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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[Date] [Month] 2022

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Date

Master’s Project submitted in partial fulfillment of the requirements for the Master of Environmental Management Degree in the Nicholas School of the Environment, Duke University May 2020

**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 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 architecture and environmental conditions. In plantations, crown phenotype is developed as a mixed realization of individual crown ideotype, silviculture treatments, and environmental variables. The crown ideotype largely determines plant’s leaf area, meaning that it defines a plant’s ability to intercept light and transpire. Different planting density can modify such innate crown architecture by changing interaction between individual trees. 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 be paused 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 ideotypes, 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 differently across treatments?

This study 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 density and genotype had significant effects on sapflux, sapwood, sapflow, and transpiration responses to VPD and REW. The interactions between density, genotype and time were responsible for greater variation in transpiration properties but not sapwood properties. Sapwood area and growth rate were consistently highest in broadest-crowned genotype. Distinctions were found only between the broadest-crowned genotype and narrow-crowned genotype, where they exhibited lower transpiration and transpiration responses to REW and VPD when planting in a density unfavorable of their crown ideotype.

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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 elasticity that raises research interests (Samuelson et al., 2013; Shimizu & Sebbenn, 2008). Transpiration has been studied extensively as a quantitative measure evaluating demonstrated traits, with heavy research focuses on 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). Water moves passively from soil into the atmosphere through plant root, xylem, and shoots, carrying necessary nutrients and supports photosynthesis (Sinha, 2004). Transpiration is defined as the amount of water used in this process (Hanrahan, 2011). Transpiration 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 changes with tree characteristics as the conductance of water flow varies with each tree’s physiology and morphology (Kimball, 2007). Specific 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, ), an important measure of plant growth and productivity as it determines light interception and transpiration (Vose & Allen, 1988; Wright *et al*., 2004). Although crown traits are innate with a tree’s genetic entry, crown traits can be influenced by environmental factors (Carbaugh, 2015).

Spacing is a common silvicultural practice to achieve various management objectives. Spacing regimes affect transpiration 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 proven 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). Strongly and positively related to transpiration, vapor pressure deficit (VPD, in kPa) measures ambient air water potential as the difference in water content between ambient air and fully saturated air (Lawrence, 2005). Relative extractable water (REW, as a ratio of water contents) 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, rate, and suspension of transpiration 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 stomata conductance to reduce or stop transpiring, at the same time pausing photosynthesis (Agurla *et al*., 2018; Oren *et al*., 1999). Thus, transpiration can behave drastically different with periodic variation in water availability.

Transpiration can be either estimated or directly measured. Gauged watershed method simply subtracts runoff from precipitation to generate transpiration (Hasenmueller & Criss, 2013,). Energy balance methods such as the Penman-Monteith Equation considers transpiration as component of an integrated mass-transfer system and estimates transpiration 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 transpiration (Vose & Swank, 1992). On the other hand, direct measurements of individual tree sap flow provide the basis and references for above methods and generate most reliable results (Vose *et al*., 2003). Granier (1985) proposed sap flux density 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 sapwood 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 (in ), or how quickly water is passing through xylem. The point measurements can be scaled up spatially (tree-level and stand-level transpiration) and temporally (daily, weekly, or monthly sums) as sapflow using corresponding sapwood area.

**1.1 Goals & Objectives**

This Master’s Project (MP) exists as part of a larger project collaborated between United States Forest Service (USFS), North Carolina State University, Virginia Tech, 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* transpiration, the following hypotheses were developed:

1. Transpiration varies with genotype and planting density.
   1. Under low planting density, transpiration decreases as crown ideotypes become narrower.
   2. Under high planting density, transpiration decreases as crown ideotypes become broader.
2. Transpiration responds to VPD and REW differently with genotype and planting density.
   1. Transpiration decreases as VPD and REW decrease and increases when VPD and REW increase across genotype and planting density.
      1. Under low planting densities, with the crown ideotypes becoming narrower, transpiration sensitivity to VPD and REW increases.
      2. Under high planting densities, with crown ideotypes becoming broader, transpiration sensitivity to VPD and REW increases.

**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). 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 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 sapflux measurement. Each tree had a pair of sapflux 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). Sapflux was measured 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 sap flux density (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 and interesting patterns.

The smallest gaps (<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.

**Sapwood Area**

Tree diameter was measured in all blocks in January 2017 before sapflux measurements and again in January 2018 after sapflux 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 stands for day of year; DBH is the predicted DBH at any given time; and stand for DBHs at starting and ending points of measurement. After extracting the thickness of the bark, the calculated diurnal 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 ().

**Sapflow**

In sapflow calculation, sapflux measurements were 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. Sapflux of different orientation and depth were evaluated using paired T tests (Table 1). With an insignificant probability for probe orientation (P=0.46), 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, sapflux of the inner most sapwood area was considered half of the 20-40mm area sapflux.

The transferred sapflux of individual data points was scaled up temporally and spatially as sapflow values (in L or kg ). The 15-minute entries were summed up for each tree across daytime when photosynthetic active radiation (PAR) was above zero, as transpiration was active mostly during daytime. They were also scaled up to stand level transpiration using plot-specific sapwood area, then divided by plot size to generate sapflow per ground area (in ).

**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 equation improvised from Ritchie (1981, Equation 4):

|  |  |  |
| --- | --- | --- |
|  |  | (4) |
|  |  | (5) |

where wilting point represents the minimum soil moisture plants can endure and field capacity represents the maximum amount of water soil can hold. 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 5).

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

**Statistical Analysis**

Sapflow and sapwood area were expressed on a per unit ground area basis in statistical analysis. Analyses of variance (ANOVA) were performed to assess the variation of sapflux, sapwood area and sapflow with different genotype and planting density. The ANOVA performed on sapflux 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 sapflow was plotted by density and treatment for observation. Analyses of covariance (ANCOVA) were used to assess the overall response of daily average sapflow by week to VPD and REW among treatment groups, where VPD and REW were treated as continuous covariates. The sapflow 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 Probe Position**

Sapflux probe means were compared by orientation and by depth using paired t-tests. Sapflux 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, sapflux was averaged across probe orientation and innermost sapflux was calculated as ½ deep probe readings for sapflow calculation.

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



As shown in Figure 1, while both inner and outer probes approached similar lower values across treatments on dryer days, the pattern of inner and outer probes was drastically different between treatments. Genotype A and B showed greater inner-outer probe difference while planted in low density, while genotype C and O showed greater inner-outer probe difference when planted in high density. 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**. Inner and outer probe sapflux within each treatment (Block 4 only; I = inner probe, O = outer probe)

The ANOVA for sapflux returned significant P values for density, genotype, DOY, and the interactions between density and genotype (Table 2). Low density planting resulted in an average of 633 increase in sapflux. In pair-wise comparison, all densities and genotypes 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 sapflux followed by genotypes B, A, C across densities (Figure 2).

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



Chart, bar chart

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**Figure 2**. Adjusted means of sapflux, sapwood area, sapwood increment, and sapflow, by density and genotype.

**3.2 Variation in Sapwood Area between Treatments**

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**. Sapwood per unit ground area increment during 2017, growing season from day 152-273; DOY=day of year

The ANOVA for total sapwood area returned significant P values for density, genotype, DOY and their two-way interactions (Table 2). Low density planting resulted in an average of 8.43 decline in total sapwood area. According to pair-wise comparison (Appendix Table 2), genotype B and C were not statistically different under high planting density; genotype B and O were not statistically different under low density. Total sapwood area was highest in both genotype C and B under high planting density, followed by genotype A then O. In low planting density, genotype C remained the highest, followed by genotype A, then O and B (Table 3, Figure 2).

**Table 3**. Summary of plot level sapwood area (



The ANOVA for sapwood area increment returned significant P values for density and genotype independently (Table 2). Low density planting caused an average of 0.51 decline in sapwood area increment across genotypes. However, sapwood area increment did not yield statistically significant differences between treatments in pair-wise comparison.

**3.3 Variation in Sapflow between Treatments**

The ANOVA for sapflow returned significant P values for density, genotype, DOY, and all of their interactions (Table 2). Low density planting resulted in an average of 1.84 decrease in daily sapflow comparing to high density planting. In pair-wise comparison, under high planting density, genotype B was significantly higher than genotype A and O, which were not statistically different; and genotype A and O were significantly higher than genotype C. Under low planting density, only genotype C and O were significantly higher than genotype B in sapflow (Figure 2, 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 4). 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 4**. Daily vapor pressure deficit (VPD) and relative extractable water (REW) averaged by density during growing season. DOY: Date of year

Daily sapflow averaged by week was plotted by treatment (Figure 5). High density treatment exhibited more stable patterns than low density treatments. In high density planting, genotype A, B, C shared similar transpiration patterns. Genotype O showed statistically similar sapflow as genotype A though it deviated from the patterns of genotype A, B and C. In low planting densities, transpiration 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, genotype A, C, and O were not statistically different. Genotype A and C remained relatively constant to each other during the entire period; genotype B and O 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 sapflow values and trends.

In both densities, low 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 sapflow during week 19 in their planting density group; genotype B in high density during week 6 and genotype O during week 8 in low density reached the highest sapflow.

Chart, histogram

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**Figure 5**. Weekly sapflow average by high (upper) and low density.

**3.5 Effect of VPD and REW on Transpiration**

Comparing the weekly averages across all genotypes and densities, per unit increase in VPD results in 1.83 increase in sapflow; per unit increase in REW results in 0.17 increase in sapflow (F = 97.17, <2.2e-16). Log transformed VPD and REW were positively and linearly related to sapflow with varied slopes by treatment (Figure 6). The 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 6**. Weekly averages of sapflow per ground area against vapor pressure deficit (VPD, upper) and relative extractable water (REW)

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

**Table 4**. 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**

This MP explored and assessed the variation between silvicultural treatments in *P. taeda* sapflux, total sapwood area, sapwood increment, sapflow per ground area, and sapflow responses to VPD and REW. The study found that although density and genotype affected all above traits, the interactions between density, genotype and time created larger variation in transpiration properties but not sapwood properties. Sapwood area and growth rate were consistently highest in broadest-crowned genotype C4. C4 and narrow-crowned C3 exhibited declined overall transpiration and transpiration responses to REW and VPD when planting in a density unfavorable of their crown ideotype.

**4.1 Response Variables: Sapflux, Sapwood, and Sapflow**

Although sapflow was calculated from sapflux and sapwood area, the interactions between time, density levels and genotypes led to greater variability in transpiration properties than in sapwood properties. The ANOVA performed for sapflux and total sapwood area returned similar significance on explanatory variables, while sapflow was influenced by three more interaction terms (Table 3). Sapflux was found significantly higher in low density plantations whereas sapwood area, sapwood increment, and sapflow 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).

**4.2 Seasonality**

While C2, C3, and C4 transpired in similar patterns over the season, OP displayed higher variability. However, OP was less susceptible to the effect of planting density over the growing season potentially due to its higher genetic variability. It appeared to tolerate low water availability as it was able to transpire less than other genotypes under the driest condition during the season (Figure 5, lower). Drought resistance could be essential to genetic selection under climate change.

**4.3 Environmental Variables: VPD and REW**

Sapflow was asymptotically and positively related to both REW and VPD, where the relationships started linearly and reached certain plateaus as REW and VPD became high (Figure 6). The study’s second hypothesis was accepted where transpiration positively correlated with VPD and REW. The transpiration responses to log-transformed VPD and log-transformed REW among groups constructed different slopes and intercepts, indicating interactions between treatments and environmental covariates.

**4.4 Treatments: Density and Genotype**

As predicted in the first hypothesis, density and genotype had different effects on transpiration. Density was the primary factor affecting sapflux, sapwood, and sapflow, while genotype had varied, more subtle impact on the response variables. High density treatment appeared to experience more variability in *P. taeda* transpiration traits as it yielded more statistically distinctive groups comparing to low density treatment. The lack of interaction and competition could have restricted individual tree’s expression under low planting treatment. However, such variation was not reflected in sapwood properties. Total sapwood area and sapwood increment ranked inconsistently across treatments; C4 showed the highest total sapwood area and sapwood growth across densities. Therefore, tree size or productivity did not necessarily correspond to transpiration or planting density, instead it might be a stable trait within certain crown genotypes but exhibited large variation for other genotypes.

Great distinction was found between C4 and C3 where they almost always behaved as two extremities under each planting density. The greatest seasonal sapflow difference between planting densities was found in C3, and the least was found in C4 (Figure 2). This was also reflected in the response differences to VPD and REW between high and low planting densities. While narrow-crowned C3 exhibited the poorest responses to VPD and REW under low planting density, it was the most sensitive to changes in VPD and REW under high planting density. Whereas broadest-crowned C4 responded most positively under low planting density. This led to the partial acceptance of the study’s first hypothesis, where conflicting crown ideotype and planting density discouraged transpiration, as the difference of two genotypes out of four were supported by the statistics. In the experiment, when C3 and C4 were planted in the density encouraging the opposite crown ideotype, the genotypes consistently demonstrated lower sapflow and transpiration responses to VPD and REW. This partially confirmed our sub-hypotheses under hypothesis 2 where conflicting crown ideotype and planting density reduced transpiration sensitivity to VPD and REW. (Appendix Table 4 & 5). This was because narrow-crowned C3 thrived under high planting density that supported its crown development but struggled under low planting density; similarly, broadest-crowned C4 thrived under low planting density but struggled under high planting density. However, these conclusions only apply to selected genetic entries, more quantitative measurements such as LAI are needed for assessing the relationship between crown size and transpiration.

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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

