**[This writing sample only contains Introduction and Method section given the project is still ongoing]**

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

By

Azura Liu

Dr. Sari Palmroth, Advisor

Dr. Christopher Maier, Advisor

8/26/2022

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**

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 individual crown ideotype and environmental factors (primarily through silviculture treatments). Vapor pressure deficit (VPD) determines the strength of the force pulling water 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. This Master’s Project (MP) assessed the variation in *P. taeda* water use concerning genetic variation in crown ideotypes, planting densities, VPD, and REW. With the overall objective of examining variation in *P. taeda* transpiration, two questions were explored in this study:

1. Does transpiration differ with genotype and planting density?
2. How does transpiration respond to VPD and soil moisture across 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. Transpiration responses 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 as a continuous covariate and REW as a discrete covariate using ANCOVA.

**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), *P. taeda* contributes over 2 billion tons of annual above-ground biomass (Oswalt et al., 2019) and supports the timber industry generously. Having established through an extensive range of site conditions, the 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 tree 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. 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, m^2/m^2), 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 meet 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 (Carlson et al., 2009; Harms, Whitesell, & DeBell, 2000)—vice versa for low planting density. 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).

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, VPD measures ambient air water potential as the difference in water content between ambient air and fully saturated air (Lawrence, 2005). REW is converted from total soil water content that only represents the water available for plants (Granier et al., 1999). VPD and soil moisture 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 closing their stomata to stop transpiring, at the same time pausing photosynthesis (Agurla et al., 2018). 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 for above methods and generates 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, g H2O/m^2/s), or how quickly water is passing through xylem using the empirical function Fd = 119 \*k^1.231, where k is the flow index calculated from the temperature differential between heated and non-heated probes. The point measurements can be scaled up spatially (tree-level and stand-level transpiration) and temporally (daily, weekly, or monthly sums) 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 Master Project focuses on the Virginia site intending to assess the variation in *P. taeda* water use with the overarching goal of better understanding of *P. taeda* physiology.

To understand how *P. taeda* transpiration is affected by crown architecture and planting density, accounting for variation in VPD and REW, the following hypotheses were developed:

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

**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 & 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 TPA and low density at 250 TPA. Each high density plot measures a total of 404.43 m^2 (18.3 \* 22.1 m) and each low density plot measures 134.505 m^2 (18.3 \* 7.35 m).

Within each block contains eight plots of different treatment combination (one clone planted at one density). The experiment of the measured block was divided into four systems and was set up as in 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 crowned clones. Seedlings were planted in 2009.

Sapflux was measured as raw k values using Granier ‘s thermal probe at stand age 8-9 (2016-2017) in block 4. 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.

Tree diameter (cm) was measured for all trees 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.

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.

**Data Cleaning**

The 15-minute-interval raw k-values generated from thermal probes were transformed into sap flux density using Granier’s Equation (Fd = 119 \*k^1.231). Data was broken down, visually presented, and checked for erroneous and interesting patterns.

**Gap-filling**

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 provided by Dr. Maier as:

dbhrel = 1 / (1+(b0\*(exp(-b1\*DOY))))

DBH = ((DBH1-DBH0)\*dbhrel)+DBH0

Where: b0=61.3445, b1=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; DBH0 and DBH1 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 was calculated.

**Sapflow**

In sapflow calculation, sapflux measurements were multiplied by sapwood area, where the outer probes measure the outer 20mm of sapwood and the inner probe measures the inner 20-40mm. When sapwood depth exceeds 40mm, sapflux of the inner most sapwood area was considered half of the 20-40mm area sapflux.

The transferred sapflux (g H2O/m-2/s-1) of individual data points was scaled up temporally and spatially as sapflow values (L or kg H2O). Sapflow between north-facing and south-facing directions was evaluated using a paired T test. With an insignificant probability (P>0.05), the direction of probe was thus ignored for the rest of the analysis. 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 (L/m^3).

**REW**

Daily volumetric soil moisture was transferred into REW to achieve normalization. REW was calculated 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 (REW = (soil moisture - minimum soil moisture) / (maximum soil moisture – minimum soil moisture), Ritchie, 1981).

**Seasonality**

REW was averaged across densities to address density and soil moisture interactions (Figure 1). Weekly transpiration averages were calculated to assess the seasonal trend. REW values below 0.4 were categorized as dry soil condition, values between 0.4-0.6 as intermediate soil condition, and values above 0.6 as wet soil condition.

Chart, scatter chart

Description automatically generated

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

**Statistical Analysis**

Linear models were developed to test my objectives respectively. Whether transpiration vary with genotypes and planting densities or not was tested using a two-way analysis of variance (ANOVA). Analyses of covariance (ANCOVAs) were used to test the response to VPD under different levels of REW among treatment groups, both for the entire growing season and for different periods broken down by seasonality. REW was treated as a categorical covariate. The significance level of P<0.05 was used to determine significant effects. All models consider time (DOY) as an independent variable and Block as a random effect. All data analysis were performed in Microsoft Excel and RStudio (4.1.3).

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