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

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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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**Executive Summary**

Loblolly pine (*Pinus taeda,* or *P. 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 variables. In plantations, crown phenotype is developed as a mixed realization of environmental variables, individual crown ideotype and silviculture treatments. 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 VPD, REW, genetic variation in crown ideotypes, and planting densities. With the overall objective of examining variation in *P. taeda* transpiration between silvicultural treatments, two questions were explored in this study:

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

This study examined sapflux data from an established experiment where four crown ideotypes were planted at 250 trees per acre (TPA) and 750 TPA 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 responses across eight treatment combinations were compared throughout the growing season and by seasonal trends: 1) with VPD and REW as a continuous covariate using analysis of covariance (ANCOVA), 2) directly using the analysis of variance (ANOVA).

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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*, or *P. taeda*) contributes over 2 billion tons of annual above-ground 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 integrate 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 abiotic and biotic factors including water content in the air, water availability, individual tree crown architecture, and planting density. 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) measures ambient air water potential as the difference in water content between ambient air and fully saturated air (Lawrence, 2005). Relative extractable water (REW) is converted from total soil water content that only represents the water available for plants (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 changes with tree characteristics as the conductance of water flow varies with each tree’s physiology and morphology (Kimball, 2007). Crown architecture, or ideotype, is restricted to 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 defines 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 it is 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; on the opposite, low planting density promotes broad crown development and individual tree size (Carlson *et al*., 2009; Harms, Whitesell, & DeBell, 2000). Past study has proven that 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 comparing to high planting density (Albaugh *et al*., 2019; Akers *et al*., 2012).

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 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 (Fd, ), or how quickly water is passing through xylem using Granier’s Equation (1). 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 (three planting densities), site (North Carolina Coastal Plain, Virginia Piedmont, and Brazil), and genetic (six genotypes) 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 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. 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 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 Setup**

The experiment was designed as a block plot with a split-split plot design replicated four times (four blocks). Silviculture is the main plot treatment and spacing and genetic entry are the split plots treatments (Albaugh *et al*., 2018). Silviculture treatment includes two densities of planting: high density at 750 trees per acre (TPA) and low density at 250 TPA. Each high density plot measures a total of 404.43 (18.3 \* 22.1 m) and each low density plot measures 134.505 (18.3 \* 7.35 m). Within each block contains eight plots of different treatment combination (one clone planted at one density). Measurements were focused on block 4. The experiment of block 4 was divided into four systems and was set up Table 1:

**Table 1**. Experimental Setup



where 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. Seedlings were planted in 2009.

Block 4 was measured for sapflux and REW while all blocks were measured for diameter at breast height (DBH). Sapflux was measured 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. DBH (in cm) was measured for all blocks at the beginning and the end of each experimental year. Daily volumetric soil moisture (% water per volume soil) was available for block 4 for each treatment plot. Additional data related to the weather parameters were obtained from on-site weather station that applied to all treatment plots.

For the purpose of this study, the dataset was restricted to growing season (Day 152-273, June-September of 2017) when trees are most active (Lyu *et al*., 2020).

**2.3 Data Analysis**

The analysis had assigned treatment groups where H and L represents high (750 TPA) and low planting density (250 TPA), and A, B, C, O represents genotype C2, C3, C4, and OP, respectively.

**Sapflux**

The 15-minute-interval raw k-values generated from thermal probes were transformed into sap flux density 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 checked for erroneous and interesting patterns.

The smallest gaps (<5 entries) in 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**

Daily tree diameters were calculated using the set of empirical equations as:

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

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

where: =61.3445, =0.0235; dbhrel is an explanatory variable standing for a tree’s relative size (Vastaranta *et al*., 2011); 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 3). With an insignificant probability for probe orientation, probes of opposite directions were averaged for the rest of the analysis; with a remarkably significant probability 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 (L or kg ). The 15-minute entries were summed up for each tree across daytime when photosynthetic active radiation (PAR) is above zero, as transpiration is 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 ().

**REW**

REW was calculated 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).

**Seasonality**

The entire growing season data was broken down into weekly summaries as the environmental conditions shift during the season. Statistical analyses were performed on both weekly averages and daily values. Weekly average sapflow was plotted by density and treatment for observation.

**Statistical Analysis**

Sapflow and sapwood area were expressed on a per unit ground area basis in statistical analysis. Analyses of covariance (ANCOVA) were used to assess the response of weekly average sapflow to VPD and REW among treatment groups, where VPD and REW were treated as continuous covariates. Paired T-tests were used to test whether probe orientation and depth affect daily sapflux readings. Two-way analyses of variance (ANOVA) were performed to assess the variation of sapwood area and sapflow with different genotype and planting density. The significance level of P<0.05 was used to determine significant effects. Time (day or week) was considered an independent variable and block was considered a random effect in models. All data analysis were performed in Microsoft Excel and RStudio (4.1.3).

**3 Results**

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

VPD was inversely related with REW throughout the growing season (Figure 1). Valleys of VPD and peaks of REW signified the occurrence of raining events, followed by steady increase in VPD and decrease in REW as the water content in both air and soil declined. 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).

Chart, scatter chart

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**Figure 1**. Daily REW averaged by density during growing season. DOY: Date of year

Logged VPD and REW were positively and linearly related with sapflow with varied slopes by treatment (Figure 2 & 3). All treatments showed similar responses to REW and VPD as covariates, where significant differences existed between genotype A and C, B and C, C and O, and between high and low planting densities (Figure 4).

Chart, scatter chart

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**Figure 2**. Weekly averages of sapflow per ground area and VPD (on a log scale)

Chart, scatter chart

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**Figure 3**. Weekly averages of sapflow per ground area and REW (on a log scale)

Chart, bar chart, waterfall chart

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**Figure 4**. Effect of REW and VPD between genotype treatments and between density treatments

In pair-wise comparison, VPD and REW had similar effects on planting density within the same genotype. 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 between planting densities (Table 2). 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 most negatively in 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 more negatively to REW than genotype C (Table 3).

**Table 2**. Effect of REW and VPD as covariates between planting densities; significance codes: 0 ‘\*\*\*’ 0.001 ‘\*\*’ 0.01 ‘\*’ 0.05 ‘.’ 0.1 ‘ ’)



**Table 3**. Effect of REW and VPD as covariates between genotypes



**3.2 Variation in Sapflux Probe Position**

Sapflux between north and south facing probes were not as nearly significant (P = 0.46) as those between inner and outer probes were (P<2.20E-16, Table 4). As in Method, sapflux was averaged across probe orientation for any further analysis and inner sapflow was calculated as ½ outer probe readings.

**Table 4.** Probability table of paired t-tests in sapflux



As shown in Figure 5, while both inner and outer probes approached similar lower values across treatments, 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. From the greatest to smallest differences, the genotypes rank O, C B, A in high planting density and B, A, O C in low planting density.

A picture containing chart

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**Figure 5**. Inner and outer probe sapflux within each treatment (Block 4 only; I = inner, O = outer)

**3.3 Variation in Sapwood Area between Treatments**

Genotype A, B, C shared similar growth curve while genotype O experienced accelerated rate of growth during growing season regardless of planting density (Figure 6).

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**Figure 6**. Sapwood per unit ground area increment during 2017, growing season from day 152-273

The two-way ANOVA for total sapwood area returned significant P values for density, genotype, DOY and their two-way interactions (Table 6). Low density planting resulted in an average of 8.43 decline in total sapwood area. 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 which were not significantly different (Table 4 & 5). The treatments’ sapwood area on day 152 ranked differently from that on day 273 as treatments possessed different growth rates.

**Table 4**. Summary of plot level sapwood area



**Table 5**. Pair-wise comparison for total sapwood area



The two-way ANOVA for sapwood area increment returned significant P values for density and genotype independently (Table 6). Low density planting caused an average of 0.51 decline in sapwood area increment across genotypes. In the order of greatest sapwood area increment to the least sapwood area increment, the genotypes were ranked as C, O, B, A under high planting and as C, O, A, B under low planting density. However, sapwood area increment did not yield statistically significant differences between treatments in pair-wise comparison.

**3.4 Variation in Sapflow between Treatments**

The two-way ANOVA for sapflow returned significant P values for density, genotype, DOY, and all of their interactions (Table 6). Low density planting resulted in an average of 1.84 decrease in daily sapflow comparing to high density planting. Under high planting density, sapflow in genotype A and O were not statistically different; genotype B was significantly higher than genotype A and O, and genotype A and O were significantly higher than genotype C. Under low planting density, genotype A, C and O were significantly higher than genotype B in sapflow (Figure 7).

**Chart, bar chart

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**Figure 7**. Adjusted means of transpiration across genotypes and densities.

The ANOVA performed for total sapwood area returned similar significance for the independent variables without the three-way interaction (Table 6); there were neither interaction between specific genotype, density level, and DOY in total sapwood linear model. Despite that DOY was no longer a term in sapwood increment ANOVA, there were even fewer interactions where only density and genotype affected girth growth respectively.

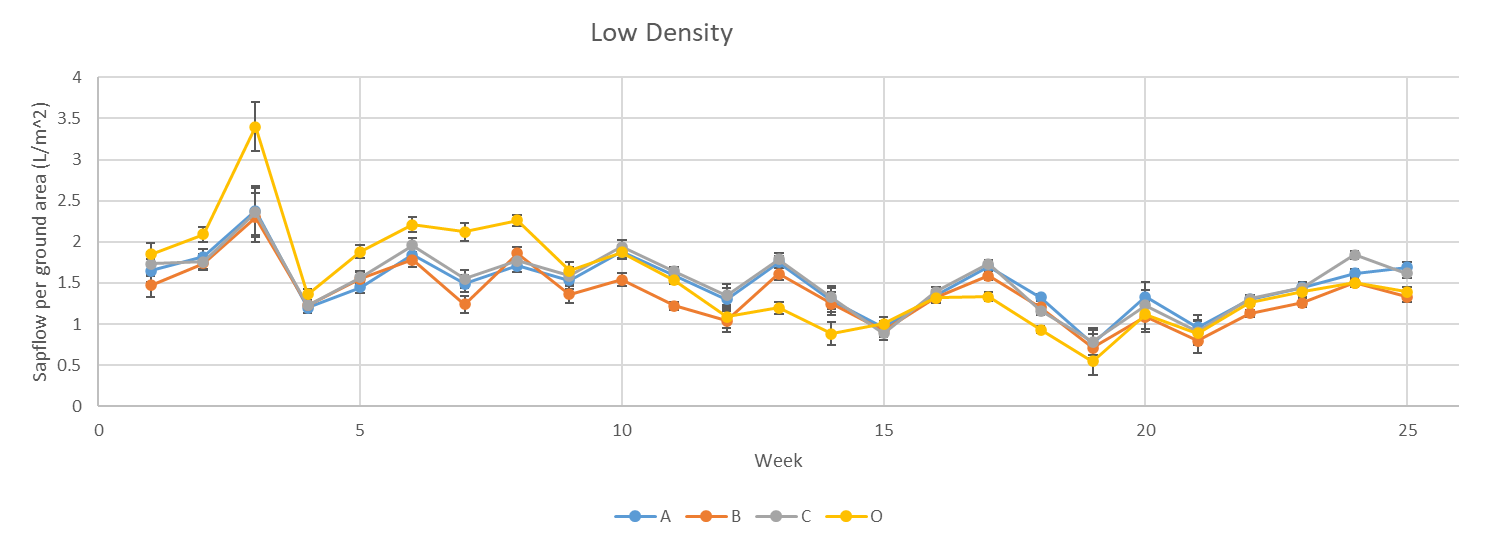
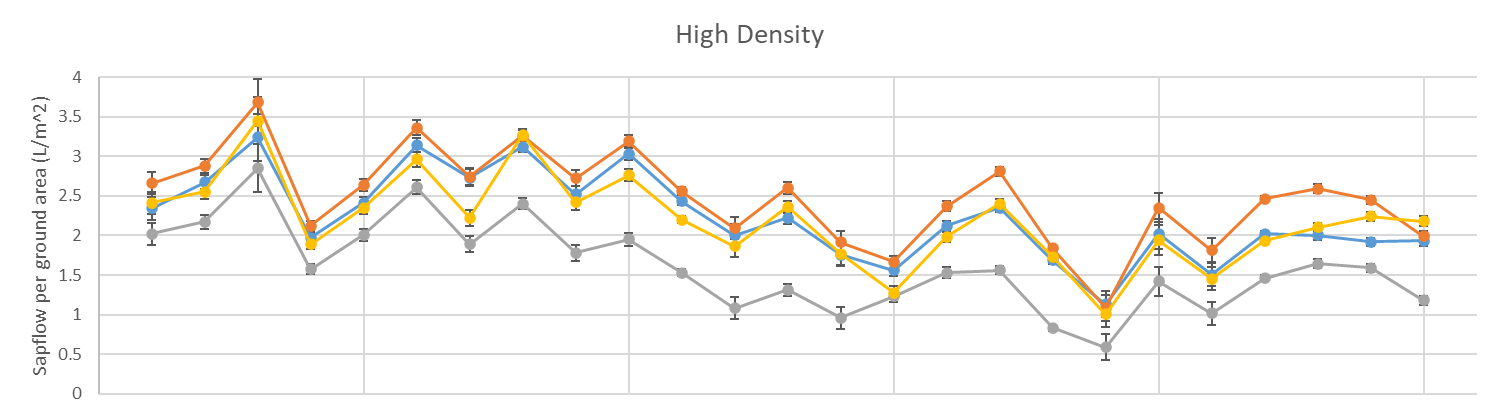
**Table 6**. Summary table: effects of density, genotype, DOY, and their interactions on sapflow, total sapwood area, and sapwood area increment



**3.5 Seasonal Trend**

High density treatment exhibited more distinct sapflow patterns than low density treatments (Figure 8). In high density planting, genotype A, B, C remained relatively constant to each other where B>A>C. Genotype O possessed statistically similar sapflow as genotype A. In low planting densities, the genotypes behaved differently during the first and second half of the growing season, 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 from 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 to each other.

In both densities, low values tended to cluster at the valleys while high values exhibited more variation at the peaks. Genotype C in high density and genotype O in low density reached the lowest sapflow during week 19 in their planting density group; genotype in high density and genotype O in low density reached the highest sapflow during week 3. Genotype O exhibited larger variation comparing to other genotypes regardless of density.

** **

**Figure 8**. Weekly sapflow average by high and low density.

**4 Discussion**

As summarized in Table 7, this MP explored and assessed the variation between silvicultural treatments in sapflow responses to VPD and REW, sapflow per ground area, total sapwood area, and sapwood increment. The study suggested that *P. taeda* performed better when planting density supported their innate crown ideotypes in terms of transpiration but not necessarily for sapwood area.

**Table 7**. Genetic entry ranking by response variable. Genotypes were ranked from the highest value to the lowest under each category; red rectangles indicate pairs or groups that were not statistically different.



**4.1 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. The study’s first hypothesis was accepted where transpiration positively correlated with VPD and REW. The transpiration responses to log-scaled VPD and log-scaled REW among groups constructed different slopes (Figure 2 & 3), indicating interactions between treatments and environmental covariates. Narrow-crowned C3 responded to VPD and REW most positively under high planting density, whereas broadest-crowned C4 responded most positively under low planting density. This confirms our sub-hypotheses under hypothesis 1 where conflicting crown ideotype and planting density reduced transpiration sensitivity to VPD and REW.

Within the same genotype between two planting densities, the differences in sapflux response to both VPD and REW ranked in the same order, where the greatest differences were found in narrow-crowned C3, and the lowest differences were found in broadest-crowned C4. 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. Because high density sapflows were significantly higher than low planting density sapflows, the difference between planting densities within C3 was enhanced by the lower sapflow under low density; the difference between planting densities within C4 was reduced by the lower sapflow under high density.

**4.2 Response Variables: Sapwood and Sapflow**

Although sapflow was calculated with sapwood area, the interactions between density levels and genotypes led to greater variability in transpiration properties than in sapwood area properties. In the experiment, when genotypes were planted in the density encouraging the opposite crown ideotype, the genotypes consistently demonstrated lower response to VPD and REW, and lower sapflow per ground area. This led to the acceptance of the study’s second hypothesis, where conflicting crown ideotype and planting density discouraged transpiration. Although total sapwood area and sapwood increment ranked inconsistently across treatments, sapflow and sapwood increment ranked the same under low planting density; broadest-crowned C4 possessed the highest total sapwood area and sapwood increment across densities. This means that tree size or productivity does not necessarily correspond to transpiration or density, instead it may be a stable trait with certain crown genotypes but exhibits large variation for other genotypes.

**References**

Agurla, S., Gahir, S., Munemasa, S., Murata, Y., & Raghavendra, A. S. (2018). Mechanism of Stomatal Closure in Plants Exposed to Drought and Cold Stress. *Advances in experimental medicine and biology, 1081*, 215–232. https://doi.org/10.1007/978-981-13-1244-1\_12

Akers, M., Kane, M., Teskey, R., Daniels, R., Zhao, D., & Subedi, S. (2012). The effects of planting density and cultural intensity on loblolly pine crown characteristics at age twelve. In *Biennial Southern Silvicultural Research Conference* (p. 1).

Albaugh, T. J., Maier, C. A., Campoe, O. C., Yáñez, M. A., Carbaugh, E. D., Carter, D. R., ... & Fox, T. R. (2020). Crown architecture, crown leaf area distribution, and individual tree growth efficiency vary across site, genetic entry, and planting density. *Trees*, *34*(1), 73-88.

Brender, E.V., Belanger, Roger, P., & Malac, B.F. (1981). *Choices in Silviculture for American* Forests (pp. 144-163). Society of American Foresters.

Carlson, C. A., Fox, T. R., Creighton, J., Dougherty, P. M., & Johnson, J. R. (2009). Nine-year growth responses to planting density manipulation and repeated early fertilization in a loblolly pine stand in the Virginia Piedmont. *Southern Journal of Applied Forestry*, *33*(3), 109-114.

Curtis, P. S., P. J. Hanson, P. Bolstad, C. Barford, J. C. Randolph, H. P. Schmid, and K. B. Wilson (2002), Biometric and eddy-covariance based estimates of annual carbon storage in five eastern North American deciduous forests, Agric. For. Meteorol., 113(14), 319, doi:10.1016/S0168-1923(02)00099-0.

de Oliveira, R. K., Higa, A. R., Silva, L. D., Silva, I. C., & Gonçalves, M. D. P. M. (2018). Emergy-based sustainability assessment of a loblolly pine (Pinus taeda) production system in southern Brazil. *Ecological Indicators*, *93*, 481-489.

Dickmann, D. I. (1985). The ideotype concept applied to forest trees. *Attributes of trees as crop plants*, 89-101.

Freeman, S. (2014). *Biological science*. United States of America: Pearson. pp. 765–766. ISBN 978-0-321-74367-1.

Granier A, Bréda N, Biron P, Villette S (1999) A lumped water balance model to evaluate duration and intensity of drought constraints in forest stands. Ecol Model 116:269–283. https://doi.org/10.1016/ S0304-3800(98)00205-1

Granier, A. (1985). Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. In *Annales des Sciences forestières* (Vol. 42, No. 2, pp. 193-200). EDP Sciences.

Hanrahan, G. (2011). *Key concepts in environmental chemistry*. Academic Press.

Hasenmueller, E. A. , & Criss, R. E. (2013). Water Balance Estimates of Evapotranspiration Rates in Areas with Varying Land Use. In (Ed.), Evapotranspiration - An Overview. IntechOpen. https://doi.org/10.5772/52811

Hubbard, R. M., Hakemada, R., & Ferraz, S. (2015, December). Effects of Planting Density on Transpiration, Stem Flow and Interception for Two Clones Differing in Drought Tolerance in a High Productivity Eucalyptus Plantation in Brazil. In *AGU Fall Meeting Abstracts* (Vol. 2015, pp. H11G-1430).

Jasechko, S., Sharp, Z. D., Gibson, J. J., Birks, S. J., Yi, Y., & Fawcett, P. J. (2013). Terrestrial water fluxes dominated by transpiration. *Nature*, *496*(7445), 347-350.

Lawrence, M. G. (2005). The relationship between relative humidity and the dewpoint temperature in moist air: A simple conversion and applications. *Bulletin of the American Meteorological Society*, *86*(2), 225-234.

Lee, X., Massman, W., & Law, B. (Eds.). (2004). *Handbook of micrometeorology: a guide for surface flux measurement and analysis* (Vol. 29). Springer Science & Business Media.

Lu, P., Urban, L., & Zhao, P. (2004). Granier's thermal dissipation probe (TDP) method for measuring sap flow in trees: theory and practice*. ACTA BOTANICA SINICA-ENGLISH EDITION-, 46*(6), 631-646.

Lyu, J., He, QY., Yang, J. *et al.* Sap flow characteristics in growing and non-growing seasons in three tree species in the semiarid Loess Plateau region of China. *Trees* **34**, 943–955 (2020). https://doi.org/10.1007/s00468-020-01972-1

Ma, S. Y., D. D. Baldocchi, L. K. Xu, and T. Hehn (2007), Inter-annual variability in carbon dioxide exchange of an oak/grass savanna and open grassland in California, Agric. For. Meteorol., 147(34), 157171, doi:10.1016/j.agrformet.2007.07.008.

Martin, T. A., Johnsen, K. H., & White, T. L. (2001). Ideotype development in southern pines: rationale and strategies for overcoming scale-related obstacles. *Forest Science*, *47*(1), 21-28.

Monteith, J., & Unsworth, M. (2013). *Principles of environmental physics: plants, animals, and the atmosphere*. Academic Press.

Oren, R., Sperry, J.S., Katul, G.G., Pataki, D.E., Ewers, B.E., Phillips, N. and Schäfer, K.V.R. (1999). Survey and synthesis of intra- and interspecific variation in stomatal sensitivity to vapour pressure deficit. *Plant, Cell & Environment 22*(12): 1515-1526.

Reichstein, Markus & Ciais, Philippe & Papale, Dario & Valentini, Riccardo & SW, Running & Viovy, Nicolas & Cramer, Wolfgang & Granier, A. & Ogée, Jérôme & Allard, Vincent & Aubinet, Marc & Bernhofer, Christian & Buchmann, Nina & Carrara, Arnaud & Grünwald, Thomas & Heimann, Martin & Heinesch, Bernard & Knohl, Alexander & Kutsch, Werner Leo & Zhao, Mysis. (2007). Reduction of ecosystem productivity and respiration during the european summer 2003 climate anomaly: A joint flux tower, remote sensing and modelling analysis. *Global Change Biology, 13*(3), 634-651.

Ritchie, J. T. (1981). SOIL WATER AVAILABILITY. *Plant and Soil*, *58*(1/3), 327–338. http://www.jstor.org/stable/42933793

RStudio Team. (2015). *RStudio: Integrated Development Environment for R*. Boston, MA. Retrieved from http://www.rstudio.com/

Samuelson, L. J., Eberhardt, T. L., Bartkowiak, S. M., & Johnsen, K. H. (2013). Relationships between climate, radial growth and wood properties of mature loblolly pine in Hawaii and a northern and southern site in the southeastern United States. *Forest ecology and management, 310*, 786-795.

Shimizu, J. Y., & Sebbenn, A. M. (2008). Espécies de Pinus na silvicultura brasileira. *Pinus na silvicultura brasileira. Colombo: Embrapa Florestas*, 49-74.

Sinha, R. K. (2004). *Modern Plant Physiology*. Alpha science international Ltd.

Souza, B. M., de Aguiar, A. V., Dambrat, H. M., Galucha, S. C., Tambarussi, E. V., da Silva Sestrem, M. S. C., ... & Longui, E. L. (2022). Effects of previous land use on genotype-by-environment interactions in two loblolly pine progeny tests. *Forest Ecology and Management*, *503*, 119762.

Valentini, R., Matteucci, G., Dolman, A. J., Schulze, E. D., Rebmann, C., Moors, E. J., Granier, A., Gross, P., Jensen, N. O., Pilegaard, K., Lindroth, A., Grelle, A., Bernhofer, C., Grünwald, T., Aubinet, M., Ceulemans, R., Kowalski, A. S., Vesala, T., Rannik, U., Berbigier, P., … Clement, R. (2000). Respiration as the main determinant of carbon balance in European forests. *Nature, 404*(6780), 861–865. <https://doi.org/10.1038/35009084>

Vose, J. M., & Allen, H. L. (1988). Leaf area, stemwood growth, and nutrition relationships in loblolly pine. *Forest science*, *34*(3), 547-563.

Vose, J. M., Harvey, G. J., Elliott, K. J., & Clinton, B. D. (2003). Measuring and modeling tree and stand level transpiration. *Phytoremediation: transformation and control of contaminants*, 263-282.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., ... & Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, *428*(6985), 821-827.

Zhang, Y. J., Rockwell, F. E., Graham, A. C., Alexander, T., & Holbrook, N. M. (2016). Reversible leaf xylem collapse: a potential “circuit breaker” against cavitation. *Plant Physiology*, *172*(4), 2261-2274.