Other studies have found that density differences either from the initial planting spacing or from later thinning result in alterations in the components of the water balance across different ecosystems. For example, thinning by 50% reduces the  $T_{\text{stand}}$  from 44% to 21% in *Chamaecyparis obtusa* and *Cryptomeria japonica* (TATEISHI et al., 2015) and in semi-arid regions thinning reduced  $T_{\text{stand}}$  of *Pinus halepensis* by 40% (DEL CAMPO et al., 2014, GONZALEZ-SANCHIS et al., 2015). LAI is directly related to  $T_{\text{stand}}$  (WHITEHEAD and BEADLE, 2004; LIANG et al., 2015; ZHU et al., 2015,). SUN et al. (2015) simulated thinning through a reduction of LAI by 20% to 80% of original levels and obtained an increase in water yield ranging from 3% to 13% in a simulation applied to the entire US territory. In *Chamaecyparis obtusa* density reduction from 2,400 to 1,300 plants  $ha^{-1}$  increased TF by 21% (NANKO et al., 2015).

The negative SWB values observed in this study probably resulted from the use of deep ground water, because *Eucalyptus* trees often have deep root systems (e.g. CHRISTINA et al., 2010; LACLAU et al., 2013). At a density of 2.949 Urograndis plants per hectare, high water consumption (ET 44% greater than PPT) resembled what was observed with *E.camaldulensis* at a density of 2.500 plants  $ha^{-1}$  in India (CALDER et al., 1997), where ET was 62% greater than PPT, on average. Mendham et al. (2011) reported that, because the depth of the root system of *Eucalyptus globulus*, there was a reduction in groundwater that would probably not be replenished in a following rotation, thus affecting the timber productivity in that site. Yin et al. (2015) calculated that, among species inhabiting semi-arid environments,

up to 45% of the ET comes from deep water reservoirs. Lower P rates also directly affect the ratio ET/P (XIE et al., 2014), because ET in plants with elevated LAI remains high even with reduced water availability. Christina et al. (2015) identified that after the canopy closure at about 2 years-old in *Eucalyptus grandis* and under similar weather conditions to those we observed, approximately 20% of  $T_{\text{stand}}$  water comes from depths greater than 10 m. Approximately, 1% to 3% of  $T_{\text{stand}}$  comes from the water table after canopy closure. This strategy has been identified in a number of tropical species as an adaptation to water stress (CANADELL et al., 1996; MARKEWITZ et al., 2010).

During the first 12-month study period, the aridity index (PET/P) was twofold higher than the historic average of 0.81. This ratio is directly associated with the potential impact of plant cover on the water available for the streamflow (ZHOU et al., 2015). In areas where the ratio is higher than one, i.e., under water deficit, the degree of change in soil cover impacts more than what is observed in humid areas. Thus, in face of extreme climate events, the use of adaptive management practices becomes even more important (HOLLING, 1978).

The management of planted forests aims to obtain greater productivity and profitability at the end of the rotation period. When the productivity expected from tropical sites was lower because of less intensive forestry practices (e.g., fertilization, intensive weed control) and lower requirement for value-added materials ( $<20 \text{ m}^3 \text{ ha}^{-1} \text{ yr}^{-1}$ ), the optimum density was higher ( $>1600 \text{ trees ha}^{-1}$ ) (SCHONAU; COETZEE, 1984; BALLONI and SIMÕES, 1980; BREDEKAMP, 1987). However, recent studies point to a density of 1,000 to 1,500 trees per hectare as optimal for volume and profit generation for the industries of energy and cellulose (BERNARDO et al., 2004; STAPE; BINKLEY, 2010; GONÇALVES et al., 2014; HAKAMADA et al., 2014). In the present study, conducted during two years of high water deficit, densities below 1,028 allowed for a ratio of  $\text{ET/P} < 1$ . Thus, in face of current climate change issues, it is recommended that densities equal to or below  $1,028 \text{ trees ha}^{-1}$  should be strongly considered. This practice should increase water yield and reduce the potential for water stress (WHITE et al., 2009, HAKAMADA et al., 2016), in addition to maintaining maximum productivity and profitability at the end of rotation (BERNARDO et al., 2004; STAPE et al., 2010; GONÇALVES et al., 2014; HAKAMADA et al., 2014). In an older aged forest of *E.camaldulensis*, Horner et al. (2009) observed that mortality was near zero in densities between 600 and 1,000

trees per hectare, whereas at higher density, tree survival was highly impacted after 30 years. Similar results were found in highly productive forests in Brazil, where densities above 2,000 trees per hectare presented high mortality rates at the peak of LAI, at approximately 3 years of age (HAKAMADA et al., 2014).

How general would these patterns be for other clones at this site, or for these clones at other sites? We evaluated only two clones widely planted in Brazil, but other seven clones were planted in the same trial (no published). All of the nine clones showed the same trend in total stand stemwood biomass, i.e., the higher the planting density, the higher the biomass accumulation. Additionally, this research platform (TECHS, see site description session) has other two trials with the same clones planted in very similar conditions of soil and climate (no published). The two clones in these two other sites evaluated presented exactly the same patterns of growth compared with our field trial. Therefore, we have a reasonable degree of confidence to say our results are consistent and represents a general response of wood growth and soil water balance to planting density for this edafoclimatic condition. However, we recommend future research evaluating soil water balance for the same genotypes in other contrasting climate and soil sites.

Corroborating our hypothesis, water balance components were affected by planting density in a high productive *Eucalyptus* clonal plantation. ET was in average of the two clones 30% greater in the highest density treatment (2,949 stem ha<sup>-1</sup>) compared with the lowest density (591 stem ha<sup>-1</sup>). However, greater difference between lower and higher densities was observed for Urograndis (49%), a non-drought tolerant genotype. The similarity in ET and SWB between the two contrasting hydrological years studied, allowed us to corroborate our second hypothesis, that the drought tolerance level would be related with the effect of planting density in soil water balance. Normally the decision of choosing a planting density is empirically made. Based on our results, the trending short rotation system should not be planted in a density lower than 1,028 hectares in very high potential productivity sites, with a special attention for non-drought tolerant species or genotypes. This decision can alleviate water use by *Eucalyptus* trees and could be a strategy for enhance the balance between wood production and maintenance of ecosystems services in forest plantations.

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## 5. Hypothesis, conclusions and recommendations

Our thesis aimed to evaluate the response of *Eucalyptus* clonal plantation to manipulation of spacing and genetic on physiological traits and soil water balance. In the first study, we discussed the relationship of planting density and different genotypes to water stress, giving a physiological character to the discussion. The second study addressed the efficiency of water use, a key issue for physiological ecology, but also of practical interest in the forest hydrology and forestry. Finally, the third study dealt with the forest hydrology purest way, evaluating the effect of planting density on soil water balance. We had five hypothesis and all of them were corroborated:

- 1) Study 1: Individual trees growing on stands with higher biomass production, regardless of tree genotype and stocking, are subjected to lower leaf water potential, resulting in a trade-off between plant productivity and potential water stress. **CORROBORATED.**
- 2) Study 2: Water use efficiency will not be affected by planting density. Increment in leaf area index because of higher planting density will lead to largest increase in water use and a proportional increase in timber production. **CORROBORATED.**
- 3) Study 2: However, different genotypes will have different levels of transpiration and water use efficiency. **CORROBORATED.**
- 4) Study 3: Increasing planting density will increase evapotranspiration for both clones, explained by the increase in leaf area index (LAI) in higher densities. **CORROBORATED.**
- 5) Study 3: The effect will be greater for the drought sensitive clone Urograndis, once its stomatal conductance is less sensitive than Grancam to the water deficit in the system, in our case, controlled by planting density. **CORROBORATED.**

This study led us to many applicable aspects for silviculture management. First of all, the relationship found between potential water stress and productivity gives us an alert: very high levels of productivity – the main objective of breeding programs and silvicultural practices – should not be the goal, but genotypes with drought tolerance and average productivities. Two similar studies evaluating traits related with drought tolerance were made with eucalypt in the last 2 years (ARNDT et al., 2014; PFAUSTCH et al., 2016) and found very similar results: high growth trees led to high susceptibility to drought. In terms of spacing recommendations, for the region studied, the reduction from 3.333 and 1.667 to 1.111 trees per hectare, three very used stockings, reduced leaf water potential in 19% and 13%, respectively. So, spacing adjustment can be used to reduce water stress in similar conditions.

The second study showed we need to analyze both water use and water use efficiency instead of trying to get the highest water use efficient genotype or silvicultural practice. If only water use efficiency is considered, and considering a small scale plantation, problems with high water use might lead to low hydrosolidarity. We found that the same level of productivity can be reached reducing transpiration (when changing Grancam by Urophylla).

We also found that optimum density to achieve the highest level of productivity used to be higher ( $>1600$  trees  $ha^{-1}$ ). However, recent studies point to a density of 1,000 to 1,500 trees per hectare as optimal for volume and profit generation for the industries of energy and cellulose. In the present study, densities below 1,000 allowed for a ratio of  $ET/P < 1$ . On the other hand, when planting more than 1 thousand trees per hectare the balance was negative, indicating it can consume the deep water more than opened spacings. Thus, in face of current climate change issues, it is recommended that densities equal to or below 1,000 trees  $ha^{-1}$  should be strongly considered. This practice should increase water yield and reduce the potential for water stress (chapter 2), in addition to maintaining maximum productivity and profitability at the end of rotation (STAPE et al., 2010; HAKAMADA et al., 2014).

Our thesis fulfilled some gap knowledges in the relationship between genotypes and spacings in very high productivity plantations, but opened many others. For example, we cannot predict the response of the interaction genotype x spacing in a very dry area or an area with shallow soils. A few genotypes were evaluated and many new species that have been introduced in breeding programs might have

different behaviors. It is also difficult to suggest how is the behavior by the end of rotation. Studies linking leaf and watershed levels are suggested to integrate many scales.

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