Impacts of Genetic Variation and Silvicultural Treatments on Loblolly Pine Water Use

By

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Masters project submitted in partial fulfillment of the requirements for the Master of Environmental Management degree in the Nicholas School of the Environment of  
Duke University

**Executive Summary**

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12/11/2022

Approved

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Date

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**Executive Summary**

Loblolly pine (*Pinus taeda*) is of high ecological and economical value in the U.S. for its abundance and rapid growth. *P. taeda* has adapted to a wide range of sites, exhibiting considerable variation in its physiology and morphology. In efforts of understanding such variation, transpiration has become a major study focus for its integral role in tree growth and survival. Past studies have developed methods of quantifying tree transpiration and explored the relationships between transpiration and plastic traits such as productivity observed in *P. taeda*. Understanding elements that affect transpiration provides an opportunity to explain and model *P. taeda* physiological and morphological variation for better forest management.

Transpiration is strongly influenced by crown traits and environmental conditions. In plantations, crown phenotype is developed as a mixed realization of individual crown architecture, silviculture treatments, and environmental variables. The crown architecture largely determines plant’s crown properties, meaning that it defines a plant’s ability to intercept light and transpire. While crown architecture can be innate, different planting density can modify such innate crown architecture where low density planting promotes broad crown development and high planting density encourages narrow crown development due to competition. Vapor pressure deficit (VPD) determines the strength of the force pulling water from tree crowns into the air, while soil relative extractable water (REW) indicates water available for plants to supply transpiration. Transpiration can pause temporarily or permanently during drought conditions due to extreme water potential differences between roots and shoots. This Master’s Project (MP) assessed the variation in *P. taeda* water use concerning planting densities, genetic variation in crown architecture, VPD and REW. With the overall objective of examining variation in *P. taeda* transpiration between silvicultural treatments, two questions were explored in this study:

1. Does transpiration differ with genotype and planting density?
2. How does transpiration respond to VPD and REW across treatments?

I examined sapflux data from an established experiment where four crown ideotypes were planted at 1853 trees per hectare (TPH) and 618 TPH with four replications. Among the four genotypes chosen to represent different crown ideotypes, two genotypes represented broad crown ideotypes, one genotype represented narrow crown ideotype, and one open-pollinated family possessed the crown size between broad and narrow crown genotypes. Transpiration and related properties across eight treatment combinations were compared throughout the growing season and by seasonal trends: 1) directly using the analysis of variance (ANOVA); 2) with VPD and REW as a continuous covariate using analysis of covariance (ANCOVA).

The study found that sapflow was higher in broad-crown genotypes under low planting density but higher in narrow-crowned C3 under high planting density. Under high planting density, transpiration in high density plantations were more responsive to changes in REW across genotypes; between genotypes, the narrow-crowned C3 was more responsive to changes in VPD and REW than the broad-crown genotypes. Under low planting density, the genotypes showed similar the transpiration responses to VPD, whereas the broad-crown genotypes were found to be more sensitive to REW than C3. While many of these conclusions were genotype-specific, more crown trait measurements are necessary to explore the relationship between crown ideotypes and transpiration. Further recommendations in forest management include 1) consideration of the genetic variation in the effect of planting density on self-shading, water use efficiency, and carbon allocation; 2) the adoption of site- and genotype- specific silviculture treatments to achieve high plasticity in growth and other physiological processes.

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**1 Introduction**

As the most common softwood species in the U.S. and the most commercially important timber species in the South (Brender, Belanger, & Malac, 1981), loblolly pine (*Pinus taeda*) contributes over 2 billion tons of annual aboveground live-tree biomass (Oswalt et al., 2019) and supports the timber industry generously. Having successfully established through an extensive range of site conditions, this species demonstrates unforeseen plasticity that raised research interests (Samuelson et al., 2013; Shimizu & Sebbenn, 2008). Transpiration has been studied extensively as a quantitative measure evaluating demonstrated growth and productivity (Curtis et al., 2002; Ma et al., 2007; Reichstein et al., 2007; Valentini et al., 2000).

Plant transpiration is an integrated part of local and global carbon and hydrological cycle (Jasechko et al., 2013). When transpiration occurs, water moves passively from soil into the atmosphere through plant root, xylem, and shoots, carrying necessary nutrients and supports photosynthesis (Sinha, 2004). The amount of water used in this process is defined as sapflow (Hanrahan, 2011). Transpiration (*E*T) responds to both biotic and abiotic factors including individual tree crown architecture, planting density, water content in the air, and water availability in the soil. *E*T changes with tree characteristics as the conductance of water flow varies with each tree’s physiology and morphology (Kimball, 2007). Genetic effects influenced canopy-level gas-exchange and drought resistance, thus affecting hydraulic properties and carbon uptake (Aspinwall *et al*., 2011; Hillel *et al*., 2005). For example, stomata conductance is affected by the density, size, and degree of opening of the stomata that varies with trees (Kirkham, 2014). Crown architecture, or ideotype, is restricted to certain consistent morphological expressions such as crown size, density, branching patterns, and angle of leaves relative to each other (Dickmann, 1985; Martin, Johnsen, & White, 2001). Crown ideotype largely relates to leaf area (expressed as Leaf Area Index, or LAI, m2/m2), an important measure of plant growth and productivity as it determines light interception and *E*T (Vose & Allen, 1988; Wright *et al*., 2004). Although crown traits are innate with a tree’s genetic entry, they can be influenced by environmental factors (Carbaugh, 2015).

Spacing is a common silvicultural practice to achieve various management objectives. Spacing regimes affect *E*T by manipulating the interactions between trees. High planting density promotes competition, thus encourages narrow crown development, and reduces individual tree sizes; in contrast, low planting density promotes broad crown development and larger individual tree size (Carlson *et al*., 2009; Harms, Whitesell, & DeBell, 2000). Past study has shown that, compared to high planting density, low planting density of *P. taeda* yields greater diameter branches and stem, foliage and branch biomass, leaf area and canopy density, longer-lived crown, lower height to live crown and lower foliage to branch mass ratio (Albaugh *et al*., 2019; Akers *et al*., 2012).

Because water movement follows a high to low water potential gradient, moving from wet to dry locations, the water potential between shoots and roots becomes the primary driving force of water movements in trees (Freeman, 2014). *E*T is strongly and positively related to vapor pressure deficit (VPD, in kPa), which measures ambient air water potential as the difference in water content between ambient air and fully saturated air (Lawrence, 2005). Relative extractable water (REW, unitless) is an estimate of the plant water availability and can be calculated from measured volumetric soil water content (Granier et al., 1999). VPD and REW together decide the action and rate of *E*T by modifying the water potential gradient along the soil-plant-atmosphere continuum. Permanent cavitation of water-transporting xylems can occur when extreme water potential difference breaks the water continuum, resulting in declining water conductance that restricts tree growth and maintenance (Zhang *et al*., 2016). To avoid xylem cavitation, plants can cope with restricted water supply by declining their stomatal conductance to reduce or stop transpiring, at the same time pausing photosynthesis (Agurla *et al*., 2018; Oren *et al*., 1999). Thus, *E*T can behave drastically different with periodic variation in water availability.

Transpiration (*E*T) can be either estimated or directly measured. Gauged watershed method simply subtracts runoff from precipitation to generate *E*T (Hasenmueller & Criss, 2013,). Energy balance methods such as the Penman-Monteith Equation considers *E*T as component of an integrated mass-transfer system and estimates *E*T from stomatal conductance (Monteith & Unsworth, 1990). The eddy covariance and flux gradient method calculates flux by computing the covariance between fluctuations in vertical wind velocity and fluctuations of transferred properties such as heat and moisture (Lee & Law, 2004); there are also various hydrological models for estimating *E*T (Vose & Swank, 1992). On the other hand, direct measurements of individual tree sapflow provide the basis and references for above methods and generate most reliable results (Vose *et al*., 2003). Granier (1985) proposed a method to estimate sap flux density (*Fd*, in g H2O m-2 s-1) as a function thermal conductivity. A thermal sensor with two probes, one electrically heated at upper position and one at ambient temperature at lower position, is inserted into the xylem of a tree trunk where water transportation occurs (Liu, Urban & Zhao, 2004). The heat dissipated by the upper probe is cooled by water movement within the stem. The temperature differences between the upper and lower probes can therefore be transformed into sap flux density, or how quickly water is passing through xylem. The point measurements can be scaled up spatially (tree-level and stand-level *E*T) and temporally (daily, weekly, or monthly sums) as per ground unit sapflow (*Fs*, in L m-2) using corresponding sapwood area.

**1.1 Goals & Objectives**

This Master’s Project (MP) exists as part of a larger project collaborated between North Carolina State University, Virginia Tech, United States Forest Service (USFS), and Federal University of Santa Catarina, Brazil. The larger study is a long-term silviculture, site, and genetic experiment in efforts to comprehend *P. taeda* physiology. This MP focused on the Virginia site. With the overarching goal of better understanding *P. taeda* *E*T, the following questions and hypotheses were developed:

1. Does *Et* differ with genotype and planting density?

H1: under high planting density, *Et* is not different between genotypes; under low planting density, *Et* is higher in broad-crown genotypes.

1. How does transpiration respond to VPD and REW across treatments?

H2: *Et* in high density plantations are more responsive to changes in REW.

H3: under high density planting, the transpiration responses to VPD and REW are similar across genotypes; under low density plantation, *Et* in broad-crown genotypes are more sensitive to changes in VPD and REW.

**2 Material and Methods**

**2.1 Study Area**

Three experimental sites were established in the larger study, including one in the Piedmont (Reynolds Homestead Center, Critz, Virginia, northern edge of *P. taeda* range), one located on the coastal plain (Bladen lakes, NC, a typical *P. taeda* site), and one far away from *P. taeda* range (Paraná, Brazil). The data analyzed in this MP solely came from the Piedmont site in Virginia. Although slightly outside of the northern range of P. taeda, the species has established successfully in the region.

**2.2 Data Source and Experiment Design**

The larger experiment was designed as a block plot with a split-split plot design replicated three or four times. Seedlings were planted in 2009. Silviculture (operational/intensive fertilization) was the main plot treatment and spacing and genetic entry were the split plots treatments (Albaugh *et al*., 2018). The split plot treatments included six genetic entries and three densities of planting.

This MP used data from intensive silviculture treatment with four genetic entries and two planting densities from the Virginia site. The treatments were replicated four times (four blocks). Within each block contained eight plots of different treatment combination (one clone planted at one density). Sapflux (*Fd*) measurements were focused on block 4.

In this experiment, genetic entry was the main plot treatment and spacing was the split plot treatment. Four genotypes were chosen: OP, C2, C3, and C4. According to Carbaugh (2015), OP stands for an open-pollinated family and C refers to clones. C3 is considered narrow crown genotype whereas C2 and C4 are considered broad crown genotypes. The narrow crown genotype possesses smaller branch diameter, branch length, and crown volume than the broad crown genotypes. Within the broad crown genotypes, C4 has a slightly larger crown volume than C2. The OP family shares similar branch characteristics to the broad crowned clones with a crown volume in between of broad and narrow crown clones. The genotypes were planted in high density at 1853 trees per hectare (TPH) and in low density at 618 TPH. Each high density plot measured a total of 404.43 (18.3 \* 22.1 m) and each low density plot measured 134.505 (18.3 \* 7.35 m). The analysis had assigned treatment groups where H and L represents high and low planting density, and A, B, C, O represents genotype C2, C3, C4, and OP, respectively.

**2.3 Measurements and Statistical Analysis**

**Sapflux**

Sapflux (*Fd*) was measured in Block 4 using Granier ‘s thermal probe at stand age 8-9 (2016-2017). Briefly, within each treatment plot, eight trees were selected for *Fs* measurement. Each tree had a pair of probes inserted from 0-20 mm (shallow) on the north and south side of the tree. Two of the trees in each plot had an additional probe inserted from 20-40mm (deep). The measurement was made every 30 seconds and then averaged over a 15-minute time step. For the purpose of this study, the dataset was restricted to growing season (Day 152-273, June-September of 2017) when trees were most active (Lyu *et al*., 2020).

The 15-minute-interval raw k-values generated from thermal probes were transformed into *Fd* using Granier’s Equation:

|  |  |  |
| --- | --- | --- |
|  |  | (1) |

where k is the flow index calculated from the temperature differential between heated and non-heated probes. Data was broken down, visually presented, and inspected for erroneous or missing data. The smallest gaps of missing data (<5 entries) from the raw data were interpolated. Relatively small gaps (<48 entries, or half day) of individual probes were filled in with simple linear regression between probes for each 15-minute entry. Gaps larger than 48 entries were gap-filled after calculating daily sums using simple linear regression between probes for each daily sum.

Sapflux (*Fd*) of different orientation and depth were evaluated using paired T tests (Table 1). With an insignificant probability for probe orientation (P=0.46), *Fd* of probes of opposite directions were averaged for the rest of the analysis; with a remarkably significant probability (P<0.001) for probe depth, when sapwood depth exceeds 40mm, *Fd* of the inner most sapwood area was considered half of the 20-40mm area. The 15-minute entries of *Fd* were summed up for each tree across daytime when photosynthetic active radiation (PAR) was above zero, then summed across trees for plot level *Fd.*

**Sapwood Area**

Tree diameter was measured in all blocks in January 2017 before *Fd* measurements and again in January 2018 after *Fd* measurements concluded. Daily tree diameters were calculated using site-specific equations as:

|  |  |  |
| --- | --- | --- |
|  |  | (2) |

|  |  |  |
| --- | --- | --- |
|  |  | (3) |

where: =61.3445, =0.0235; dbhrel is an explanatory variable standing for a tree’s relative size (Tim Albaugh, personal communication); DOY is day of year; DBH is the predicted DBH at any given time; and are the DBH at starting (time 0) and ending points (time 1) of measurement, respectively. After extracting the thickness of the bark, the calculated seasonal sapwood diameters were then transformed into sectioned sapwood areas corresponding to probe depths: outer 20mm, 20mm-40mm, and >40mm areas. The total sapwood area by ground area of each treatment plot was calculated (cm3 m-2).

**Sapflow**

Sapflux (*Fd*) by sapwood section from Block 4 was scaled up temporally and spatially as plot-level sapflow using per unit ground sapwood area calculated for all plots. In sapflow calculation, *Fd* was multiplied by their corresponding sapwood area, where the outer probes measure the outer 20mm of sapwood and the inner probe measures the inner 20-40mm. The 15-minute entries were again summed up for each tree across daytime for sapflow. They were also scaled up to stand level *E*T using plot-specific sapwood area, then divided by plot size to generate sapflow per ground area (*Fs*, L m-2).

**Environmental Variables**

Daily volumetric soil moisture (as % water per volume soil) was available in Block 4 for each treatment plot. REW was calculated from volumetric soil moisture using the equation improvised from Ritchie (1981):

|  |  |  |
| --- | --- | --- |
|  |  | (4) |

To achieve normalization, daily volumetric soil moisture was transferred into REW as the ratio of the difference between each soil moisture entry and minimum soil moisture during our time of interest, and the difference between maximum soil moisture and minimum soil moisture (Equation 4).

Additional weather parameters including VPD and PAR were obtained from on-site weather station that applied to all treatment plots.

**Statistical Analysis**

Sapflow (*Fs*) and sapwood area were expressed on a per unit ground area. Analyses of variance (ANOVA) was used to assess the variation of *Fd*, sapwood area and *Fs* with different genotype and planting density. The ANOVA performed on *Fd* was a pseudo-ANOVA where individual trees were treated as replicas, as the data was only available in Block 4. The entire growing season data was broken down into weekly summaries as the environmental conditions shift during the season. Weekly average *Fs* was plotted by density and treatment for observation. Analyses of covariance (ANCOVA) were used to assess the overall response of daily average *Fs* by week to VPD and REW among treatment groups, where VPD and REW were treated as continuous covariates. The *Fs* response curves to VPD and REW were compared between treatments using ANOVA.

The significance level of P<0.05 was used to determine significant effects. Time (DOY or week) was considered an independent variable and block was considered a random effect in statistical models. All data analysis were performed in Microsoft Excel and RStudio (4.1.3).

**3 Results**

**3.1 Variation in Sapflux**

Sapflux (*Fd*) probe means were compared by orientation and by depth using paired t-tests. *Fd* between north and south facing probes were not statistically different (P = 0.46) whereas those between inner and outer probes were significantly different (P<0.001, Table 1). As mentioned in Methods, *Fd* was averaged across probe orientation and innermost *Fd* was calculated as ½ deep probe readings.

**Table 1.** Probability table of paired t-tests in sapflux



As shown in Figure 1, while both inner and outer probes approached similar lower *Fd* values across treatments on dryer days, the pattern of inner and outer probe *Fd* was drastically different between treatments. *Fd* in genotype A and B showed greater inner-outer probe difference when planted in low density, while *Fd* in genotype C and O showed greater inner-outer probe difference when planted in high density. *Fd* in genotype C exhibited the least variation between high and low planting densities comparing to other genotypes.

Chart

Description automatically generated with low confidence

**Figure 1**. Seasonal sapflux for inner and outer probes within each treatment (Block 4 only; I = inner probe, O = outer probe)

The ANOVA for *Fd* returned significant P values for density, genotype, DOY, and density and genotype interaction (Table 2). *Fd* in high density planting was on average 633 g H2O m-2 s-1 lower than in low density planting. In pair-wise comparison, *Fd* for all treatments were statistically different except for low density planted genotype B and O (Appendix Table 1). Ranking was consistent with genotypes where genotype O had the highest *Fd* followed by genotypes B, A, C across densities (Figure 2).

**Table 2**. Probability table: effects of density, genotype, DOY, and their interactions on sapflux, total sapwood area, and sapflow; sapflux ANOVA was performed within block 4.



Chart, bar chart

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**Figure 2**. Adjusted means of sapflux within Block 4, by density and genotype.

**3.2 Variation in Sapwood Area**

The genotypes shared similar growth patterns during growing season regardless of planting density (Figure 3). Under high density planting, genotype O exhibited lower sapwood area while genotype A, B, C clustered together. Under low density planting, genotype C exhibited higher sapwood area than the other three. Genotype B showed the greatest sapwood area difference between high and low planting density while genotype O showed the least.

**Chart

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**Figure 3**. Total sapwood area by treatment during 2017, growing season from day 152-273; DOY=day of year. Each high density (solid line) plot measured 404.43 and each low density (dashed line) plot measured 134.505 .

The ANOVA for plot level sapwood area returned significant P values for density, genotype, DOY and their two-way interactions (Table 2). Low density planting sapwood area was on average 8.43 cm2 m-2 lower than that of high density planting. According to pair-wise comparison (Appendix Table 2), sapwood area of genotype B and C were not statistically different under high planting density; sapwood area of genotype B and O were not statistically different under low density. Plot level sapwood area was highest in both genotype C and B under high planting density, followed by genotype A then O. In low planting density, sapwood area in genotype C remained the highest, followed by genotype A, then O and B (Table 3, Figure 4).

**Table 3**. Summary of plot level sapwood area (cm2 m-2



Chart, bar chart

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**Figure 4**. Adjusted means of sapwood area and sapflow for all blocks, by density and genotype.

**3.3 Variation in Sapflow**

The ANOVA for sapflow (*Fs*) returned significant P values for density, genotype, DOY, and all of their interactions (Table 2). Low density planting *Fs* was on average of 1.84 L m-2 lower than high density planting *Fs*. In pair-wise comparison, under high planting density, *Fs* in genotype B was significantly higher than in genotype A and O, which were not statistically different; and *Fs* in genotype A and O were significantly higher than in genotype C. Under low planting density, only genotype C and O were significantly higher than genotype B in *Fs* (Figure 4, Appendix Table 3).

**3.4 Seasonal Trend**

Average daily VPD ranged from 0 to 1.59 kPa for all stands throughout the growing season. REW was averaged by planting density (Figure 5). High density REW appeared to decline faster than low density when REW was low and VPD was high.

Chart, line chart, histogram

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**Figure 5**. Daily vapor pressure deficit (VPD) and relative extractable water (REW) averaged by density during growing season. DOY: Date of year

Daily *Fs* averaged by week was plotted by treatment (Figure 6). High density treatments exhibited more stable *Et* patterns between genotypes than low density treatments. In high density planting, genotype A, B, C shared similar *Et* patterns. Genotype O showed statistically similar *Fs* as genotype A though it deviated from the patterns of genotype A, B and C. In low planting densities, *Fs* between different genotypes tended to cluster closely. The genotypes behaved differently during the first and second half of the growing season under low planting density, although over the entire growing season, *Fs* in genotype A, C, and O were not statistically different. *Fs* of genotype A and C remained relatively constant to each other during the entire period; genotype B and O *Fs* exhibited higher variation compared to other genotypes for the first half of the season, especially that genotype O started off the growing season transpiring significantly more than other genotypes. From week 15, the four genotypes displayed similar *Fs* values and trends.

In both densities, low *Fs* values tended to cluster at the valleys when days were dry, while high values exhibited more variation at the peaks during wet conditions. Genotype C in high density and genotype O in low density reached the lowest *Fs* during week 19 in their planting density group; genotype B in high density during week 6 and genotype O in low density during week 8 reached the highest *Fs*.

Chart, histogram

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**Figure 6**. Weekly sapflow average by high (upper) and low density.

**3.5 Effect of VPD and REW**

Comparing the weekly average *Fs* across all genotypes and densities, per unit increase in VPD resulted in 1.83 L m-2 increase in *Fs*; per unit increase in REW results in 0.17 L m-2 increase in *Fs* (F = 97.17, <2.2e-16). Log transformed VPD and REW were positively and linearly related to *Fs* with varied slopes by treatment (Figure 7). *Fs* between treatments responded to VPD and REW similarly, where significant differences existed between genotype A and C, B and C, C and O, and between high and low planting densities (Appendix table 4 & 5).

Chart, scatter chart

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**Figure 7**. Weekly averages of sapflow per ground area against vapor pressure deficit (VPD, upper) and relative extractable water (REW)

Sapflow response to VPD and REW differed with different treatments (Table 4). The ANOVA on the intercepts and slopes of models between *Fs* and VPD and between *Fs* and REW returned significant probability values for density, genotype, and their interactions (Table 5). In pair-wise comparison, within the same genotype, *Fs* responses to VPD and REW were similar between planting densities. Genotype B exhibited the greatest difference in *Fs* response rates to VPD and REW between planting densities while VPD and REW did not affect genotype C *Fs* differently between planting densities (Appendix Table 4 & 5). Genotype B and C showed the greatest difference in their *Fs* responses to environmental variables: *Fs* ingenotype B increased the most with the increase in VPD and REW, and *Fs* in genotype C increased the least under high planting density, and they behaved the exact opposite under low planting density. However, under high planting density, genotypes A and O showed similar *Fs* response to VPD and REW. In low planting density, only genotype B *Fs* responded less to REW than genotype C.

**Table 4**. Mean intercepts and slopes of sapflow responses to vapor pressure deficit (VPD, upper) and relative extractable water (REW) across treatments.



**Table 5**. Probability table on the slopes and intercepts of models between 1) Sapflow and vapor pressure deficit (VPD) 2) Sapflow and relative extractable water (REW).



**4 Discussion**

**4.1 The Effect of Density and Genotype**

The first hypothesis of this study was partially accepted that transpiration (*Et*) was higher in broad-crown genotypes under low planting density. Under low planting density, the narrow-crowned C3 transpired significantly less than the other three genotypes, whereas C2, C4, and OP showed similar sapflow (*Fs*). Contrary to the expectation of finding no differences in *Et* between genotypes in high density plantation, the narrow-crown genotype displayed higher *Et* than the other three.

**Density**

Sapflux (*Fd*) was consistently higher in low density plantations whereas sapwood area and *Fs* were found significantly higher in high density plantations. This can be explained by the bigger-sized trees in low density plantation, where both inner and outer probes were inserted near newly grown, active xylems, while smaller trees had their probes closer to heartwood section which did not transport water (Sperry, 2003).

High density treatment appeared to experience more *E*T variability between genotypes comparing to low density treatment. This agreed with Hakamada et al. (2020) where higher variation in *E*T was found in higher planting densities between Eucalyptus clones especially when water was not limiting. The lack of interaction and competition for resources might have restricted individual tree’s physiological responses under low planting treatment.

**Genotype**

Over the season, C2, C3, and C4 transpired in similar patterns, whereas OP displayed higher variability. While it is commonly agreed that genetic diversity facilitates plant adaptation to varied conditions (McNulty et al., 1997; McDowell et al., 2008), Aspinwall et al. (2011) found that plant physiological processes did not correspond to genetic uniformity. This means that unlike this study, the clones might display higher within-genotype variation in *E*T than the genetically diverse open-pollinated family. At the same time, my finding was supported by Yáñez (2015), who studied the same experiment for both the VA and NC sites. Yáñez only found the variation in growth, crown development, and physiology between genotypes at the VA site potentially due to higher site quality; the clones at the VA site also showed higher productivity than the open-pollinated family. Therefore, the clones might only exhibit higher plasticity under site- and genotype-specific silviculture treatment.

Transpiration (*E*T) in narrow-crowned C3 was the most sensitive to planting density while *E*T in the broadest-crowned C4 was not affected. Presumably, the lower leaf area found in C3 led to lower *E*T under low planting density and the leaf area of C4 was restricted under high planting density. This scenario was plausible as long as crown closure had not been achieved in the narrow-crown genotype at the stage (stand age 9) of the experiment and broad-crown genotypes possessed higher leaf area consistently. Radtke and Burkhart (1999) estimated *P. taeda* crown closure by age 12 regardless of spacing regimes although more initial spacing could delay the occurrence of stem inflection. However, the higher *E*T found in C3 under high density could not be attributed to higher leaf area. Carbaugh (2015) proposed that low density planting reduced self-shading in narrow-crown genotypes whereas high density planting encouraged self-shading in broad-crown genotypes. In this situation, higher leaf area was not a necessary condition for narrow-crown ideotypes to transpire more than broad-crown ideotypes, with or without crown closure.

**Density X Genotype**

Although *Fs* was a product of *Fd* and sapwood area, the effect of density on genotypes were similar in *Fd* and sapwood area but not in *Fs*. *Fs* was more susceptible to the effect of density-genotype interaction. C3 and C4 showed the highest total sapwood area under high planting density and C4 showed the highest total sapwood area under low planting density. Stem size or productivity did not necessarily correspond to *E*T or planting density, instead, variation in water use efficiency (Hubbard et al., 2020) and carbon allocation (King et al., 2008) among genotypes were expected and needed to be examined.

**4.2 Transpiration Responses to VPD and REW**

The study’s second hypothesis was accepted where *Et* in high density plantations were more responsive to changes in REW across genotypes. While VPD was the same for all treatments, water became limiting at a faster rate in high density plantations when REW becomes low (Figure 5) as more trees took up water faster. The first part of the third hypothesis was rejected where the transpiration responses to VPD and REW were expected to be similar across genotypes under high planting density. Under high planting density, the result showed the narrow-crowned C3 to be more responsive to changes in VPD and REW than the broad-crown genotypes. This related to the varied effect of planting density on self-shading between crown ideotypes as discussed earlier. The second part of the third hypothesis was partially accepted where originally, higher rate of *Et* in broad-crown genotypes was expected with changes in VPD and REW under low planting density. Rather, only the transpiration responses to VPD were not different among genotypes, whereas the broad-crown genotypes were more sensitive to REW than C3 under low planting density. Yáñez (2017) confirmed that crown traits in broad-crown genotypes were more subjected to the effect of planting density, site, and silvicultural intensity in the same experiment. Without self-shading occurring at low planting density, the higher leaf area in the broad-crown ideotypes led to higher *Et* and greater *Et* increase with per unit increase in REW.

**5 Conclusion and Recommendations**

This MP explored the variation between crown ideotype and spacing in *P. taeda* water use concerning the effects of VPD and REW. The study found that sapflow (*Fs*) was higher in broad-crown genotypes under low planting density but higher in narrow-crowned C3 under high planting density. Transpiration (*Et*) in high density plantations were more responsive to changes in REW across genotypes. Under high planting density, the narrow-crowned C3 was more responsive to changes in VPD and REW than the broad-crown genotypes. Under low planting density, the transpiration responses to VPD were not different among genotypes, whereas the broad-crown genotypes were more sensitive to REW than C3. However, many of these conclusions were genotype-specific, more quantitative measurements such as leaf area are needed for assessing the relationship between crown ideotypes and *E*T. In addition, the variation in the effect of density on self-shading, water use efficiency, and carbon allocation between crown ideotypes need to be considered when developing forest management objectives. Site- and genotype- specific silviculture treatments are recommended to achieve high plasticity in growth and other physiological processes.

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**Appendix**

**Table 1**. Pairwise comparison in the effect of density and genotypes on sapflux ()



**Table 2**. Pairwise comparison in the effect of density and genotypes on sapwood area (



**Table 3**. Pairwise comparison in the effect of density and genotypes on sapflow (



**Table 4**. Pairwise comparison in the effect of REW and VPD as covariates on transpiration between planting densities



**Table 5**. Pairwise comparison in the effect of REW and VPD as covariates on transpiration between genotypes

