The effect of soil nutrients and moisture during ontogeny on apparent wood density of *Eucalyptus grandis*

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Knowledge of the effect of soil nutrients, such as K and Na and their interaction with water availability, on the growth and wood properties of the eucalypts, is needed to increase the productivity of commercial plantation forests in Brazil that generate employment and taxes. The present study evaluates the apparent wood density (at 12% wood moisture) of *Eucalyptus grandis* trees at 12, 24, 36 and 48 months old under ambient and lower than ambient (66%) rainfall conditions and K and Na nutrient enrichment. The treatments were two water availability (100 and 66% of the rainfall) and the three nutrient treatments were: K (4.5 kmol/ha), Na (4.5 kmol/ha) and a control (natural conditions). The apparent wood density of samples at breast height (1.3 m) was determined by X-ray densitometry and digital images. Increased nutrients at all four ages and water availability at 36 and 48 months reduced apparent wood density in *E. grandis* trees, however, effects of nutrients are lower under water availability reduction. The radial profile of wood density was higher in four-year-old trees, but there was radial variation in apparent wood density at all ages. These findings predict that, under water stress, apparent wood density will not decline in commercial *E. grandis* plantations fertilized with potassium. The use of sodium, as a substitute of potassium, should consider their negative impacts on wood density of *Eucalyptus grandis* trees.

In Brazil, eucalyptus species are usually cultivated in soils with low fertility, potassium and other minerals scarcity and water deficit¹. The expansion of forest plantations in the country depends on the understanding of the interaction between minerals, such as sodium and potassium, and water availability, and their effect on the seasonality of growth and wood quality.

The total or partial replacement of potassium by sodium in forest plantations has practical and scientific interest². Potassium sources, with sodium in their composition, require less energy in the fertilizer production and, therefore, reducing its final prices. These minerals affect the tree water balance and increase water use efficiency under water stress and improve drought resistance¹⁴.

Because wood is a product of tree growth, factors that affects the growth rate can also affect wood anatomy and therefore wood density and other physical properties. Thus, the application of silvicultural traits in a changing environmental conditions have consider the potential impacts on wood quality.

As well as volume productivity, the wood properties are important factors in forest management, since its affects the transformation process and quality of wood products. In pulp and paper industry wood density is an important attribute for yield and quality of pulp and paper products⁵⁶.

However, the effects of mineral fertilizers in wood density of young eucalyptus trees is poorly studied and with controversial results, indicating an increase⁷, decrease or no effect on apparent wood density with moisture content between 12 and 15%⁸. The mineral fertilization effect on the anatomical, physical and mechanical properties of the wood of eucalyptus trees with high growth rates needs further study⁹.

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Climate change and increased eucalyptus stands have reduced water availability. Water stress can be alleviated with silvicultural practices such as proper fertilization. The tree growth under water stress is usually studied in controlled environment to evaluate the wood quality and, specifically, its apparent wood density ratio between mass and volume, at 12% wood moisture. Destructive methods do not allow a precise evaluation of small specimens, such as the density between growth rings. On the other hand, non-destructive methods, such as radiographic X-ray, can determine the apparent wood density quickly, with greater accuracy and efficiency in data processing. X-ray densitometry characterizes wood quality in terms of its apparent density and has been used to evaluate the effect of eucalyptus tree deterioration in response to white-rot fungi, detecting core-sapwood and effect of forest management on the wood properties, annual biomass production and relation with wood anatomical structure. The apparent wood density of E. grandis, from two to 20 years-old, was 0.46 to 0.80 g/cm³ in the pith and bark, respectively. Digital radiography uses X-ray densitometry to obtain the image of the internal wood structure in relation to its chemical composition, density and moisture content. This methodology accurately detects intr and inter-growth ring variations and can be used to interpret tree growth response to change in climatic conditions, fertilization and other silvicultural practices, and carbon fixation on wood quality.

The wood density is related to mechanical properties, dimensional stability, water flow, carbon fixation, and climate change. Hence, precise methods for its determination, such as X-ray densitometry, are becoming more important.

Given the widespread practice of incorporating high amounts of K during the fertilization of Eucalyptus plantations in water-deficit regions and the potential use of Na as a substitute for K, a split plot experiment of 34% of the data processing was initiated in 2010, in Itatinga, Brazil, to evaluate the interactive effects between K/Na fertilization, water availability, and stand age, on tree growth and wood properties. Here, the objective was to evaluate the effects of K and Na fertilization under water reduction and non-water reduction conditions on the apparent wood density (at 12% wood moisture) using radiographic method, in E. grandis trees from 1 to 4 years after planting.

We intend, therefore, to answer the following questions:

How do additions of K and Na to tree fertilization affect apparent wood density?
How do effects of K and Na supply on wood properties change under rainfall reduction?
The effects of fertilization and water regimes change according tree age?

**Results**

The apparent wood density (at 12% wood moisture) in the first two years of evaluation was similar between treatments (nutrition and water availability). In the first year, Na nutrition decreased significantly the apparent wood density only under normal water availability (Na+/−R). At 24 month old, differences were not significant. K and Na nutrition decreased significantly the apparent wood density at 36 and 48 months only under normal water availability. Under conditions of rainfall reduction, effects of K and Na on apparent density were no significant over time. The reduction of wood density were higher in Na than K treatments. A reduction of water availability increase significantly wood density only under K or Na nutrition (Table 1).

The minimum and maximum apparent wood density with 12, 24 and 36 months old was similar between treatments and ranged from 0.19–0.39 to 0.61–0.82 g/cm³, respectively. At 48 months old, minimum and maximum apparent density reached lowest values under K and Na nutrition, respectively, regardless of water availability (Table 1).

In absence of significant interaction between nutrition and water availability in each age, in general, the results indicate that K and Na nutrition decreased significantly apparent wood density from 24 to 48 months old (Table 1). These effects were stronger in Na nurtured trees. Likewise, reduction of water availability increased significantly apparent wood density.

The first distinct growth ring, formed in the 24th month and representative of the cambial age of eucalyptus trees, was identified in the wood cross section (Fig. 1). The apparent wood density, formed at 24, 36 and 48 months varied in this cross section under rain reduction, between 85 and 100% of its radius. The radial variation of the apparent wood density showed a same pattern in all ages and treatments lower density (0.35–0.50 g/cm³) in the region near the pith with stabilization in the intermediate region (0.50–0.55 g/cm³) and increase in the external region wood, near to the bark (0.55–0.70 g/cm³). However, radial profiles showed a more homogenous wood density along radial direction in non-water stressed trees.

The digital images of the wood cross section of the E. grandis trees, with 24 and 36 months, showed distinct fibrous zones formation (growth bands), mainly in the treatments with rain reduction and Na/−A and K/−A The (Fig. 2). The formation of such fibrous zones indicates the presence of false growth rings, that confound dendrochronology studies.

**Discussion**

Variations in the minimum and maximum apparent wood density are due to the alternation of cell regions with high wall fraction and smaller pore area with those with low wall fraction and large pore area. Anatomical and wood formation characteristics varied with the rain exclusion, with changes in fiber and vessel growth due to the lower cambial activity and, consequently, the trunk diameter growth of the trees. The apparent wood density of the E. grandis trees, at the 12th, 24th, 36th and 48th months old, was lower than that of Eucalyptus grandis x urophylla; E. botryoides; E. camaldulensis; E. cypellocarpa; E. globulus; E. grandis; E. maculate; E. melliodora; E. nitens; E. ovata; E. polyanthemos; E. propinqua; E. regnans; E. resinifera; E. robusta; E. rudis; E. saligna; E. sideroxylon; E. tereticornis and E. viminalis with five to seven years old evaluated with X-ray methodology and with the same moisture content (12% wood moisture). This difference is due to changes in the meristem and the...
mechanical and physiological requirements of the tree development process, represented by increased fiber wall thickness and reduced vessel frequency and diameter, as the mature wood is formed in the trunk of E. grandis and E. grandis × urophylla tree. The density increase over time is due to the xylem production with thin cell exchange, called juvenile wood, during the beginning of the secondary growth with more robust cells produced, forming the mature wood.

The desired apparent wood density varies according to its use, therefore, the increase of this parameter in wood due to environmental stress and the application of potassium and sodium may be positive or not. The increase in the apparent wood density is due to changes in fiber morphology, such as an increase in the cell wall fraction.

The most homogeneous wood formation with K and Na application was also reported with the potassium application in Eucalyptus sp. stands without water stress. This increases the osmotic potential and cellular expansion and, consequently, the cambium activity, altering the wood anatomical characteristics.

The highest density of 48-month-old eucalyptus trees in the control treatment and with rainfall reduction may be related to genetic, environmental and tree age factors. Increased density in regimes with lower rainfall

Table 1. Mean, maximum and minimum apparent wood density (mean ± standard error) of E. grandis trees at four ages in treatments (a) C/+R, without fertilization at 100% rainfall; (b) Na/+R, sodium fertilization (4.5 kmol/ha) at 100% rainfall; (c) K/+R, potassium fertilization (4.5 kmol/ha) at 100% rainfall; (d) C/−R, without fertilization at 100% rainfall; (e) Na/−R, sodium fertilization (4.5 kmol/ha) at 66% rainfall; (f) K/−R, potassium fertilization (4.5 kmol/ha) at 66% rainfall. Means followed by the same letter, per column and age, do not differ by the Tukey test (α = 0.05).

| Treatment | Age (months) | Apparent wood density (at 12% wood moisture) (g/cm³) |
|-----------|-------------|----------------------------------------------------|
|            | Mean        | Maximum | Minimum |
| C/+R       | 0.49 ± 0.04 b | 0.73 ± 0.06 a | 0.33 ± 0.06 a |
| Na/+R      | 0.42 ± 0.04 a | 0.66 ± 0.11 a | 0.25 ± 0.05 a |
| K/+R       | 0.43 ± 0.03 ab | 0.63 ± 0.04 a | 0.27 ± 0.05 a |
| C/−R       | 0.41 ± 0.02 a | 0.66 ± 0.05 a | 0.24 ± 0.05 a |
| Na/−R      | 0.37 ± 0.05 a | 0.60 ± 0.05 a | 0.19 ± 0.08 a |
| K/−R       | 0.41 ± 0.03 a | 0.62 ± 0.02 a | 0.25 ± 0.07 a |
| C/+R       | 0.48 ± 0.01 a | 0.65 ± 0.04 a | 0.35 ± 0.01 a |
| Na/+R      | 0.45 ± 0.01 a | 0.61 ± 0.04 a | 0.30 ± 0.06 a |
| K/+R       | 0.45 ± 0.02 a | 0.62 ± 0.02 a | 0.30 ± 0.06 a |
| C/−R       | 0.48 ± 0.01 a | 0.65 ± 0.02 a | 0.33 ± 0.02 a |
| Na/−R      | 0.46 ± 0.02 a | 0.68 ± 0.02 a | 0.31 ± 0.03 a |
| K/−R       | 0.46 ± 0.01 a | 0.67 ± 0.08 a | 0.31 ± 0.02 a |
| C/+R       | 0.54 ± 0.01 b | 0.72 ± 0.04 a | 0.39 ± 0.01 a |
| Na/+R      | 0.50 ± 0.01 a | 0.71 ± 0.04 a | 0.36 ± 0.06 a |
| K/+R       | 0.50 ± 0.02 a | 0.82 ± 0.02 a | 0.29 ± 0.06 a |
| C/−R       | 0.55 ± 0.01 b | 0.76 ± 0.02 a | 0.37 ± 0.05 b |
| Na/−R      | 0.52 ± 0.02 ab | 0.79 ± 0.02 a | 0.34 ± 0.06 a |
| K/−R       | 0.54 ± 0.01 b | 0.80 ± 0.08 a | 0.34 ± 0.02 ab |
| C/+R       | 0.55 ± 0.02 a | 0.87 ± 0.04 b | 0.31 ± 0.03 a |
| Na/+R      | 0.50 ± 0.01 c | 0.82 ± 0.08 c | 0.35 ± 0.02 ab |
| K/+R       | 0.53 ± 0.02 b | 0.89 ± 0.00 c | 0.31 ± 0.05 b |
| C/−R       | 0.55 ± 0.02 a | 0.89 ± 0.06 ab | 0.36 ± 0.03 a |
| Na/−R      | 0.53 ± 0.01 a | 0.90 ± 0.06 ab | 0.36 ± 0.02 ab |
| K/−R       | 0.54 ± 0.01 ab | 0.90 ± 0.04 a | 0.34 ± 0.02 ab |

Table 2. Apparent wood density (mean ± standard error) (g/cm³) of eucalyptus trees with four ages, per nutritional treatment and water availability. Means followed by the same letter, per column and age, do not differ by the Tukey test (α = 0.05).

| Treatment | Apparent wood density (at 12% wood moisture) (g/cm³) |
|-----------|----------------------------------------------------|
|            | 12 months | 24 months | 36 months | 48 months |
| Control    | 0.39 ± 0.05 a | 0.46 ± 0.01 a | 0.54 ± 0.01 b | 0.55 ± 0.01 c |
| Na        | 0.42 ± 0.03 ab | 0.46 ± 0.02 a | 0.52 ± 0.03 a | 0.53 ± 0.01 b |
| K        | 0.45 ± 0.05 b | 0.46 ± 0.01 a | 0.52 ± 0.02 a | 0.53 ± 0.01 a |
| 48% rainfall | 0.40 ± 0.04 a | 0.46 ± 0.02 a | 0.54 ± 0.02 b | 0.54 ± 0.01 b |
was also reported for *Eucalyptus* sp. trees, being explained by the fiber wall thickness and associated to the plants nutritional state and the water availability\(^9,^{40}\). The trees synthesize more simple sugars by photosynthesis with increasing cambial activity and cellulose biosynthesis and these molecules are incorporated as microfibrils, thickening the secondary wall\(^{37,41}\).

**Figure 1.** Radial profile of the apparent wood density at breast height diameter of the *E. grandis* tree trunks at 48 month in the treatments (a) C/+R, without fertilization at 100% rainfall; (b) Na/+R, sodium fertilization (4.5 kmol/ha) at 100% rainfall; (c) K/+R, potassium fertilization (4.5 kmol/ha) at 100% rainfall; (d) C/−R, without fertilization at 66% rainfall; (e) Na−R, sodium fertilization (4.5 kmol/ha) at 66% rainfall; (f) K−R, potassium fertilization (4.5 kmol/ha) at 66% rainfall.
The more variable apparent wood density of eucalyptus trees in water stress treatments, regardless of nutrition, is due to the wall fiber thickening near the bark due to the smaller cambium activity with osmotic potentials and, consequently, lower cell expansion.

The cambial activity variation rate in response to climatic conditions explains the radial profile of the apparent wood density due to the increase in this parameter in the pith to bark direction and juvenile wood formation with intra- and inter-annual density variation\textsuperscript{16,42,43}. The densitometric profile of eucalyptus trees at four ages differed

**Figure 2.** Digital X-ray images of the wood cross sections of *E. grandis* trees at 12, 24, 36 and 48 months old in the treatments (a) C/+R, 100% rainfall control; (b) Na/+R, sodium at 100% rainfall; (c) K/+R, potassium at 100% rainfall; (d) C/−R, control at 66% rainfall; (e) Na/−R, sodium at 66%; (f) K/−R, 66%, potassium at 66% rainfall.
from that obtained for adult trees (five to seven years) and can be explained by mechanical and physiological requirements resulting from the tree development process with increased fiber wall thickness and reduction of the pore frequency and diameter, comparing juvenile and mature wood in eucalyptus trees14,18,34.

The different contrasts in the *E. grandis* wood at four ages in the treatments are due to the transverse dimensions (width, lumen diameter and wall thickness) of the wood tissue cells and the attenuation intensity of X-ray bundles that run longitudinally in wood sample every 50 μm44. The high precision of the images shows a darker coloration due to the smaller attenuation of the X-ray bundles in the longitudinal and radial parenchyma and the lumen cells of the vessels (mainly)20. The characteristic white spots in the cells of the longitudinal parenchyma indicate the presence of tilose at higher density, filling its lighter color with limits defined by the high X-rays attenuation in the reading process. The light tissue coloring (fibers) indicates narrow fibers with thick walls and reduced lumen. The vessels obstruction by tilose, gums, etc. reduces the treatability, permeability and capillary movement of water in the wood and the drying processes45.

In the treatments without rain reduction, the digital wood images by X-ray irradiation made possible to demarcate and measure the width of the growth rings, being an important tool for dendrochronology. Soil treatments with rainfall reduction, the false ring formation does not allow to count the rings with precision, resulting from the water stress to the plants with fibrous zones formation in more than one period per year, making dendrochronology studies difficult37,46.

The mean apparent wood density at 12% wood moisture of *E. grandis* trees was lower with mineral nutrition in all ages and 100% rainfall at 36 and 48 months. The wood apparent tree density at 36 and 24 months was similar in treatments with water availability and nutrition. The apparent wood density of trees after 48th month was greater with reduced water availability. The eucalyptus wood trees should be continuously evaluated to detect changes in their anatomical structure, density and other properties in K and Na fertilization situations, and water restriction to characterize wood for different uses. The effect of silvicultural practices in forest plantations with water stress and submitted to nutrition with K and Na can be evaluated with digital X-ray images.

In normal conditions of water availability our results indicate detrimental effects of K and Na nutrition in wood density, mainly in older trees. In the context, which a higher trees planted area and climate change will increase water scarcity in many water-limited regions, the effects of fertilizers on wood density of *E. grandis* could be less severe. Effects of K nutrition are negligible and Na nutrition can slightly decrease wood density. Nevertheless, the beneficial effects of K and Na increase tree growth, even under a water availability reduction1,4,26,47, largely compensate for the loss in wood quality for pulp and paper production. These findings predict that, under water stress, apparent wood density will not decline in commercial *E. grandis* plantations fertilized with potassium. The use of sodium, as a substitute of potassium, should consider its negative impacts on wood density of *Eucalyptus grandis* trees.

**Methods**

**Characterization of the area, selection and cutting of trees.** The experiment was installed in the municipality of Itatinga, São Paulo State, Brazil (23°00′40.51″S and 48°44′10.92″W). The climate of the region is humid mesotherm (Cfa), according to Köeppen, with rainfall and average annual temperature of 1,635 mm and 26.2 °C and 28.6 °C in the cooler and hotter months, respectively. The soil is of the type dystrophic yellow red latosol with medium texture and lithology composed by sandstone, Marília formation, of the Bauru group. The soil chemical attributes, in the implantation of the experiment, were characterized up to six meters deep26. The cation exchange capacity in the soil were 0.02 cmolc·kg⁻¹ at 5 cm depth and 0.01 cmolc·kg⁻¹ between 0.05 and 6 meters depth, indicating severe K and Na deficiencies throughout the profile. The experiment was implemented in May 2010 with *E. grandis* clone spaced 3 × 2 m. The basic fertilization was done at planting, with 2,000 kg of dolomitic limestone/ha and fertilized with 75 kg of P₂O₅, 80 kg of N (NH₄(SO₄)₂) and 20 kg of FTE (BR-12) per hectare in all treatments.

The experiment was developed in subdivided blocks, split-plot type. First, each block contained a different age, these blocks were divided by the fertilization type, then, there was a division into two types of water availability, totaling 24 plots. The treatments were: Four different ages (i): 12; 24; 36 and 48 months-old; Three fertilizer doses (ii): 0 (control); 4.5 kmol/ha and 4.5 kmol/ha in KCl and NaCl form, respectively, applied three months after planting and two water availability (ii): 100% and 63% of rainfall (artificial exclusion by soil cover supported by wires and covering 37% of the total soil surface (Fig. 3). These gutters were supported on wood cuttings (height from 1.5 to 0.30 m) in slope to withdrawal the rainwater, preventing the arrival of water in the soil. Leaves and branches, retained in the plastic canvas, were removed periodically.

Four eucalyptus trees were selected per treatment (nutrition and water availability) at 12th, 24th, 36th and 48th months old, totaling 96 trees to determine their apparent wood density (at 12% wood moisture). The basal area of the trees selected was similar to that of the plantation.

**Radial profiles of apparent wood density.** Four wood discs were sectioned at 1.3 m (DBH) of the trees selected at the four ages analyzed. Samples (20 × 10 mm, width x thickness) were cut from these disks, glued on a wooden support and a 2.0 mm thick sample sectioned radially in parallel double circular sawing equipment. The radial sections were conditioned in an air-conditioning chamber (20 °C, 60% relative humidity and 12% wood moisture) for twenty four hours13.
The 2.0 mm thick wood samples were inserted with the cellulose acetate calibration scale into the shielded compartment of the Faxitron X-ray digital equipment LX-60 calibrated for automatic reading (30 Kv, 19 seconds). The digital images, with ultra-contrast and resolution, were saved in TIFCOM format. The apparent wood density diametrical profiles were constructed with the digital images in grayscale and calibration analyzed in ImageJ software. This allowed determining the radial values of wood bulk density (every 50 μm) obtained by the software and transferred to the spreadsheet.

Digital images from the methodology applied to the 2.0 mm thick samples of the wood were obtained from twin samples (0.5 cm thick) cut from the DAP of the trunk of eucalyptus trees at four ages and six treatments, sanded, scanned and put in 20°C, 24h, 60% R.H. and 12% wood moisture conditions.

The apparent wood density (at 12% wood moisture) according to the nutritional treatments (control, Na and K) and water availability (66 and 100% rainfall) and interaction (nutrition x water availability) were submitted to variance analysis (ANOVA) and the Tukey test at 95% probability with the SAS program.

The apparent wood density (mean, minimum and maximum) was determined with the minimum and maximum values of each tree per treatment for the four ages evaluated.

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Author contributions
V.R.C. and R.C.L. conducted the experiment and statistical analyses; M.T.F. and P.G.S. analyzed the results; A.J.V.Z., J.C.Z., M.T.F., P.G.S., R.C.L. and V.C.R. wrote the manuscript and reviewed the final manuscript.

Competing interests
The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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