

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

By

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

[I/WE] certify the following:

Does this proposed MP involve human subjects research? ☐ Yes ☒ No

If yes, has an approved IRB protocol been obtained? ☐ Yes ☐ No

Does this proposed MP involve the use of animals in research? ☐ Yes ☒ No

If yes, has an approved IACUC protocol been obtained? ☐ Yes ☐ No

Does this proposed MP involve signing a non-disclosure agreement? ☐ Yes ☒ No

If yes, does the advisor have a signed copy? ☐ Yes ☐ No

Student Signature:  Date: 7/4/2022

Advisor Signature:  Date: 7/4/2022

Part I: Scope of Work

Introduction

Water moves passively from soil into the atmosphere through the root, xylem, and shoots of trees, carrying necessary nutrients and supports photosynthesis (Sinha, 2004).

Transpiration is defined as the amount of water used in this process (Hanrahan, 2011).

Plant transpiration is an integrate part of local and global carbon and hydrological cycle (Jasechko et al., 2013).

Transpiration responds to both biotic and abiotic factors including individual tree crown architecture, planting density, water content in the air, and soil moisture.

Transpiration changes with tree characteristics as the conductance of water flow varies with each tree's morphology and physiology (Kimball, 2007). Crown architecture, or ideotype, is restricted to consistent morphological expressions including crown size, density, branching patterns, angle of leaves relative to each other, etc. (Dickmann, 1985; Martin, Johnsen, & White, 2001). Although it is innate with a tree's genetic entry, the traits can be influenced by environmental factors (Carbaugh, 2015). Crown ideotype largely defines leaf area, an important measure of plant growth and productivity as it determines light interception and transpiration (Vose & Allen, 1988; Wright et al., 2004).

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 and reduces individual tree sizes (Carlson et al.,

2009; Harms, Whitesell, & DeBell, 2000), thus encourages narrow crown development—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 dryer places, the low water potential in the ambient air becomes the primary driving force deciding how much water is pulled out through leaf stomata (Freeman, 2014). This water potential, or how “wet/dry” the air is, can be expressed as vapor pressure deficit (VPD, is measure of how much water is in the air versus the maximum amount of water vapor that can exist in that air or fully saturated air) (Lawrence, 2005).

Soil water availability is another critical consideration especially when we are facing the challenge of climate change, as drought is the primary factor contributing to reduced productivity and increased mortality (Allen et al., 2010). Zhao and Running (2009) estimated a drought-induced reduction of 0.55 petagram carbon in global net primary productivity from 2000 to 2009. Plants can cope with restricted water supply by closing their stomata to stop transpiring, at the same time pausing photosynthesis (Agurla et al., 2018). Permanent cavitation of water-transporting xylems can occur when extreme water potential difference “breaks” the water continuum, consequently, reduces a tree’s ability to

conduct water, to grow and to survive (Zhang et al., 2016). Thus, transpiration can be affected by stomata closure drastically under different water availabilities, expressed as soil moisture measurements. The amount of water available to plants can be expressed as soil moisture percentage.

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 affects the temperature of the lower probe. The temperature differences between the two probes can therefore be transformed into sap flux density, or how quickly

water is passing through xylem using the empirical function $F_d = 119 * k^{1.231}$, where F_d is sap flux density ($\text{g H}_2\text{O}/\text{m}^2/\text{s}$) and k is the flow index calculated from the temperature differential between heated and non-heated probes. From the point measurements, we can scale it up to tree-level and stand-level transpiration using sapwood area.

Goals & Objectives

In this MP, we will test two biotic factors that theoretically affect transpiration: crown architecture and planting density. Four genetic entries are chosen to represent different crown architectures that represent narrow and broad crown ideotypes. We are interested in how water costs might differ for each ideotype and planting density, along with their interactions. We also want to test the variation in responses to environmental factors. We will evaluate whether there is a difference in how each treatment group respond to VPD; because the stomata closure response can change transpiration drastically, we are also interested in how *P. taeda* transpiration responds to VPD with seasonal changes in soil moisture.

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 further understand *P. taeda* physiology. This Master Project focuses on the Virginia site intending to assess the variation in *P. taeda* water use.

Goals:

- To enhance the understanding of *P. taeda* physiology.

Objective:

- To understand how *P. taeda* transpiration is affected by crown architecture and planting density, accounting for variation in environmental parameters (VPD, soil moisture, and light availability), in efforts to answer the following sub questions:

1. Does transpiration vary with genotypes and planting densities?
2. Does transpiration respond to VPD differently with different genotypes and planting densities?
3. Does response to VPD vary with different levels of soil moisture between genotypes and planting densities?

Methods and Sources of Support

Study Area

Three experimental sites were established in the larger study, including one in the Piedmonts (Reynolds Homestead Center, 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 (Renova Forest, 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 Piedmont.

Data Source & Experiment Setup

The experiment is a block plot with a split-split plot design replicated 4 times (4 systems); silviculture (low/high intensity) is the main plot and spacing and genetic entry are the split plots (Albaugh et al., 2018). With a total of eight plots, each plot contains one treatment (one clone planted at one density). The experiment was set up as the chart below:

Table 1. Experimental Setup

System	Genotype (crown architecture variation)	Density (trees/acre)
1	C4	750
	OP	250
2	C4	250
	OP	750
3	C2	250
	C3	250
4	C3	750
	C2	750

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 share 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 at stand age 8-9 (2016-2017). Sapflux data was provided by USFS Research Biological Scientist Chris Maier (additional MP advisor). 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.

Additional data related to the weather parameters were either directly recorded from the site or obtained from on-site weather station.

Workflow

Data Cleaning: The 15-minute-interval raw k-values generated from thermal probes have been transformed into sap flux density using Granier's Equation ($F_d = 119 * k^{1.231}$). Data have been visually presented and checked for error and interesting patterns.

Small gaps (<48 entries, or half day) of individual probes will be filled in with simple linear regression between probes for each 15-minute entry. The transformed sap flux of individual data points will be scaled up temporally (daytime, 24-hour period). It will also be scaled up spatially to stand level transpiration using plot-specific sapwood area. Gaps bigger than 48 entries will be gap-filled using simple linear regression between probes for each daily sum.

Data analysis: The true means of treatments will be compared using a Two-Way ANOVA (Sap flux ~ genotype + density). Then, I will assess the differences of responses to VPD between treatments using a linear model (Sap flux ~ VPD + 8 treatment groups), and how the responses to VPD change with soil moisture between treatments with another linear model (Sap flux ~ VPD + 8 treatment groups + 2 soil moisture levels).

Analysis phases: Phase I of the analysis is to find and test the best approach with one month's data. Phase II is to expand this approach to a larger range of data—as large as time permits.

Data would be analyzed primarily using Microsoft Excel and R. Limited python will be applied.

Expected Results and Format of Report

The study aims to model treatment differences in terms of *P. taeda* water use, in the hope of contributing to further studies within the larger project. This study will identify

water use efficient P. taeda genotype(s) and help direct future studies that ultimately try to explain the differences in P. taeda productivity between locations.

The result of this project will be delivered in the format of a report and a presentation. The report shall be written professionally and scientifically; it will be submitted to the Nicholas School as the final MP report. A presentation will be given to the Nicholas School audiences and/or any individual interested in such topic during the MP Symposium on December 1st, 2022. The presentation shall be relatively succinct and intelligible to the general public, including sufficient background information and appealing graphic presentations of the study outcome.

The anticipated outcome, in addition to MP, is a potential manuscript submitted to a top professional journal.

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Faculty

Sari Palmroth (primary advisor), Nicholas School of the Environment, Duke University

Christopher Maier (additional advisor), Research Biological Scientist/Team Leader at

Southern Research Station, U.S. Forest Service

Part II: Project Timeline

<i>Time</i>	<i>Action/Due</i>
Academic year	<ul style="list-style-type: none"> • Accepting project as assistantship
2020-21	<ul style="list-style-type: none"> • Developing advisory team and plan for MP
<i>Initial setup</i>	<ul style="list-style-type: none"> • Complete supplemental reading related to the project: <ul style="list-style-type: none"> ◦ Previous publications on the project ◦ Additional papers on tree physiology (especially on sap flux) • Understand study design and background information • Data cleaning
Fall 2021	<ul style="list-style-type: none"> • Data exploration: visually present the data and look for interesting patterns or abnormalities
<i>Analysis</i>	<ul style="list-style-type: none"> • Eliminate errors and gap fill • Narrow down analysis direction and statistical methods; devise the scope of MP • Project prospectus due on 10/29
Spring 2022	<ul style="list-style-type: none"> • Work plan due on 2/1
<i>Analysis</i>	<ul style="list-style-type: none"> • Literature review • Re-run data cleaning procedure with newly possessed coding skills

	<ul style="list-style-type: none"> • Statistical analysis • Project status presentation
Summer 2022	<ul style="list-style-type: none"> • Statistical analysis • Expanding analysis to larger range of the dataset as much as I can. Ideally perform the analysis on an entire year of data
Fall 2022	<ul style="list-style-type: none"> • Wrap-up analysis
<i>Wrap-up and writing</i>	<ul style="list-style-type: none"> • Written draft of Final Report due on 9/30 • Develop good visual presentations of the study outcome • Revised draft due on 10/31 • 10-Line Abstract due to Student Services on 11/18 • Submission of final MP to iThenticate for scanning on 11/28 • Present MP (Symposium) on 12/1 • Final MP due on 12/8 <ul style="list-style-type: none"> ○ Upload to DukeSpace ○ Submit signed executive summary to Student Services via the DukeBox Upload Window

Part III: Team Charter

Team Roles and Responsibilities

Member	Role/Title	Responsibilities/Expectations
Azura Liu	<i>MEM-ESC/MF Student</i>	<ul style="list-style-type: none">• Conduct analysis and complete the MP• Schedule meetings and ensure communication
Sari	<i>Master's Advisor,</i>	<ul style="list-style-type: none">• Primary advisor
Palmroth	<i>NSOE Professor/Faculty</i>	<ul style="list-style-type: none">• Assist student with administrative requirements• Provide guidance on study design and direction• Give feedback and insight on analysis completed by student
Christopher	<i>Member of the Advisory</i>	<ul style="list-style-type: none">• Additional advisor
Maier	<i>Team,</i> <i>USFS Research Biological</i> <i>Scientist</i>	<ul style="list-style-type: none">• Supply data and background information necessary for the project• Provide guidance on study design and direction• Give feedback and insight on analysis completed by student

Regular Meeting Schedule

The MP team meets on a weekly to bi-weekly basis based on the progress student has made. Student meets one or both advisors according to each member's schedule.

Team Expectations

Team members will communicate efficiently through email, the primary method of communication. Meetings are conducted primarily via Zoom, but the team tries to meet in person at NSOE or USFS research station as possible.

Team Purpose and Mission

The team aims to complete the MP in reasonable timeframe. Beyond the MP requirement, we will take advantage of the marvelous dataset and explore as much as possible. The outcome of the study shall aid understanding in loblolly pine physiological mechanisms and production.

Top Priorities and Goals: As someone who never performed research before, the student takes the project as a valuable learning experience, understanding the logic and obtaining necessary skills for research as diving deeper. The top priority and goal of the advisors is to provide appropriate guidance and advice so student can learn efficiently and yield a high-quality MP in time.

Team Cohesion and Conflict Resolution

When conflicts emerge, team members will communicate honestly and openly regarding responsibilities. If there are disagreements in analysis approaches, statistical methods, etc., the team members will each present their thoughts and decide on what is best. Additional help from other faculties (e.g. Statistics professor) can be sought if agreement cannot be achieved.

Additional Resources: available at Duke Graduate School Student Resources

(<https://gradschool.duke.edu/student-life/student-resources>)