Interactions between the Mechanical and Hydraulic Properties of Eucalyptus Trees Under Different Environmental Conditions of Fertilization and Water Availability

Charlene Arnaud, Loïc Brancheriau, Sylvie Sabatier, Christine Heinz, Gilles Chaix, and Mario Tomazello-Filho

Water and potassium are limiting factors for tree growth. Their influence on morphology and anatomy are well known, but their combined effects on tree growth remain to be clarified. A single clone of Eucalyptus grandis was analyzed under different treatments of rainfall exclusion and fertilization. Eight trees per treatment were sampled to investigate the influence of these treatments on the morphological, mechanical, and anatomical traits. The results showed that the fertilization mainly influenced the morphological and anatomical characteristics. Considering the difference between the fertilized and non-fertilized trees, the function of mechanical support was provided through increasing the diameter and the stiffness. Considering the fertilized trees, two different mechanisms occurred inducing a high stiffness: the wood density was higher for trees with rainfall exclusion; the specific modulus was higher for trees with a normal water supply.

Keywords: Tree morphology; Wood anatomy; Mechanical properties; Rainfall exclusion; Potassium fertilization; Eucalyptus

Contact information: a: UMR AMAP, CIRAD, University of Montpellier, 34398 Montpellier, France; b: UR BioWooEB, CIRAD, Univ Montpellier, 34398 Montpellier, France; c: UMR AGAP, CIRAD, University of Montpellier, 34398 Montpellier, France; d: ESALQ, University of São Paulo, Department of Forest Sciences, Piracicaba, SP 13418-900, Brazil; * Corresponding author: loic.brancheriau@cirad.fr

INTRODUCTION

Over the next decade, global climate change will lead to a rise in average temperatures as well as an increase in the frequency and intensity of drought occurrences (IPCC 2013). These phenomena will likely have an impact on the growth and phenology of plants as well as on wood production and quality. Therefore, within a global change context it will be crucial to understand the effects of environmental changes on plant growth to better anticipate potential changes in forest populations (Fonti and Jansen 2012).

Eucalyptus is an important species used in the paper pulp industry as well as for charcoal production. Eucalyptus plantations represent approximately 8% of forest plantations worldwide (Battie-Laclau et al. 2013). Because of climate change, the geographical movement of humid zones as well as an increase in the intensity and length of droughts have reduced the available area suitable for growing eucalyptus (Almeida et al. 2010; Booth 2013). In addition to climatic factors, nutrients, such as nitrogen, phosphorus, and potassium, are also crucial for plant growth. This is particularly the case in tropical and sub-tropical areas, where soils are often significantly weathered; potassium...
is a crucial factor for crop and forest productivity (Triple et al. 2006; Sardans and Peñuelas 2015). It is therefore important to select and analyze functional traits of trees linked to wood production that are likely to be affected by environmental change in the context of forestry practices.

Environmental fluctuations due to global climate change can be expected to decrease radial wood growth and lead to lower wood production (Lebourgeois et al. 2010; Michelot et al. 2012). These fluctuations can also modify wood quality in terms of anatomical and physical traits (Olson and Rosell 2013; Pfautsch et al. 2016). Recent studies on eucalyptus have shown optimization of primary growth and leaf morphology and anatomy following potassium supply (Laclau et al. 2009; Christina et al. 2011; Battie-Laclau et al. 2016). The main results showed an increase in above ground primary production and an increase in leaf area index, in which the latter was due to an increase in leaf lifespan (Laclau et al. 2009). Leaves were thicker, lived longer, and possessed larger intercellular spaces in the parenchyma (Battie-Laclau et al. 2014a). Physiological models have also shown the positive effects of potassium fertilization. Gross primary production, photosynthetic light use efficiency, tree height, trunk diameter, and leaf area index are all substantially reduced when the potassium supply is limited (Christina et al. 2015).

Little is known about how xylem plasticity varies at the intra-specific level in terms of resistance to drought combined with potassium availability (Schreiber et al. 2011). Potassium ions have a direct impact on wood formation via the increased osmotic potential of cambium cells, increased cell expansion, and an increase in vessel diameter (Fromm 2010). This process might play a pivotal role in eucalyptus trees, where fast growth and long distances from soil water supply to leaves constrain the hydraulic architecture. Furthermore, potassium might also have effects on photosynthesis and wood production, especially under drought conditions (Koch et al. 2004; Petit et al. 2010). The main objective of this study was to evaluate the effect of rainfall exclusion and potassium fertilization on the morphological, mechanical, and anatomical traits of Eucalyptus trees.

**EXPERIMENTAL**

**Materials**

**Study site and treatments**

The study site (managed under the aegis of the Escola Superior de Agricultura “Luiz de Queiroz” [Luiz de Queiroz College of Agriculture] – ESALQ) was located near the city of Itatinga in São Paulo State (SP) in southern Brazil (23° 02’ S, 48° 38’ W, and 850 m above sea level). A highly productive clone of *Eucalyptus grandis* used by the Suzano Company (Salvador, Brazil) was planted in June 2010 with 144 trees per plot interspaced by distances of 2 m × 3 m. The experimental treatments included fertilization regimes with two water supply regimes (37% rainfall exclusion and no-exclusion) (Battie-Laclau et al. 2014b). Rainfall exclusion was initiated in 2010 and continued for the entire experiment. Strips of plastic tarpaulin were installed at a height of 0.5 m to 1.6 m to exclude rainfall (Fig. 1). Potassium (17.4 g/m²) was added to the site as KCl only once at three months after planting.

In this study, four treatments were considered: Control, with no potassium and no rainfall exclusion; RExclusion, with no potassium and with rainfall exclusion; Fertilization&RExclusion, with potassium fertilization and rainfall exclusion; and fertilization, with potassium fertilization and no rainfall exclusion.
Methods

Experiment setup

In May 2015, eight trees per treatment were selected and felled (total of 32 trees). For each treatment, trees were grouped according to eight class diameters measured at a height of 1.3 m (diameter at breast height: DBH). One tree was selected from each class diameter to ensure that trees of all diameters were represented in the study. Discs of wood were removed from the base of each tree crown, which was defined as the point of insertion of the most basal leaf-bearing branch. The sub-canopy position on the trunk was selected for its known plasticity in response to environmental variations (Petit et al. 2010; Plavcová and Hacke 2012). A cube was then cut from each disc (1 cm$^3$) for the anatomical and mechanical properties’ measurements and stored at 20 °C ± 2 °C and 65% ± 5% air humidity. The cubes were removed ensuring that the wood samples did not include noticeable areas of reaction wood. The radial positions of the samples were at identical positions (distance relative to the bark) for each disc to compare the wood from different trees at the same point of development. The functional traits measured were grouped as the morphological, mechanical, and anatomical parameters, and their determination was detailed in the following subsections.

Morphology

After felling, the tree height (H) was measured and the slenderness (SL) was approximated from the height/diameter ratio based on the DBH.

Mechanics

The wood cubes were first weighed, and then the volume was calculated with a caliper. Ultrasound measurements (CIRAD, Montpellier, France) were made in the longitudinal direction of the wood cubes to determine the ultrasonic velocity ($V_L$) (Rakotovololonalimanana et al. 2015). The pressure between the transducers and the sample (without wet coupling) was sufficient to neglect the effect of lateral motion in the modulus of elasticity (MOE) determination. The density ($\rho$), the longitudinal elastic modulus (MOE), and the specific modulus (modulus divided by density, MOE / $\rho$) were then calculated according to Eq. 1.
The measurement was repeated three times for each sample and the average value of $V_L$ was used in the computation of the $MOE$:

$$MOE = \rho \times V_L^2$$ (1)

**Anatomy**

Transverse sections were made from each cube following ultrasound measurements using a sliding wood microtome (PFM Medical, Merignac, France) (section thickness 15 µm to 25 µm). The sections were cleared and stained using the following protocol: bleach (8%, 20 min), distilled water (two rinses), acetic acid (10%, 5 min), and toluidine blue (1%, Merck, Darmstadt, Germany) prepared with ethanol (5 min). The wood sections cut with the microtome were then dehydrated in three successive ethanol baths for 5 min each (concentration of ethanol in volume of 70%, 90%, and 100%), then cleared in Histo-Clear (National Diagnostics, Atlanta, USA) (5 min) and mounted on glass slides using HistoMount (Thermo Fisher Scientific, Waltham, USA). Images were captured at 5× magnification using an Olympus BX60 light microscope equipped with an Olympus DP71 camera (Olympus, Rungis, France). Three images were made per slide. The measurements were made using ImageJ software (version 1.51, Wayne Rasband, Research Services Branch, National Institute of Mental Health, Bethesda, MD, USA) and colour thresholding to determine the mean lumen surface ($V_{area}$) and mean lumen diameter ($V_{diam}$). The vessel frequency ($V_{num}$) was calculated from the entire field’s transverse sections using the “cell counter” plug-in. Theoretical specific conductivity ($K_{th}$) was determined according to the Hagen-Poiseuille equation (Tyree and Zimmermann 2002). The average hydraulic diameter of the vessels ($D_h$, Eq. 2) was calculated from measurements of the diameters ($D$) and the number of measured vessels ($N$). The theoretical hydraulic conductivity ($K_{th}$ in kg.s$^{-1}$.m$^{-1}$.MPa$^{-1}$, Eq. 3) was then calculated from $D_h$, the vessel frequency ($V_{num}$), and the water viscosity ($\eta = 1.002 \times 10^{-9}$ MPa.s):

$$D_h = \left(\frac{\sum_D^4}{N}\right)^{0.25}$$ (2)

$$K_{th} = \frac{D_h^4 \pi}{128\eta} \times V_{num} \times 1000$$ (3)

**Data analysis**

Statistical analysis were carried out with R software (version 3.2.1, R Foundation for Statistical Computing, Vienna, Austria). The differences between treatments were analyzed with a multiple comparisons test of Wilcoxon. The statistical level of significance was 5%. The results of the tests were presented in Figures and Tables with letters associated to the groups identified by the Wilcoxon test. The Pearson correlation coefficient was computed to estimate the strength of dependence between the ratio of sampling height to tree height and the measured parameters. Analyses of covariance (ANCOVA) were used to test the influence of the sampling position of the wood discs on the measured parameters.

**RESULTS AND DISCUSSION**

The effect of fertilization was clearly shown on the morphology of the trees compared to the effect of water exclusion (Table 1). The fertilized trees had a height and a diameter larger than the others. These results on the tree development of *Eucalyptus*
grandis were consistent with the growth enhancement observed in other studies. Studies on eucalyptus (Laclau et al. 2009; Battie-Laclau et al. 2013, 2014a, 2014b, and 2016; Christina et al. 2011, 2015) have shown evidence of primary growth optimization due to potassium supply several months after planting. Thirty-six months after planting, Laclau et al. (2009) observed an increase in net aboveground primary production, mainly due to an increase in the leaf area index. Modelling of these trees based on physiological processes has shown evidence of the positive effects of potassium fertilization on primary growth. Figure 2 shows the box plots of tree slenderness according to the different treatments. The control plot was characterized via a high variability of this parameter, and the Wilcoxon test did not clearly separate the fertilized trees from the others. However, the slenderness of the fertilized trees tended to be lower (Fig. 2). Thus, the secondary growth of the trees was increased by the fertilization. The increase of diameter reinforced the mechanical stability of the trees.

**Table 1. Functional Traits Measured According to Treatments**

<table>
<thead>
<tr>
<th>Property</th>
<th>Control</th>
<th>RExclusion</th>
<th>Fertilization&amp; RExclusion</th>
<th>Fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>14.7 a*</td>
<td>15.6 a</td>
<td>19.5 b</td>
<td>20.3 b</td>
</tr>
<tr>
<td>Diameter DBH (cm)</td>
<td>9.3 a</td>
<td>9.6 a</td>
<td>12.9 b</td>
<td>14.4 b</td>
</tr>
<tr>
<td>Slenderness</td>
<td>163.6 a</td>
<td>164.2 b</td>
<td>151.7 ab</td>
<td>142.3 a</td>
</tr>
<tr>
<td>$\rho$ (kg/m$^3$)</td>
<td>548 a</td>
<td>538 a</td>
<td>596 b</td>
<td>554 a</td>
</tr>
<tr>
<td>MOE (MPa)</td>
<td>13828 a</td>
<td>13810 a</td>
<td>15573 b</td>
<td>15967 b</td>
</tr>
<tr>
<td>MOE/$\rho$</td>
<td>25.2 a</td>
<td>25.6 a</td>
<td>26.2 a</td>
<td>28.8 b</td>
</tr>
<tr>
<td>$V_{num}$ (N/mm$^2$)</td>
<td>27.1 a</td>
<td>24.2 a</td>
<td>16.3 b</td>
<td>14.1 b</td>
</tr>
<tr>
<td>$V_{area}$ (µm$^2$)</td>
<td>7744.9 a</td>
<td>7602.4 a</td>
<td>13278.9 b</td>
<td>14459.2 b</td>
</tr>
<tr>
<td>$V_{diam}$ (µm)</td>
<td>123.5 a</td>
<td>124.0 a</td>
<td>163.9 b</td>
<td>172.2 b</td>
</tr>
</tbody>
</table>

*Values in the table are mean values; letters correspond to groups from the Wilcoxon test.

**Fig. 2.** Box plot of tree slenderness according to treatments (letters correspond to groups obtained by the Wilcoxon test)
The sampling height varied according to the trees, ranging from 74.7% of the total tree height for the control plot, 74.5% for the plot with rainfall exclusion, 70.4% with fertilization and rainfall exclusion, to 65.3% for the fertilized plot. This difference in sampling height was mainly explained by the size of the crown, which was larger for the fertilized trees. Tree size is known to have a close connection with the hydraulic properties. Studies of the relationship between the conduit dimensions and stem size have shown that the vessel diameter scales predictably with the stem length or diameter (Anfodillo et al. 2006). However, Pfautsch et al. (2016) showed that climate is a dominant factor in controlling the diameter and frequency of vessels in sapwood of eucalyptus. This was in contradiction with recent meta-analyses that suggested the vessel diameter was a function of tree height and not climate (Olson and Rosell 2013; Olson et al. 2014). Figure 3 shows the correlation coefficients between the ratio of the sampling height to the tree height and the measured properties. These correlations were significant, and the distance to the apex was taken into account in the analysis. An analysis of covariance (ANCOVA), with the effect of fertilization, water exclusion, and the distance to the apex, showed that this latter variable was not significant in the study of these effects (Table 2). The same conclusion was obtained if the height ratio (ratio of sampling height to tree height) was considered in the ANCOVA as a categorical explanatory variable. Thus, this variable was excluded from further analysis.

As for the morphology parameters, the vessel characteristics and hydraulic conductivity were mainly driven by the fertilization (Table 1). Fertilized trees had larger vessels (average diameter and area of 124 µm and 7,700 µm², respectively, for the non-fertilized plots, and 168 µm and 13,900 µm², respectively, for the fertilized ones) but less frequent vessels than non-fertilized trees (average frequency of 25.7 per mm² for the non-fertilized plots and 15.2 per mm² for the fertilized ones).
Table 2. Analysis of Covariance According to the Measured Property*

<table>
<thead>
<tr>
<th>Property</th>
<th>Variables</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \rho )</td>
<td>Treatments; Distance to apex</td>
<td>7.02</td>
<td>0.0028</td>
</tr>
<tr>
<td>MOE</td>
<td>Treatments</td>
<td>13.70</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MOE/( \rho )</td>
<td>Treatments</td>
<td>15.51</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>( V_{area} )</td>
<td>Treatments; Distance to apex</td>
<td>7.60</td>
<td>0.0012</td>
</tr>
<tr>
<td>( V_{diam} )</td>
<td>Treatments; Distance to apex</td>
<td>13.35</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>( V_{num} )</td>
<td>Treatments; Distance to apex</td>
<td>19.34</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>( K_{th} )</td>
<td>Treatments</td>
<td>8.89</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

The theoretical hydraulic conductivity was also particularly high in the case of fertilization (247.0 kg.m\(^{-1}\).MPa\(^{-1}\).s\(^{-1}\) and 273.6 kg.m\(^{-1}\).MPa\(^{-1}\).s\(^{-1}\) for fertilized trees with and without rainfall exclusion, respectively; 153.9 kg.m\(^{-1}\).MPa\(^{-1}\).s\(^{-1}\) and 143.9 kg.m\(^{-1}\).MPa\(^{-1}\).s\(^{-1}\) for the control plot and with rainfall exclusion only, respectively) (Fig. 4). The rainfall exclusion effect can be attenuated in this case as a result of the use of stored water in deep soil (Battie-Laclau et al. 2014b). The soil water content (up to 7-m to 8-m-deep) was reduced to zero two years after planting. As a result, root networks developed deeper within the soil to mitigate the effects of drought; this was observed for all treatments (Laclau et al. 2009). After three and a half years, the roots were already 15.8-m-deep (85% of the total tree height), which corresponded to the depth of the water table at the site (Christina et al. 2011).

Fig. 4. Box plot of theoretical hydraulic conductivity (\( K_{th} \)) according to the treatments (letters correspond to groups obtained by the Wilcoxon test)
The effect of rainfall exclusion on the mechanical parameters was marked if one considered the fertilized trees (Fig. 5). The wood density was higher for the trees fertilized with water exclusion (596 kg/m³ and an average of 547 kg/m³ otherwise, Fig. 5a). However, the longitudinal MOE was similar between the fertilized trees with or without water exclusion (average of 15,770 MPa, Fig. 5b). The specific modulus was high for fertilized trees (without rainfall exclusion, MOE/ρ of 28.8, Fig. 5c).

**Fig. 5.** Box plot of mechanical properties according to the (letters correspond to groups obtained by the Wilcoxon test): (a) density, (b) modulus of elasticity, and (c) specific modulus

The values of the elastic modulus and the specific modulus measured for eucalyptus in this study were consistent with values in the literature. According to the values in Cirad’s Tropix database, *E. grandis* has an elastic modulus of 15,200 MPa and a specific modulus of 23.4. The hybrid, *E. grandis × urophylla*, has an elastic modulus and specific modulus of 15,000 MPa and 27, respectively (Hein *et al.* 2016). Considering the difference between
the fertilized and non-fertilized trees, the function of mechanical support was provided by increasing the diameter (implying a higher moment of inertia) and the MOE values. If the trees fertilized with or without water were considered, two different mechanisms occurred that both induced high MOE values. For rainfall exclusion, the wood density was more resistant to the cavitation phenomenon. A higher wood density implied a higher MOE. For a normal water supply, the specific modulus (intrinsic rigidity of the material) was particularly high. The specific modulus is related to the microfibril angle of the S2 cell wall layer as well as the length of the fibers (Christensen-Dalsgaard et al. 2008). These fiber characteristics were altered in this case to lead to a higher MOE. These results were in agreement with the findings of Christensen-Dalsgaard et al. (2008) who reported that hydraulic and mechanical stress adaptations may be interrelated, and thus physiological responses are complex balances rather than pure optimizations.

CONCLUSIONS

1. The variability of the morphological and anatomical parameters measured were mainly influenced by the fertilization factor. This effect was considered taking into account the evolution of the water storage depth in the soil. An interaction existed between the effects of potassium fertilization and water stress regarding the mechanical properties. The effect of water availability was increased for the fertilized trees, inducing differences in wood density and in microfibril angle.

2. Considering the difference between the fertilized and non-fertilized trees, the function of mechanical support was provided through increasing the diameter and the stiffness. Considering the fertilized trees, with or without rainfall exclusion, two different mechanisms occurred that both induced a high stiffness (MOE values). For rainfall exclusion, the wood density was stronger. For a normal water supply, the fiber characteristics were altered.

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