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

By

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12/15/2022

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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**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 productivity in *P. taeda*. Understanding factors that affect transpiration provides an opportunity to explain and to 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 densities 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 the soil through 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 related to 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 genotypes were planted at 1853 trees per hectare (TPH) and 618 TPH with four replications. Among the four genotypes chosen to represent different crown genotypes, two clones represented broad crown genotype, one clone represented narrow crown genotype, 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: 1) directly using the analysis of variance (ANOVA); 2) with VPD and REW as a continuous variables using analysis of covariance (ANCOVA).

Based on this study, 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 was 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 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, additional crown trait measurements are necessary to explore the relationship between crown architecture 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) adoption of site-specific silviculture treatments.

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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 (*Fs*, 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.

Transpiration (*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 vary with trees (Kirkham, 2014). Crown architecture 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 architecture largely relates to leaf area, an important measure positively related to plant growth and productivity as it determines light interception and *E*T (Albaugh et al., 1998; Vose & Allen, 1988; Wright *et al*., 2004). When grown in the open, broad-crown genotypes are expected to have higher leaf area and broader crown, hence higher rate of transpiration (Vose & Allen, 1988). 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 studies have 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 *Fs* 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 *Fd*, 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 *Fs* (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 *E*T 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: *E*T in high density plots is 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 plots, *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). Transpiration (*Et*) was measured as saflow (*Fs*) in 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).

**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 (outer) on the north and south side of the tree. Two of the trees in each plot had an additional probe inserted from 20-40mm (inner). 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, the *Fd* of the outer 0-20cm xylem was greater than *Fd* at 20-40 cm. 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 per unit ground area of each treatment plot was calculated by dividing total sapwood area by plot area (cm3 m-2).

**Sapflow**

Sapflux (*Fd*) by sapwood section scaled up temporally and spatially to estimate sapflow (*Fs*). Here *Fs* was calculated by multiplying *Fd* measured in Block 4 by sapwood area of all the plots, where the outer probes measure the outer 20mm of sapwood and the inner probe measures the inner 20-40mm. When sapwood depth exceeded 40mm, *Fd* of the inner most sapwood area was considered half of the 20-40mm area. The 15-minute entries were summed up for each tree across daytime for *Fs*. Plot-level *E*T was estimated as *Fs* divided by plot size to generate *E*T per ground area (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 C2 and C3 showed greater inner-outer probe difference when planted in low density, while *Fd* in C4 and OP showed greater inner-outer probe difference when planted in high density. *Fd* in C4 exhibited the least variation between high and low planting densities comparing to other genotypes.

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**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 C2 and OP (Appendix Table 1). Ranking was consistent with genotypes where OP had the highest *Fd* followed by C3, C2, C4 across densities (Figure 2).

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



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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, OP exhibited lower sapwood area while the three clones clustered together. Under low density planting, C4 exhibited higher sapwood area than the other three. C3 showed the greatest sapwood area difference between high and low planting density while OP showed the least.

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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 plot measured 404.43 and each low density 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 C3 and C4 were not statistically different under high planting density; sapwood area of C3 and OP were not statistically different under low density. Plot level sapwood area was highest in both C4 and C3 under high planting density, followed by genotype C2 then OP. In low planting density, sapwood area in C4 remained the highest, followed by C2, then OP and C3 (Table 3, Figure 4).

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

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**Figure 4**. Adjusted means of sapwood area and sapflow per ground area 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, C3 had the highest *Fs* while C4 had the lowest; *Fs* in C2 and OP were not statistically different. Under low planting density, only C4 and OP had significantly higher *Fs* than C3 (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.

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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, C2, C3, and C4 shared similar *Et* patterns. OP showed statistically similar *Fs* as C2 though it deviated from the patterns of genotype C2, C3, and C4. 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 C2, C4, and OP were not statistically different. *Fs* of C2 and C4 remained relatively constant to each other during the entire period; *Fs* inC2 and OP exhibited higher variation compared to other genotypes for the first half of the season, especially that OP 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. C4 in high density and OP in low density reached the lowest *Fs* during week 19 in their planting density group; C3 in high density during week 6 and OP in low density during week 8 reached the highest *Fs*.

**Chart, histogram

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**Figure 6**. Daily sapflow average week 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, P <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 C2 and C4, C3 and OP, C4 and OP, and between high and low planting densities (Appendix table 4 & 5).

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

Transpiration (*Et*) 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 interaction (Table 5). In pair-wise comparison, within the same genotype, *Fs* responses to VPD and REW were similar between planting densities. C3 exhibited the greatest difference in *Fs* response rates to VPD and REW between planting densities while VPD and REW did not affect C4 *Fs* differently between planting densities (Appendix Table 4 & 5). C3 and C4 showed the greatest difference in their *Fs* responses to environmental variables: *Fs* inC3 increased the most with the increase in VPD and REW, and *Fs* in C4 increased the least under high planting density, and they behaved the exact opposite under low planting density. However, under high planting density, C2 and OP showed similar *Fs* response to VPD and REW. In low planting density, only C3 *Fs* responded less to REW than C4.

**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**

H1 was partially rejected that transpiration (*Et*) was found higher in broad-crown genotypes under both planting density. Contrary to the prediction of finding no difference in *Et* between genotypes in high density plots, 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. In this study, narrow-crowned C3 displayed higher *Et* than the other three. *Et* in 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 while the leaf area and *E*T 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 across density treatments. Radtke and Burkhart (1999) estimated *P. taeda* crown closure by age 12 regardless of spacing regimes although more initial spacing between trees could delay the inflection age in stem growth. However, the higher *E*T found in C3 under high density could not be attributed to higher leaf area. As found in this study, Carbaugh (2015) reported higher leaf area in broad-crown genotypes than in narrow-crown genotypes at stand age 5 for the same experiment. He 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 genotypes to transpire more than broad-crown genotypes, with or without crown closure.

A significant genotype x density interaction was found in *Et* as measured sapflow (*Fs*). Although *Fs* was a product of sapflux (*Fd*) and sapwood area, the effect of density between genotypes were similar in *Fd* and sapwood area but not in *Fs*. *Fd* was consistently higher in low density plots. This can be explained by the bigger-sized trees in low density plots, 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). Sapwood area was consistently higher in high density plots where 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. However, the effect of genetic entry on *Fs* was not consistent across density treatments. Stem size or productivity thus 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 to assess the relationship between *Et* and productivity. Assuming similar carbon allocation mechanisms across genotypes, the sapwood increment to *E*T ratio across the season represented stem water use efficiency (cm2 L-1). Although under both densities, C4 had the highest sapwood increment to *E*T ratio followed by OP, C4 exhibited higher sapwood increment to *E*T ratio in high density plots while the other three genotypes showed higher sapwood increment to *E*T ratio in low density plots (Table 6). This suggested that water use efficiency was subjected to genotype x density interaction as well (F = 6.06, P<0.01). If carbon allocation patterns differed by genotype, further research must examine the total biomass or carbon ratio across genotypes to estimate treatment effects on water use efficiency. A smaller stem in one genotype could be attributed to more carbon partitioned into the crown or root instead of lower productivity (Litton, Raich, & Ryan, 2007).

**Table 6**. Means and standard errors of sapwood increment (cm2 m-2) to sapflow (L m-2) ratio across densities and genotypes.



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 growth response triggered by well-drained soil at the site; 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.

**4.2 Transpiration Responses to VPD and REW**

I found that *Et* in high density plots were more responsive to changes in REW across genotypes (H2). While VPD was the same for all treatments, water became limiting at a faster rate in high density plots when REW becomes low (Figure 5) as more trees took up water faster. The first part of H3 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, narrow-crowned C3 was more responsive to changes in VPD and REW than the broad-crown genotypes. The second part of H3 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 given higher estimated leaf area (Carbaugh, 2015). Rather, the broad-crown genotypes were only more sensitive to REW than C3 under low planting density, whereas the transpiration responses to VPD were not different among genotypes. This related to the varied effect of planting density on self-shading between crown genotypes as discussed earlier. Self-shading restricted transpiration in broad-crown genotypes, allowing narrow-crown genotypes to transpire more under high 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 at low planting density, the higher leaf area in the broad-crown genotypes led to faster decline in *Et* with decreasing REW.

**5 Conclusion**

This MP explored the variation between crown architecture 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 architecture, *E*T, and productivity. In addition, the variation in the effect of density on self-shading, water use efficiency, and carbon allocation between genotypes need to be considered when developing forest management objectives. The effect of site also needs to be considered given strong site-specific behaviors exhibited between treatments.

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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

