Impacts and Management of Foliar Pathogens of Eastern White Pine (Pinus strobus) in the Northeastern United States

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IMPACTS AND MANAGEMENT OF FOLIAR PATHOGENS OF EASTERN WHITE PINE

(*PINUS STROBUS*) IN THE NORTHEASTERN UNITED STATES

BY

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DISSERTATION

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

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in

Natural Resources & Environmental Studies

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TABLE OF CONTENTS

ACKNOWLEDGMENTS ............................................................................................................. III
LIST OF TABLES ....................................................................................................................... VIII
LIST OF FIGURES ..................................................................................................................... IX
ABSTRACT ............................................................................................................................... XII

CHAPTER 1: Variability of sapwood area, water content, density, and thermal diffusivity and their relative influence on estimates of sap flow ........................................... 1

Abstract ..................................................................................................................................... 1
1.1 Introduction .......................................................................................................................... 2
1.2 Methods .............................................................................................................................. 7
  1.2.1 Study site and field methods ......................................................................................... 7
  1.2.2 Measurements of stem geometry ................................................................................. 9
  1.2.3 Determination of MC, $\rho_d$, and D ............................................................................ 11
  1.2.4 Sap flow measurements .............................................................................................. 12
  1.2.5 Quantifying variability of the sapwood ....................................................................... 13
  1.2.6 Temporal and spatial variability of sapwood properties ............................................. 14
  1.2.7 Sensitivity analysis ....................................................................................................... 14
1.3. Results .............................................................................................................................. 16
  1.3.1 Stem geometry and variability of the sapwood area .................................................... 16
  1.3.2 Temporal and spatial variability of sapwood properties ............................................. 22
  3.3.3 Sap flow sensitivity analysis ....................................................................................... 27
1.4 Discussion ........................................................................................................................... 30
1.5 Conclusion .......................................................................................................................... 35

CHAPTER 2: Impacts of White Pine Needle Damage on seasonal litterfall dynamics and wood growth of eastern white pine in northern New England ........................................... 37

Abstract ..................................................................................................................................... 37
2.1 Introduction .......................................................................................................................... 38
2.2 Methods .............................................................................................................................. 41
  2.2.1 Study sites ................................................................................................................... 41
  2.2.2 Litterfall and foliar nitrogen ....................................................................................... 43
2.2.3 Dendrochronology sampling and processing .................................................. 45
2.2.4 Growth decline analysis .................................................................................... 46
2.3 Results ................................................................................................................. 48
  2.3.1 Seasonal changes in litterfall abundance ......................................................... 48
  2.3.2 Foliar litter nutrient dynamics ......................................................................... 51
  2.3.2 WPND-induced growth declines ...................................................................... 53
2.4 Discussion ............................................................................................................. 57
2.5 Conclusion .......................................................................................................... 63

CHAPTER 3: Impacts of White Pine Needle Damage on water use, gas exchange, and the allocation of non-structural carbohydrates ......................................................... 64
  Abstract .................................................................................................................. 64
  3.1 Introduction ........................................................................................................ 65
  3.2 Methods .............................................................................................................. 70
    3.2.1. Study sites .................................................................................................. 70
    3.2.2 Needle phenology ....................................................................................... 70
    3.2.3 Sap flow measurements .............................................................................. 71
    3.2.4. Leaf gas exchange measurements .............................................................. 72
    3.2.5 Non-structural carbohydrate sampling and processing ............................. 74
    3.2.6 Processing and analysis of sap flow data ..................................................... 75
    3.2.7 Analysis of leaf gas exchange data ............................................................... 78
    3.2.8 Analysis of non-structural carbohydrate data ............................................ 79
  3.3 Results ................................................................................................................ 80
    3.2.1 Impacts of WPND on sap flux density ......................................................... 80
    3.3.2 Leaf gas exchange and phenology ............................................................... 83
    3.3.3 Non-structural carbohydrate allocation ..................................................... 86
  3.4 Discussion .......................................................................................................... 89
  3.5 Conclusion ........................................................................................................... 95

CHAPTER 4: Thinning treatments reduce severity of foliar pathogens in eastern white pine ................................................................................................................................. 97
  Abstract .................................................................................................................. 97
  4.1 Introduction ........................................................................................................ 98
4.2 Methods ......................................................................................................................... 100
  4.2.1 Study sites .............................................................................................................. 100
  4.2.2 Experimental design .............................................................................................. 102
  4.2.3 Response variables ................................................................................................. 104
  4.2.4 Pre-treatment analyses .......................................................................................... 107
  4.2.5 Analysis of thinning effects .................................................................................... 109
4.3 Results ........................................................................................................................ 109
  4.3.1 Impacts of WPND on tree health ........................................................................... 109
  4.3.2 Response to thinning treatments .......................................................................... 114
4.4 Discussion .................................................................................................................... 117
4.5 Conclusion and recommendations ............................................................................. 120

**LIST OF REFERENCES** .................................................................................................. 121
LIST OF TABLES

Table 1.1 Geometric models used to estimate total sapwood. Models 1-6 are referred to as circular, longest diameter, shortest diameter, geometric mean diameter, arithmetic mean diameter, and quadratic mean diameter respectively. ................................................................. 11

Table 1.2 Measured values of MC, ρ, and D for eastern white pine. ......................................................... 22

Table 1.3 Measured values of MC, ρ, and D for northern red oak. ............................................................. 22

Table 1.4 Two-way factorial ANOVA results for sapwood properties per species.......................... 23

Table 2.1 Study site location, basal area (BA), trees per hectare (TPHA), and dendrochronological sampling statistics .................................................................................................................. 49

Table 2.2 Results of the D-score analysis on the high-severity tree ring chronologies......................... 54

Table 2.3 Test results from the two-factor crossed repeated measure ANOVA for the six sites. The bottom portion of the table showing all sites gives the results for the maximum likelihood model ........................................................................................................ 56

Table 3.1 Dates of the gas exchange sampling for each study site. Dates in June marked with * indicate sampling periods in which current year foliage was not measured due to insufficient needle length. ..................................................................................................................................... 73

Table 3.2 Results of the three-factor mixed model run for photosynthesis, stomatal conductance, and intrinsic water use efficiency. Time indicates the month in which measurements were collected (July or August), severity corresponds to the distinction between observed low- and high-WPND severity, and needle age differentiates between current-year and second year foliage. ................................................................................................................ 84

Table 3.3 Between-subject and within-subject results of the 3-factor repeated measures MANOVA for sugar and starch concentrations. Effects are considered significant at p < 0.001. 87

Table 4.1. Initial conditions of the two naturally established eastern white pine stands in New Hampshire measured in 2015. ................................................................................................................................. 101

Table 4.2 Post-treatment metrics of stand level stocking for control, high-density (HD), and low-density (LD) thinning plots. Means and standard error are calculated from n=6 prism points per treatment per site. ........................................................................................................ 104

Table 4.3 Summary of mixed effects models testing sources of variation on the six tree response variables of interest prior to stand thinning. The severity source consists of four ocular classes of WPND symptom (chlorosis and defoliation) severity: healthy, mild, moderate, and severe. 110

Table 4.4 Variable mean, standard deviation (n=275), and adjustment used in calculating individual trees z-scores. PCA 1 indicates the factor loadings for each variable used in calculating $z'$, but was not applied to the WPND severity parameter. ........................................ 113

Table 4.5 Summary statistics of the mixed effect model testing sources of variation on $\Delta z'$ in the years following thinning treatments. .......................................................................................... 115
LIST OF FIGURES

Fig. 1.1 Diagram showing the orientation of eight radii for disk samples relative to the major axis and geometric center. Photo on the right shows the outlined sapwood area of a disk using ImageJ, note the large variation in sapwood thickness about the perimeter of the disk. 

Fig. 1.2 Allometric relationships for bark thickness (A) and sapwood area (B) for eastern white pine at DBH. Mean bark thickness was determined from n=8 measurements per disk, error bars show the standard error. Reported sapwood is the AAsw derived from image analysis. Shaded area for each curve denotes the 95% confidence interval.

Fig. 1.3 Disk coefficient of variation (CV) for radii (A), sapwood thickness (B), and the pith offset (C) as a function of m measured eccentricity in eastern white pine. Fitted lines are simple linear regressions and the shaded area denotes the 95% confidence interval.

Fig. 1.4 Simple linear regressions of model estimated sapwood area (Asw) as a function of the actual sapwood area (AAsw) of eastern white pine. (A) The circular model showing the eight total fits, one for each radii measurement. (B) Longest diameter model. (C) Shortest diameter model. (D) Geometric mean diameter model. (E) Arithmetic mean diameter model. (F) Quadratic mean diameter model. Dashed line is the 1:1 line for AAsw and shaded region of regression lines indicate the 95% confidence interval.

Fig. 1.5 Values of R² derived from simple linear regressions of AAsw to Asw estimated using the circular model based on measurements of sapwood depth, bark thickness, and DBH of eastern white pine. Core replicates indicate the number of radii averaged within the circular model to calculate Asw. Number above boxes denote the number of possible combinations of radii at each level of replication.

Fig. 1.6 Relative error in the estimate sapwood area (Asw) in relation to the number of core replicates used on eastern white pine disks. Points show the mean values derived from the combination analysis and bars indicate the minimum and maximum relative error.

Fig. 1.7 Variation in sapwood water content (MC) over the course of the 2015 growing season for red oak and white pine. Open and closed symbols represent the DBH and stump positions respectively. Error bars show standard deviation.

Fig. 1.8 Variation in sapwood density (ρ) over the course of the 2015 growing season for red oak and white pine. Open and closed symbols represent the DBH and stump positions respectively. Error bars show standard deviation.

Fig. 1.9 Variation in sapwood thermal diffusivity (D) over the course of the 2015 growing season for red oak and white pine. Open and closed symbols represent the DBH and stump positions respectively. Error bars show standard deviation.

Fig. 1.10 Sapwood water content (MC) as a function of sapwood density (ρ). Circles and squares denote white pine and red oak respectively, open and closed shapes denote measurements obtained from the breast height and stump position respectively. Shaded region shows the 95% confidence interval of the second-order polynomial fit.

Fig. 1.11 Estimates of sap flux density (Js) in red oak (A) and white pine (B) for three days in early June. Each color indicates the substitution of sapwood properties measured on a single sampling date and applied to the raw data.
Fig. 1.12 Variance in \( J_D \) estimated from substituting the sapwood properties derived from the four collection dates as a function of \( J_{\text{max}} \) for northern red oak. Each shape represents a different tree replicate and the DBH/stump position is denoted by open and closed fill respectively.

Fig. 1.13 Variance in \( J_D \) estimated from substituting the sapwood properties derived from the four collection dates as a function of \( J_{\text{max}} \) for eastern white pine. Each shape represents a different tree replicate and the DBH/stump position is denoted by open and closed fill respectively.

Fig. 1.14 The relative error associated with assumptions of constant MC, \( \rho_d \), and D sampled for a given month by species and location of sapwood core extraction (DBH, stump). Error rates are relative to setting the mean value of MC, \( \rho_d \), and D across all months, reported in Table 1.2 and Table 1.3. Data includes 95 and 79 days for red oak and white pine respectively.

Fig. 2.1 Map of the New England states showing the location of the eight US Forest Service White Pine Needle Damage (WPND) monitoring sites assessed in this study. All sites except for DUR were sampled for tree ring analysis. Sites DUR, FOX, MEF, and BTH were sampled for litterfall during the 2014-2016 growing seasons.

Fig. 2.2 Mean monthly litterfall totals of eastern white pine during the 2014-2016 growing seasons ± 1SE. These data are normalized using the total basal area of white pine for each plot. Values with the same letter are not significantly different between months within the measured year (\( \alpha = 0.05 \)).

Fig. 2.3 Foliar N content (%) measured in litter samples collected throughout the 2014 growing season. The horizontal line within the box indicates the median, boundaries of the box indicate the upper and lower quartile, and the whiskers indicate the highest and lowest values of the results. Values with the same letter are not significantly different (\( \alpha = 0.05 \)).

Fig. 2.4 The estimated nitrogen flux (g N m\(^{-2} \)) as white pine litter for each study site throughout the 2014 growing season. Error bars show the propagated standard error derived from monthly litterfall and foliar N measurements.

Fig. 2.5 Master chronologies for low-severity (black) and high-severity (red) WPND-infected eastern white pines by site from 1960-2015. Shaded area around each time series shows ± 1 SE. Arrows within each panel indicate the year of \( D_{\text{max}} \) in the high severity chronologies.

Fig. 2.6 Pre-outbreak (2000 to year prior to \( D_{\text{max}} \)) and post-outbreak (year of \( D_{\text{max}} \) to last year of growth) basal area increment (cm\(^2\)) in low severity (closed circles) and high severity (open circles) chronologies across the six study sites ±1SE.

Fig. 3.1 Daily total sap flux density at FOX (A) and MEF (B) between May and August 2014. Black and red timeseries denote mean values (\( n =4 \)) of low-severity and high-severity trees respectively. Ribbons about each timeseries show the standard deviation. Dashed lines indicate the limits of the discrete time periods relative the WPND-induced defoliation event beginning in mid-June.

Fig. 3.2 Total daily sap flux density as a function of ETo and VPD and sites FOX (A) and MEF (B) respectively. Point shapes are coded according to the relative timing relating to the WPND-induced defoliation event. Open and filled points represent the high- and low-severity trees respectively. Shaded area along the fitted curve indicates the 95% confidence interval.
Fig. 3.3 The ratio of daily sap flux density ($J_d$ high-severity: $J_d$ low-severity) for each discrete time period relating to the relative timing of WPND-induced defoliation at study sites FOX (A) and MEF (B). A change in connecting letters indicates a significant difference ($\alpha = 0.05$) determined using the Wilcoxon method across each pair. ................................................................. 83

Fig. 3.4 Photosynthesis (A), stomatal conductance ($g_s$), and intrinsic water use efficiency (iWUE) over time for current year (light-green) and second year (dark-green) needles. “NM” indicates dates in which the current-year needles could not be measured due to insufficient needle length in the month of June. Boxes marked with * indicate a significant difference between needle age class within each severity class at the corresponding date via a pooled t-test ($\alpha = 0.05$) .................................................................................................................. 85

Fig. 3.5 Length of the current year needles over time. Sigmoidal curve fit using a 4-parameter probit model, $R^2 = 0.937$ ................................................................................................................................. 86

Fig. 3.6 Mean (n = 6) starch and sugar content for the low- (open circle) and high-WPND-severity (filled circle) trees across the five tissue types and three sampling periods. Error bars show standard deviation and * represents a significant difference between WPND-severity class within a given sampling date. ...................................................................................................... 88

Fig. 3.7 Ratio of allometrically-scaled sugar and starch content (kg) to basal area increment (BAI at DBH, cm$^2$) of the current (2015) and previous year (2014) wood growth. Letters indicate the results of a t-test ($\alpha = 0.05$) performed between WPND-severity class, where a change in lowercase letters denotes a significant difference when applying 2014 relative growth rate and uppercase letter corresponds to the 2015 relative growth rate within each tissue type. ................................................................. 89

Fig. 4.1 Diagram of the blocked experimental design at the white pine stands in southern NH. Black circles show the orientation of the three replicated prism subplots within the treatment plots used to select trees for trait measurements ........................................................................................................... 103

Fig. 4.2 Post hoc analysis (Tukey HSD) of LCR, crown diameter, and DBH as a function of WPND severity class. Values with the same letter are not significantly different between severity classes ($\alpha=0.05$). ................................................................................................................................. 111

Fig. 4.3 Principal component analysis of the seven z-score relativized traits grouped according to WPND severity in two dimensions. The mean z-score eigenvector is plotted as a supplemental variable, but not factored into in the analysis. .................................................................................................................. 113

Fig. 4.4 Mean health index score ($z'$) as a function of WPND severity class. Positive $z'$ represents trees that are more diseased than the average, while negative values are healthier. ........................................ 114

Fig. 4.5 Changes in $z'$ in the first two years of thinning treatments. Horizontal line within the box indicates the median, boundaries of the box indicate 25$^{th}$ and 75$^{th}$ percentile of the four treatment blocks. Dashed line at zero indicates no change from the pre-treatment year, positive values of $\Delta z'$ indicate trees are more stressed on average, negative values are healthier. ........................................... 116

Fig. 4.6 Differences in WPND severity between treatments for each year of the study. Values represent block means and standard error. Values followed by the same letter are not significantly different within a given year ($\alpha=0.05$). ........................................................................................................ 116
ABSTRACT

IMPACT AND MANAGEMENT OF FOLIAR PATHOGENS OF EASTERN WHITE PINE
(Pinus strobus) IN THE NORTHEASTERN UNITED STATES

By

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University of New Hampshire, May 2018

White Pine Needle Damage (WPND) is a complex of foliar fungal pathogens currently impacting forests in the Northeastern US. Since ca. 2009, chlorosis and defoliation caused by WPND has been observed in stands of eastern white pine (Pinus strobus L) throughout the region. A changing climate, notably warmer temperatures and higher than average spring precipitation in the region are thought to be exacerbating the establishment and spread of these native pathogens. The goals of this research are to enhance the understanding of the timing and magnitude of WPND-induced defoliations across infected stands, assess the physiological response of trees affected by chronic needle loss, quantify the resulting growth reductions, and explore management options using silvicultural tools. This research builds upon previous work that has examined the epidemiology of the diseases’ causal agents and is the first to address aspects of WPND as it relates to tree and forest health.

The first chapter of this work investigates methodology regarding sap flux measurements used for estimating whole-tree transpiration rates, which are later applied in the third chapter of this dissertation. I assess the variability of physical properties of the sapwood, consisting of the
estimates of the sapwood area based on six different geometric models, as well as the spatial and temporal variability of sapwood water content, density, and thermal diffusivity. Results indicate that the commonly applied circular model used for estimating total sapwood area tends to overestimate by 10% or more depending on the degree of replication used in determining sapwood thickness at a given radius. Additionally, it was found that sapwood water content and thermal diffusivity are sensitive to changes over time, resulting in significant error rates when assuming a single seasonal value. This data highlights the need for adequate replication of core samples used to derive parameters relating to the sapwood for estimating sap flux.

Chapter two presents research findings of a multi-year litter trapping study and the results of a growth analysis using tree ring data obtained from sites throughout WPND-infected stands in northern New England. It was found that defoliations in June and July within diseased stands accounted for 47% of the total annual litterfall between 2014 and 2016. Foliage cast prior to the natural abscission in October was found to have significantly higher concentrations of nitrogen, thus fundamentally altering the timing and amount of nutrient deposition in diseased stands. Repeated defoliations were shown to induce significant reductions in wood growth initiating 2007-2009, resulting in declines in basal area increment of 25-73% compared to pre-outbreak growth rates.

Chapter three address several aspects of tree physiology as it relates to WPND-induced defoliation. A subtle, yet significant decline in transpiration was measured in trees of high-infection severity compared to reference low-severity trees. However, disease severity was not found to influence leaf level gas exchange nor non-structural carbohydrate allocation to various storage tissues throughout the growing season. These findings indicate that diseased trees are not
likely to be carbon limited, thus are compromising wood growth at the expense of storing of photosynthate for other uses.

Chapter four applies the current knowledge regarding the spread and dispersal of WPND fungi and tests the use of thinning as a silvicultural tool for mitigating the negative impacts of disease. Replicated plots of different residual densities (14 and 25 m² ha⁻¹) are used to evaluate the impacts of defoliation on common tree metrics to develop an index score for monitoring treatment response over time. Within the first two years following stand thinning, residual trees were found to increase in vigor and a reduction in WPND severity of 35% was noted in plots thinned to 14 m² ha⁻¹. Based on these findings, it is recommended that infected stands or stands at risk of infection should be maintained at densities equivalent to or below to B-line of the eastern white pine stocking guide.
CHAPTER 1

VARIABILITY OF SAPWOOD AREA, WATER CONTENT, DENSITY, AND THERMAL DIFFUSIVITY AND THEIR RELATIVE INFLUENCE ON ESTIMATES OF SAP FLOW

Abstract

The sapwood is the hydraulically active portion of outer xylem within woody plants and serves as a conduit for the movement of water from the roots to foliage. Most heat-based sap flux measurements rely on the parameterization of physical properties of the sapwood to accurately estimate the ascent of sap through the xylem. The sapwood properties that are critical for computing a sap flux density ($J_s$) include the sapwood water content, density, and thermal diffusivity. However, there is limited information on how these properties differ temporally and spatially throughout the stem. Estimates of the total cross-sectional sapwood are also necessary for scaling estimates of sap flux from individual trees to larger spatial orders. The work presented in this chapter is from two studies that address the potential variability and error associated with estimating sap flux when using increment core samples to derive the physical properties of sapwood. Breast height disk samples obtained from 30 eastern white pine were used to generate allometric equations for bark thickness and total sapwood area while assessing the accuracy of six different geometric models for predicting the true sapwood area. The variability of sapwood water content, density, and thermal diffusivity was assessed at four sampling dates between June - October 2015 as well was the variability between obtaining core samplings from two different locations on the bole of both eastern white pine and northern red oak. This research found that the assumption of a circular shape for stem geometry tends to over-
estimate the total sapwood area by 10%, while prediction models that assume an elliptical shape tend to exhibit a more consistent and higher level of accuracy. This work also reports significant changes in sapwood water content and thermal diffusivity over the course of the 2015 growing season, but no difference in sapwood density or between the location at which cores were extracted in either species. Findings from this chapter highlight the necessity for adequate core replication when deriving sapwood properties that are critical to accurate estimates of sap flux, and demonstrates that cores may be sampled from positions low on the stem to reduce destructive sampling without sacrificing measurement accuracy.

1.1 Introduction

Trees are an important component of the global water cycle as they function as a conduit in the transfer of water from the soil to the atmosphere through the process of transpiration, accounting for over 60% of the total evapotranspiration from terrestrial ecosystems (Schlesinger and Jasechko, 2014). This flow of water occurs passively across a gradient of low to high water potentials regulated via stomata that are sensitive to both evaporative demands of the atmosphere and soil water content. Transpiration is largely a biproduct of photosynthesis, as carbon dioxide can only be exchanged between leaves and the atmosphere when stomates are open. As a result, large volumes of sap move through plants relative to the amount of water required for the light-dependent reaction of photosynthesis. Greater than 99% of water transport through plants occurs within the xylem, and for tree species the outer hydroactive portion of the stem is referred to as the sapwood. Estimates of transpiration are most often conducted using heat-based sap flow sensors, where point measurements of water movement in the axial direction of a woody stem are integrated over the cross-sectional area of the sapwood. These sap flow techniques require
the parameterization of several physical properties of the sapwood to convert discrete

temperature measurements into a heat pulse velocity which can then be used to estimate different

metrics of tree water use. Among these, the sapwood area ($A_{sw}$), water content ($MC$) and dry
density ($\rho_d$) are often directly measured from core samples extracted near the measurement point
of in-situ sap flow probes. Theoretical heat-pulse methods for measuring sap flow in woody
plants include the compensation heat pulse method (CHP, Marshall, 1958), the heat ratio method
(HRM, Burgess et al., 2001), the T-max method (Cohen et al., 1981), and the Sapflow$^+$ method
(Vandegehuchte and Steppe, 2012). Each of these methods have proven to provide accurate
estimates of transpiration based on gravimetric calibrations, though the expected range of sap
velocities must be considered for a given species when choosing an appropriate method (Steppe
et al., 2010; Vandegehuchte and Steppe, 2013). Each of these methods derive a heat pulse
velocity ($V_h$), that is the speed of the heat pulse traveling in the axial direction and emitted by a
probe inserted radially into the sapwood. From $V_h$, the sap-flux density can be estimated:

$$J_s = \frac{\rho_d}{\rho_s} \left( MC + \frac{c_{dw}}{c_s} \right) V_h$$

(1)

where $J_s$ is the sap-flux density (cm$^3$ cm$^{-2}$ h$^{-1}$), $V_h$ is the heat pulse velocity (cm h$^{-1}$), $MC$ is the
sapwood water content (kg kg$^{-1}$), $c_{dw}$ is the specific heat capacity of the wood matrix (1200 J kg$^{-1}$
K$^{-1}$ at 20°C), $\rho_d$ is the dry density of the sapwood (kg m$^{-3}$), $\rho_w$ is the density of the sap (assumed
to be equivalent to water, 1000 kg m$^{-3}$), and $c_s$ is the specific heat capacity of water (4186 J kg$^{-1}$
K$^{-1}$ at 20°C).

As $J_s$ is useful for assessing transpiration rates independent of tree size because it relates

the movement of sap perpendicular to unit area of sapwood, it is the favorable metric for making

comparisons of sap flux between difference species or experimental treatments. However, when

the goal is to quantify a total sap flux ($Q$, L day$^{-1}$), estimates of sap velocity are integrated across
the total sapwood area. Thus, any error incurred in the estimate of the sapwood area of an individual tree will scale proportionately with Q. At the tree level, estimates of sapwood area are most frequently derived through obtaining increments cores from trees sampled at the point of sap flow measurement. By measuring the bark and sapwood thickness of a core sample it is possible to estimate the sapwood area as the difference of the inside-bark basal area and subtracting the estimated area of the inner heartwood. Similar to the calculation applied to compute a trees’ basal area, the most commonly used methods assume that stems are circular in cross-section. However, it has long been recognized that stem cross-sections are almost never exactly circular (Matérn, 1956; Pulkkinen, 2012). Though deviation from a circular model assumption may be small, error will be compounded when scaling to higher spatial orders (plot, catchment, landscape). Scaling transpiration estimates from individual trees is most often conducted by using estimates of sap velocity at a given diameter class and applying an allometric equation relating stem diameter to sapwood area to inventory data for the scale of interest (Schafer et al., 2002). Therefore, a high degree of precision should be desired for the sapwood area parameter, both within trees and for generating allometric relationships. It is common for sap flow studies to obtain a single core to estimate sapwood area assuming radial symmetry of sapwood depth within the stem (Chelcy R Ford et al., 2007). Here, I assert that this assumption of symmetry could introduce considerable error in calculation of sapwood area and hence Q. Within this study, the first objective is to quantify the variability of the sapwood area as a function of sapwood depth at different radii within stem cross-sections, then, to determine the optimal geometric model for predicting the true sapwood area.

Of the four heat-based sap flow methods described above, T-max and HRM require a determination of sapwood thermal diffusivity (D), the ratio of the axial sapwood thermal
conductivity (K) to volumetric heat capacity (c) in order to calculate $V_h$, based in part on measured values of MC and $\rho_d$. Therefore, the accuracy of sap flow measurements relies in part on the physical properties of the sapwood, where relatively small uncertainties can be propagated to produce significant error when scaling point measurements to tree, stand, or larger spatial orders (Hatton et al., 1995; Kumagai et al., 2007, 2005; Lu et al., 2000; Phillips et al., 1996; Schafer et al., 2002). Several studies have evaluated the induced error in sap flow measurements pertaining to variability throughout the sapwood radial profile of the sapwood (Alvarado-Barrientos et al., 2013; Cermak and Nadezhdina, 1998; Gebauer et al., 2008; Nadezhdina et al., 2002b; Poyatos et al., 2007), water content (Looker et al., 2016; Vergeynst et al., 2014), density (Bowman et al., 2005; Delzon et al., 2004; Looker et al., 2016), and thermal diffusivity (Chen et al., 2012; Looker et al., 2016; Vandegehuchte and Steppe, 2012). It is also well established that there are considerable differences in MC and $\rho_d$ between species (Kimberley et al., 2015; Ross, 2010), and thus calculated values of D are also species specific. However, only recently has a more accurate calculation of D been worked out through differentiating between bound and unbound water within the sapwood (Vandegehuchte and Steppe, 2012). Prior to this determination, D has often been assigned the nominal value of $2.5 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$ first presented by Marshall (1958), which is an intermediate thermal diffusivity of water ($1.4 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$) and dry wood ($4.0 \times 10^{-3} \text{ cm}^2 \text{ s}^{-1}$). Alternatively, D has previously been determined according to an empirical relationship between MC and $\rho_d$ to calculate K based on the work of Swanson (1983) and popularized by the application of HRM (Burgess et al., 2001). As a result of this recent refinement of D which corrects for MC, many previous sapflow studies have likely over- or underestimated heat-pulse based transpiration measurements due to assumptions or incorrect determination of this important parameter of the sapwood (Vandegehuchte and Steppe, 2012).
It is generally supported that measurements of MC and $\rho_d$ should be directly measured for heat-pulse-based sap flow studies to accurately determine $J_s$ in lieu of using literature values. This is most often performed through obtaining increment core samples of the sapwood. In this study, the spatial and temporal variability of sapwood properties estimated from core samples is assessed between two temperate tree species: northern red oak (*Quercus rubra* L.) and eastern white pine (*Pinus strobus* L.). While collecting sapwood core samples for determining MC and $\rho_d$ is not generally considered expensive or time consuming, it does require destructive sampling on the individual. There are several reasons to consider limiting the extraction of cores and thus reducing damage on trees sampled for sap flow. Sap flow measurements are typically conducted at breast height, 1.3 m above ground level, since most allometric equations relating to sapwood area for scaling point measurements of sap velocity are derived from diameter at breast height (DBH). However, destructive sampling at DBH can often be an issue in commercially valued trees, since this will create wounds in the most valuable part of the bole in addition to those created by the insertion of sap flow probes. Repeated sampling of sap flow measurements on individual trees over the course of multiple years can also limit the area available for core sampling, as probes must be relocated periodically to reduce the effects of wounds on measurement accuracy. This is especially true for smaller diameter stems, as a sufficient buffer should be applied between wounds created by sap flow probes and/or core extractions such that they don’t interfere with the ascent of sap. Hence, it may be advantageous to obtain measurements of MC and $\rho_d$ from positions on the bole other than DBH. To this end, an objective of this study was to ascertain differences in MC, $\rho_d$, and D at two locations on the main stem of our target species.
Additionally, we sought to evaluate differences among these sapwood properties over time. Several studies have confirmed seasonal and subtle diurnal changes in MC across a wide range of tree species (Gibbs, 1958; Henderson, L.G. and Choong, 1968; Lopez-Bernal et al., 2014; Vandegehuchte and Steppe, 2012; Wullschleger et al., 1996), thus assigning a MC value based on a single measurement could potentially over- or underestimate sap flow measurements when scaling to $V_h$ and $J_s$. Depending on the required accuracy of a study, this temporal variability could imply multiple collections of core samples over the course of a growing season, thus further damaging the stem while reducing the available area for continuous measurements. To accomplish this objective, we assess sapwood properties over a five-month period to quantify this variability and the potential interaction with core position. Finally, sapwood parameters measured at different times and spatial positions in this study were used to perform a sensitivity analysis using HRM sap flow data to evaluate error rates relative to the mean values determined for each species.

1.2 Methods

1.2.1 Study site and field methods

Research presented herein was conducted at two field sites located in Madbury and Durham, New Hampshire, separated by approximately 6 km. Collection of stem cross-sections (disks) for assessment of sapwood area and development of allometric models was conducted at the Kingman Farm (43° 10’ N 70° 55’ W; 45 m above sea level) in Madbury, NH. The study site is a 105 ha multi-use research facility managed by the University of New Hampshire. The forest is composed of secondary mixed conifer and deciduous stands dominated by eastern white pine, eastern hemlock (*Tsuga Canadensis* L.), red oak (*Quercus rubra* L.), and red maple (*Acer* L.).
rubrum L.). Average annual precipitation at this site is 1070 mm and distributed relatively evenly throughout the year. Mean annual temperature is 8.5 °C, with the mean monthly maximum and minimum temperatures occurring in July (21.2 °C) and January (-4.7 °C) respectively. For the purpose of this study, a pure stand of white pine was selected for destructive sampling in conjunction with a shelter-wood silvicultural treatment in which ca. 80% of the standing basal area of mature trees were removed. Disks approximately 10 cm wide at breast height were collected in June 2014 from a subset of the felled trees \( n=30 \) ranging in diameter from 23.4 to 69.1 cm.

Research relating to sapwood properties (MC, \( \rho_d \), and D) was conducted at the East Foss Farm in Durham, NH (43.2° N, -71.0° W), a secondary forest owned and managed by the University of New Hampshire. During the 2015 growing season we selected ten mature codominant trees of northern red oak and eastern white pine for obtaining increment core samples. The mean DBH for the sampled trees was 45.7 cm (SD ± 12.3) and 50.7 cm (SD ± 16.7) for red oak and white pine respectively. We collected sapwood samples at four dates over the course of the study: 4 June, 1 July, 3 August, and 12 October, hereafter referenced by their respective months. Wood samples were collected from a random radius at both DBH and stump position on each sample date using a 5.15 mm-diameter increment borer (Haglöf Sweden AB). The stump position (40-60 cm above ground level) was determined visually by assessing the location on the stem at which a significant taper occurred, below the point where the main bole would theoretically be cut for harvest. Core samples were immediately placed in plastic tubes, sealed within a polyethylene bag, and placed on ice to inhibit evaporation until measurements could be conducted in the lab.
1.2.2 Measurements of stem geometry

White pine disks were air dried and the surfaces sanded in a step wise progression using a handheld belt sander while taking care to maintain an intact the bark perimeter. The geometric center of each disk is defined here as its center of mass, rather than the origin of the pith. The geometric center was identified by suspending a weight attached at a random position on the outside perimeter of the disk and allowing it to stabilize perpendicular to a flat work bench; this was then repeated two additional times in order to find the point of intersection and the resulting centroid was marked with a pencil. The major axis of the disk (i.e. the maximum diameter) from inside the bark end to end through the center was also marked. Starting at the larger of the two radii from the center on the major axis ($r_1$), additional radii ($r_2$-$r_8$) were drawn clockwise from the center to the end of the disk at $45^\circ$ intervals, resulting in a total of eight marked radii emanating from the geometric center of the disk (Fig. 1.1).

![Diagram showing the orientation of eight radii for disk samples relative to the major axis and geometric center. Photo on the right shows the outlined sapwood area of a disk using ImageJ, note the large variation in sapwood thickness about the perimeter of the disk.](image)
Assuming the general shape of the disk is an ellipse, the diameter perpendicular to the major axis \((r_1 + r_5)\) is defined as the minor axis \((r_3 + r_7)\) and therefore the eccentricity of a disk can be calculated as the ratio of the major axis to the minor axis. At each radius, measurements were taken of the inside the bark radius (IBR), sapwood thickness, and bark thickness. For length of the IBR, the distance from center to the outside edge of the last year of wood growth was measured to 1.0 mm using a metric ruler. The sapwood-heartwood interface was visually identifiable for each disk and most often terminated on the boundary of an annual ring. The sapwood thickness from inside the bark to the heartwood boundary was measured to 0.1 mm using a digital caliper (#1277-830 WVR International, Randor, PA). Bark thickness was measured to the nearest 0.1 mm by aligning a straight-edge perpendicular to the cambium and applying a depth gauge (#368-7031 Fowler Precision, Newton, MA) to where contact at the base of the gauge was made with the outermost edge of the bark. For each measurement of sapwood thickness along the eight radii, total sapwood area was estimated using the six area models adapted from Biging and Wensel (1988) (Table 1.1). In addition to model estimates of sapwood area, actual sapwood area (AAsw) was implicitly derived by processing high-resolution photographs of disks using ImageJ software (Schneider et al., 2012) and delineating the observable sapwood to calculate the total pixels using a reference measurement within the image (Fig. 1.1). The offset of pith was also measured, defined as the absolute distance between pith and the geometric centroid. Allometric relationships to DBH were generated for total sapwood area and bark thickness using measurements of AAsw and the mean of the eight radii bark measurements for each disk.
Table 1.1 Geometric models used to estimate total sapwood. Where Asw is the estimated total sapwood area of the disk, \( r_{ib} \) is the inside the bark radius at a given radii, \( r_{sw} \) is the sapwood thickness at that radii. Models 1-6 are referred to as circular, longest diameter, shortest diameter, geometric mean diameter, arithmetic mean diameter, and quadratic mean diameter respectively.

<table>
<thead>
<tr>
<th>Model #</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( \pi \left( \frac{DBH}{2} - b_n \right)^2 - \pi \left( \frac{DBH}{2} - b_n - r_{sw} \right)^2 )</td>
</tr>
<tr>
<td>2</td>
<td>( \pi \left( \frac{r_1 + r_5}{2} \right)^2 - \pi \left( \frac{(r_1 + r_5) - (r_{sw_1} + r_{sw_5})}{2} \right)^2 )</td>
</tr>
<tr>
<td>3</td>
<td>( \pi \left( \frac{r_3 + r_7}{2} \right)^2 - \pi \left( \frac{(r_3 + r_7) - (r_{sw_3} + r_{sw_7})}{2} \right)^2 )</td>
</tr>
<tr>
<td>4</td>
<td>( \pi \left[ \frac{(r_1 + r_5) \times (r_3 + r_7)}{4} \right]^2 - \pi \left[ \frac{(r_1 + r_5) - (r_{sw_1} + r_{sw_5}) \times ((r_3 + r_7) - (r_{sw_3} + r_{sw_7}))}{4} \right]^2 )</td>
</tr>
<tr>
<td>5</td>
<td>( \pi \left[ \frac{(r_1 + r_5) + (r_3 + r_7)}{4} \right]^2 - \pi \left[ \frac{(r_1 + r_5) - (r_{sw_1} + r_{sw_5}) + ((r_3 + r_7) - (r_{sw_3} + r_{sw_7}))}{4} \right]^2 )</td>
</tr>
<tr>
<td>6</td>
<td>( \pi \left[ \frac{(r_1 + r_5)^2 + (r_3 + r_7)^2}{8} \right]^2 - \pi \left[ \frac{(r_1 + r_5) - (r_{sw_1} + r_{sw_5})}{8} + \frac{(r_3 + r_7) - (r_{sw_3} + r_{sw_7})}{8} \right]^2 )</td>
</tr>
</tbody>
</table>

1.2.3 Determination of MC, \( \rho_d \), and D

Sapwood water content (MC) and density (\( \rho_d \)) were determined for each core using gravimetric techniques \(< 1\) h from initial sample collation. The sapwood-heartwood interface was determined by observing the abrupt difference in opacity, which was conspicuous for each species. Samples were cut with a razor blade to isolate the outer sapwood from the heartwood, bark, and cambium. Length and diameter of fresh samples were measured to 0.01 mm using digital calipers, weighed to 0.001 g on an electronic balance, then dried at 70° C for 48 h and reweighed to determine sample dry weight. The MC (kg kg\(^{-1}\)) is defined as:

\[
MC = \frac{(w_f - w_d)}{w_d}
\]  \( \quad (2) \)
Where \( w_f \) and \( w_d \) are the fresh and oven dry weights (kg) of the sapwood sample respectively. The \( \rho_d \) (kg m\(^{-3}\)) was calculated as the ratio of \( w_d \) to the fresh volume. Sapwood thermal diffusivity (D) was calculating according to Vandegehuchte and Steppe (2012). D is defined as the ratio of the axial sapwood thermal conductivity (\( K \), Eqn. 3) to the product of volumetric heat capacity (\( c \), Eqn. 4) and \( \rho_d \) such that:

\[
K = K_w (MC - MC_{FSP}) \frac{\rho_d}{\rho_{cw}} + 0.04186 \left[ 21.0 - 20.0 \left( 1 - G \left( \frac{\rho_w}{\rho_{cw}} + MC_{FSP} \right) \right) \right] \tag{3}
\]

\[
c = \frac{w_d c_d + c_w (w_f - w_d)}{w_f} \tag{4}
\]

Where \( K_w \) is the thermal conductivity of water (0.5984 W m\(^{-1}\) K\(^{-1}\)), \( MC_{FSP} \) is the water content at fibre saturation point determined according to Roderick and Berry (2001), \( \rho_w \) is the density of water, \( \rho_{cw} \) is the cell wall density (1530 kg m\(^{-3}\), Kollmann and Cote, (1968)), and \( G \) is the specific gravity of the sapwood (kg m\(^{-3}\)).

1.2.4 Sap flow measurements

We estimated \( V_h \) and JS via the HRM (Burgess et al., 2001) using sensors constructed in the Asbjornsen Lab at the University of New Hampshire adapted from the protocols of Davis et al. (2012). Sensors were installed at breast height on three mature, co-dominant red oak and white pine trees adjacent to individuals sampled for core extractions. Prior to installation, bark and cambial tissue was remove from the measurement point to ensure probes were in direct contact with the xylem. A metal drill guide was placed onto the exposed area allowing for accurate spacing between sensor probes and vatical alignment in parallel with each other. Thermocouple probes were coated in petroleum jelly and positioned at a distance 0.6 cm up- and
downstream of a 3.7 cm nichrome line heater (17–20 Ω), installed perpendicular to the sapwood. A reflective radiant barrier was fixed around the sensors to inhibit potential heating via direct sunlight. Sensors were connected to a datalogger and multiplexor powered by an external 12 V battery (CR1000 and AM16/32; Campbell Scientific Inc., Logan, UT, USA). A heat pulse of 2.5 s duration was sent to the central heating probe on a 15-min interval and the change in temperature 60 s following the heat pulse was recorded for the up- and downstream thermocouple. The heat-pulse velocity was calculated as:

\[ V_h = \frac{D}{x} \ln \left( \frac{\Delta T_{\text{down}}}{\Delta T_{\text{up}}} \right) \times 3600 \]  

(5)

Where \( V_h \) is the heat-pulse velocity (cm h\(^{-1}\)), \( D \) is the calculated sapwood thermal diffusivity (cm\(^2\) s\(^{-1}\)), \( x \) is the probe spacing between thermocouples and the heating probe (cm), and \( \Delta T_{\text{down}} \) and \( \Delta T_{\text{up}} \) is the down-stream and up-stream temperature difference respectively. \( V_h \) corrections for wounding, and probe misalignment were conducted following Burgess et al. (2001). Rates of zero-flow were calibrated using a combination of night-time and meteorological data (Ambrose et al., 2010; Gotsch et al., 2014), for which we found a flat-line period for each species between the hours of 22:00-05:00 that was equivalent to flow rates measured during periods of 100% relative humidity during the day. Since the purpose of this study was primarily to quantify the influence of MC, \( \rho_d \), and \( D \) on the accuracy of sap flow estimates, we only considered measurements at a depth of 0.5 cm and extrapolated across the entire sapwood area, ignoring potential influences of the radial profile.

1.2.5 Quantifying variability of the sapwood

The accuracy Asw estimates employing the six different geometric models was evaluated by fitting simple linear regressions to AA\text{sw} based on the mean of all radii measurements. To
determine the relative and absolute error associated with using a range of radii (core) samples for estimating Asw using the most common circular model, all potential combinations of radii were assessed for each level of sample replication. Thus, the total number of possible combinations of average radii is 255 based on 8 replicates. The mean relative error was calculated as the difference between AAsw and the mean Asw of predicted combinations for a given sample replication divided by the AAsw. Disk eccentricity was evaluated as a predictor of variability in radii measurements, sapwood thickness, and the relative pith offset using simple linear regressions.

1.2.6 Temporal and spatial variability of sapwood properties

To assess the variability of MC, ρ_d, and D over time and between core positions (DBH, stump) we performed a two-factor repeated measures ANOVA to compare the mean differences between groups. Since it is well established that the sapwood properties of interest are known to vary significantly between species (Cermak and Nadezhdina, 1998; Looker et al., 2016; Phillips et al., 1996), statistical tests were run separately for red oak and white pine. Differences between factors was considered significant at α = 0.05. For assessing the relationship between MC and ρ_d, we used regression analysis assuming that the correlation between the variables will be consistent across core positions, time, and species; therefore, all core samples collected during the study were pooled for the curve fit (n=160).

1.2.7 Sensitivity analysis

Sensitivity of sap flow measurements to variability in sapwood properties was conducted by substituting mean values of MC, ρ_d, and D determined at each sample date and core position
for red oak and white pine. Estimates of D were incorporated into the calculation for \( V_h \) (Eqn. X), while MC and \( \rho_d \) were used to scale the wound-corrected \( V_h \) to \( J_s \) (Eqn. 5). \( J_s \) was integrated over a 24 h period to produce an estimate total daily sap flux density (\( J_{D_{\text{r}}} \), cm\(^3\) cm\(^{-2}\) day\(^{-1}\)) and the maximum sap flux density (\( J_{\text{max}} \)) was determined for each 24 h period. We assessed the relationship between \( J_{\text{max}} \) and \( J_{D_{\text{r}}} \), then calculated the variance for \( J_{D_{\text{r}}} \) based on the substitution of measured and calculated sapwood properties evaluated at each of the four sampling dates. This analysis allows for quantifying the resulting variability of MC, \( \rho_d \), and D as a function of \( J_{\text{max}} \), since it is expected the relative error assuming all sapwood-related variables are constant will be exacerbated at higher flow rates. \( J_s \) is then integrated over the entire measurement period to assess differences between assumptions of sapwood properties over a longer temporal scale and compare the totals estimated from core measurements taken at a single point in time to the total \( J_s \) estimated from sapwood properties using the mean values computed for each species across all four measurement dates. Since it would require instantaneous measurements of MC, \( \rho_d \), and D in conjunction with the sapflow measurements to compute a true error (the difference between observed and actual values), rather we assess overall influence of sapwood property temporal variability through analysis of the residuals using the mean values of MC, \( \rho_d \), and D determined for each species. Therefore, sapwood properties determined at each of the four sampling periods (June, July, August, October) are applied to the entire sap flow timeseries and assessed relative to the seasonal mean. The dataset of measured sap flow data used in the sensitivity analyses includes 82 days measured between 3 June and 29 August 2015.

\[
Relative \text{ error} = \frac{\sum_{x} (J_{D{\text{r}}_{x}} - J_{D{\text{r}}})}{J_{D{\text{r}}}} \left/ \frac{1}{n} \right. \quad (6)
\]
Where $J_{D,x}$ is the total daily sap flux density calculated by substituting values of MC, $\rho_{d}$, and D from a single sampling period (Jun, Jul, Aug, or Oct), $J_{D\overline{x}}$ is the total daily sap flux density calculated using the mean values of MC, $\rho_{d}$, and D for each species, and n is the number of days.

### 1.3. Results

#### 1.3.1 Stem geometry and variability of the sapwood area

Allometric relationships for eastern white pine based on DBH were derived for bark thickness and sapwood area (Fig. 1.2). Strong correlations were achieved for both variables using an exponential fit. Disk eccentricity ranged from 0.78 to 0.98 cm cm$^{-1}$, with a mean of 0.93 cm cm$^{-1}$ across all samples. The coefficient of variation (CV) of the radii measurements was found to increase significantly as a function of eccentricity, $R^2 = 0.54$, $p < 0.0001$ (Fig. 1.3A). This relationship was not significant for the CV of sapwood thickness (Fig. 1.3B). The distance from geometric center to the pith (pith offset) was found to increase with eccentricity, $R^2 = 0.17$, $p = 0.032$, such that disks that are more eccentric tended to have piths at a distance farther away from the true geometric centroid (Fig. 1.3C). All six models for estimating sapwood area high a significant correlation ($p < 0.0001$) with AAsw, though $R^2$ and y-intercept values varied (Fig. 1.4). The circular model when using only a single radii measurement was the worst predictor of AAsw, as $R^2$ value ranged from 0.70 to 0.84 (Fig. 1.4A). Alternatively, models that incorporated both the major and minor axis of disks (geometric mean, arithmetic mean, and quadradic mean diameter) each exhibited a $R^2 > 0.95$ (Fig. 1.4D-F). The best correlation of the six models was found to be using the longest diameter, $R^2 = 0.96$, however, this model tended to over-estimate the total sapwood area at the larger diameter classes and deviated the most from the AAsw 1:1 line, resulting in the highest absolute error of any model (Fig. 1.4B). Based on the strength of correlation and the lowest total absolute error, the geometric mean diameter model was
determined to be the best overall predictor of AAsw. Since models other than the circular model
are not commonly considered for estimating sapwood area, further attention was given to
estimates derived assuming a circular shape through testing the average of radii measurements
through all possible combinations. Asw was estimated for all combinations of radii and the
correlation to AAsw was assessed for each number of replicates based on the eight radii
measures in this study (Fig. 1.5). The strength of correlation ($R^2$) of Asw to AAsw was found to
increase with core replication, with the greatest difference occurring between single replication
and the average of two replicates. This increase was found to be significant up to five replicates,
at which point the $R^2$ ranged from 0.950 to 0.966. Of the possible combinations of 2 replicates ($n$
$= 28$), the highest correlation was found to be using the mean of $r_6$ and $r_8$ ($R^2 = 0.955$), however,
the combination of $r_1$ and $r_5$ (the major axis) was found to be the second-best predictor, $R^2 = 0.948$.
This finding is relevant because the major axis may be determined in the field without
destructive sampling of the stem. Although there exists only a single combination of replicates
using all eight radii, the average of these resulted in the highest level of correlation at $R^2 = 0.966$.
The mean relative error of Asw estimates using all combinations of the circular model did not
change as sample replication increased, as the sapwood area is consistently over-estimated by
10% (Fig. 1.6). However, the maximum and minimum relative error induced was reduced
significantly as sample replication increased. Maximum and minimum relative error using a
single replicate was found to be 69 and 88% respectively, while maximum and minimum relative
error at eight replicates was 13 and 21% respectively.
Fig. 1.2 Allometric relationships for bark thickness (A) and sapwood area (B) for eastern white pine at DBH. Mean bark thickness was determined from n=8 measurements per disk, error bars show the standard error. Reported sapwood is the AAsw derived from image analysis. Shaded area for each curve denotes the 95% confidence interval.
Fig. 1.3 Disk coefficient of variation (CV) for radii (A), sapwood thickness (B), and the pith offset (C) as a function of measured eccentricity in eastern white pine. Fitted lines are simple linear regressions and the shaded area denotes the 95% confidence interval.
Fig. 1.4 Simple linear regressions of model estimated sapwood area ($A_{sw}$) as a function of the actual sapwood area ($A_{Asw}$) of eastern white pine. (A) The circular model showing the eight total fits, one for each radii measurement. (B) Longest diameter model. (C) Shortest diameter model. (D) Geometric mean diameter model. (E) Arithmetic mean diameter model. (F) Quadratic mean diameter model. Dashed line is the 1:1 line for $A_{Asw}$ and shaded region of regression lines indicate the 95% confidence interval.
Fig. 1.5 Values of $R^2$ derived from simple linear regressions of AAsw to Asw estimated using the circular model based on measurements of sapwood depth, bark thickness, and DBH of eastern white pine. Core replicates indicate the number of radii averaged within the circular model to calculate Asw. Number above boxes denote the number of possible combinations of radii at each level of replication.

Fig. 1.6 Relative error in the estimate sapwood area (Asw) in relation to the number of core replicates used on eastern white pine disks. Points show the mean values derived from the combination analysis and bars indicate the minimum and maximum relative error.
1.3.2 Temporal and spatial variability of sapwood properties

The range, mean, and standard deviation of the sapwood properties of interest across core position, and sampling period are reported white pine and red oak in Table 1.2 and Table 1.3 respectively. A two-factor repeated measures ANOVA was performed on a total sample of 80 core measurements per species (2 positions × 4 sample dates × 10 replicates) for each sapwood property of interest (Table 1.4).

Table 1.2 Measured values of MC, ρ, and D for eastern white pine.

<table>
<thead>
<tr>
<th>Position</th>
<th>Date</th>
<th>n</th>
<th>MC (kg kg⁻¹)</th>
<th>Mean</th>
<th>SD</th>
<th>ρ (kg m⁻³)</th>
<th>Mean</th>
<th>SD</th>
<th>D (cm² s⁻¹)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>DBH</td>
<td>Jun</td>
<td>10</td>
<td>1.17</td>
<td>0.17</td>
<td></td>
<td>414.5</td>
<td>30.1</td>
<td></td>
<td>0.00235</td>
<td>0.00011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jul</td>
<td>10</td>
<td>1.37</td>
<td>0.21</td>
<td></td>
<td>406.4</td>
<td>31.7</td>
<td></td>
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<td>0.00010</td>
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</tr>
<tr>
<td></td>
<td>Aug</td>
<td>10</td>
<td>1.32</td>
<td>0.28</td>
<td></td>
<td>407.2</td>
<td>58.5</td>
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<td>0.00228</td>
<td>0.00014</td>
<td></td>
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<tr>
<td></td>
<td>Oct</td>
<td>10</td>
<td>1.43</td>
<td>0.26</td>
<td></td>
<td>377.5</td>
<td>35.4</td>
<td></td>
<td>0.00223</td>
<td>0.00013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>40</td>
<td>1.32</td>
<td>0.25</td>
<td></td>
<td>401.4</td>
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<td>0.00227</td>
<td>0.00013</td>
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</tr>
<tr>
<td>Stump</td>
<td>Jun</td>
<td>10</td>
<td>1.20</td>
<td>0.16</td>
<td></td>
<td>418.1</td>
<td>51.0</td>
<td></td>
<td>0.00233</td>
<td>0.00009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jul</td>
<td>10</td>
<td>1.24</td>
<td>0.20</td>
<td></td>
<td>421.3</td>
<td>33.9</td>
<td></td>
<td>0.00230</td>
<td>0.00012</td>
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</tr>
<tr>
<td></td>
<td>Aug</td>
<td>10</td>
<td>1.37</td>
<td>0.28</td>
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<td>419.7</td>
<td>42.9</td>
<td></td>
<td>0.00224</td>
<td>0.00015</td>
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</tr>
<tr>
<td></td>
<td>Oct</td>
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<td>1.39</td>
<td>0.14</td>
<td></td>
<td>394.1</td>
<td>37.8</td>
<td></td>
<td>0.00223</td>
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</tr>
<tr>
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<td>Total</td>
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<td></td>
<td>413.3</td>
<td>41.8</td>
<td></td>
<td>0.00228</td>
<td>0.00011</td>
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</tr>
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</table>

Table 1.3 Measured values of MC, ρ, and D for northern red oak.

<table>
<thead>
<tr>
<th>Position</th>
<th>Date</th>
<th>n</th>
<th>MC (kg kg⁻¹)</th>
<th>Mean</th>
<th>SD</th>
<th>ρ (kg m⁻³)</th>
<th>Mean</th>
<th>SD</th>
<th>D (cm² s⁻¹)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td>Mean</td>
<td>SD</td>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>DBH</td>
<td>Jun</td>
<td>10</td>
<td>0.68</td>
<td>0.09</td>
<td></td>
<td>573.5</td>
<td>77.5</td>
<td></td>
<td>0.00271</td>
<td>0.00009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jul</td>
<td>10</td>
<td>0.76</td>
<td>0.07</td>
<td></td>
<td>585.7</td>
<td>49.4</td>
<td></td>
<td>0.00260</td>
<td>0.00007</td>
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</tr>
<tr>
<td></td>
<td>Aug</td>
<td>10</td>
<td>0.68</td>
<td>0.10</td>
<td></td>
<td>592.0</td>
<td>38.1</td>
<td></td>
<td>0.00269</td>
<td>0.00011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oct</td>
<td>10</td>
<td>0.68</td>
<td>0.04</td>
<td></td>
<td>598.5</td>
<td>35.0</td>
<td></td>
<td>0.00269</td>
<td>0.00005</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>40</td>
<td>0.70</td>
<td>0.08</td>
<td></td>
<td>587.4</td>
<td>51.5</td>
<td></td>
<td>0.00267</td>
<td>0.00009</td>
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<tr>
<td>Stump</td>
<td>Jun</td>
<td>10</td>
<td>0.71</td>
<td>0.10</td>
<td></td>
<td>582.2</td>
<td>76.3</td>
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</tr>
<tr>
<td></td>
<td>Jul</td>
<td>10</td>
<td>0.81</td>
<td>0.09</td>
<td></td>
<td>583.7</td>
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<tr>
<td></td>
<td>Aug</td>
<td>10</td>
<td>0.70</td>
<td>0.08</td>
<td></td>
<td>618.7</td>
<td>38.4</td>
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<td>0.00266</td>
<td>0.00008</td>
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<tr>
<td></td>
<td>Oct</td>
<td>10</td>
<td>0.73</td>
<td>0.08</td>
<td></td>
<td>619.8</td>
<td>46.0</td>
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<td>0.00262</td>
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</tr>
<tr>
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<td>Total</td>
<td>40</td>
<td>0.74</td>
<td>0.10</td>
<td></td>
<td>601.1</td>
<td>66.1</td>
<td></td>
<td>0.00262</td>
<td>0.00009</td>
<td></td>
</tr>
</tbody>
</table>
There were no significant interaction effects for MC, $\rho_d$, or D between the location on the bole in which core samples were obtained and the time at which collection occurred for either species ($p > 0.535$). Simple main effects showed that the time at which sampling occurred had a significant influence on MC for both red oak ($p = 0.001$) and white pine ($p = 0.013$), while core position was also marginally significant for red oak ($p = 0.048$).

**Table 1.4** Two-way factorial ANOVA results for sapwood properties of red oak and white pine.

<table>
<thead>
<tr>
<th>Species</th>
<th>Response Variable</th>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White pine</strong></td>
<td><strong>MC (kg kg$^{-1}$)</strong></td>
<td>Time</td>
<td>0.558</td>
<td>3</td>
<td>0.186</td>
<td>3.883</td>
<td><strong>0.013</strong></td>
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<tr>
<td></td>
<td></td>
<td>Position</td>
<td>0.011</td>
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<td>0.011</td>
<td>0.227</td>
<td>0.635</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time X Position</td>
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<td>3</td>
<td>0.352</td>
<td>0.734</td>
<td>0.535</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Error</td>
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<td>72</td>
<td>0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>$\rho$ (kg m$^{-3}$)</strong></td>
<td>Time</td>
<td>12488.4</td>
<td>3</td>
<td>4162.8</td>
<td>2.450</td>
<td>0.070</td>
</tr>
<tr>
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<td></td>
<td>Position</td>
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<td>2848.9</td>
<td>1.677</td>
<td>0.199</td>
</tr>
<tr>
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<td></td>
<td>Time X Position</td>
<td>507.8</td>
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<td>169.3</td>
<td>0.100</td>
<td>0.960</td>
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<tr>
<td></td>
<td></td>
<td>Error</td>
<td>122328.3</td>
<td>72</td>
<td>1699.0</td>
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<tr>
<td></td>
<td><strong>D (cm$^2$ s$^{-1}$)</strong></td>
<td>Time</td>
<td>1.15x10$^{-7}$</td>
<td>3</td>
<td>3.84x10$^{-8}$</td>
<td>2.843</td>
<td><strong>0.044</strong></td>
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<tr>
<td></td>
<td></td>
<td>Position</td>
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<td>1.01x10$^{-10}$</td>
<td>0.008</td>
<td>0.931</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time X Position</td>
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<td>3</td>
<td>9.50x10$^{-9}$</td>
<td>0.703</td>
<td>0.553</td>
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<tr>
<td></td>
<td></td>
<td>Error</td>
<td>9.73x10$^{-7}$</td>
<td>72</td>
<td>1.35x10$^{-8}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Red oak</strong></td>
<td><strong>MC (kg kg$^{-1}$)</strong></td>
<td>Time</td>
<td>0.125</td>
<td>3</td>
<td>0.042</td>
<td>5.932</td>
<td><strong>0.001</strong></td>
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<tr>
<td></td>
<td></td>
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<td>4.060</td>
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<tr>
<td></td>
<td></td>
<td>Time X Position</td>
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<td>0.001</td>
<td>0.212</td>
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<td>Error</td>
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</tr>
<tr>
<td></td>
<td><strong>$\rho$ (kg m$^{-3}$)</strong></td>
<td>Time</td>
<td>14087.6</td>
<td>3</td>
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<td>3756.5</td>
<td>1.050</td>
<td>0.309</td>
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<tr>
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<td></td>
<td>Time X Position</td>
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<td>3</td>
<td>829.3</td>
<td>0.232</td>
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</tr>
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<td>Error</td>
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<td>72</td>
<td>3576.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>D (cm$^2$ s$^{-1}$)</strong></td>
<td>Time</td>
<td>1.62x10$^{-7}$</td>
<td>3</td>
<td>5.40x10$^{-8}$</td>
<td>7.890</td>
<td>&lt;0.001</td>
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<tr>
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<td></td>
<td>Position</td>
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<td>4.95x10$^{-8}$</td>
<td>7.230</td>
<td><strong>0.009</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time X Position</td>
<td>3.80x10$^{-9}$</td>
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<td>0.185</td>
<td>0.906</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Error</td>
<td>4.93x10$^{-7}$</td>
<td>72</td>
<td>6.85x10$^{-9}$</td>
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<td></td>
</tr>
</tbody>
</table>
Over the course of the sampling period an increasing trend in MC is noted for white pine, with a gain of 0.25 kg kg\(^{-1}\) between the month of June and October at DBH (**Fig. 1.7**). Interestingly for red oak, MC is significantly higher only for samples collected in the month of July, otherwise mean values of MC within red oak at DBH is stable at 0.68 kg kg\(^{-1}\), consistent with reported MC values of 0.69 kg kg\(^{-1}\) (Ross, 2010). We report no differences in \(\rho_d\) between core position or time for either species (**Fig. 1.8**).

**Fig. 1.7** Variation in sapwood water content (MC) over the course of the 2015 growing season for red oak and white pine. Open and closed symbols represent the DBH and stump positions respectively. Error bars show standard deviation.
Fig. 1.8 Variation in sapwood density ($\rho$) over the course of the 2015 growing season for red oak and white pine. Open and closed symbols represent the DBH and stump positions respectively. Error bars show standard deviation.

Fig. 1.9 Variation in sapwood thermal diffusivity (D) over the course of the 2015 growing season for red oak and white pine. Open and closed symbols represent the DBH and stump positions respectively. Error bars show standard deviation.

The mean calculated D across all samples for red oak and white pine is $2.67 \times 10^{-3}$ (SD $\pm 0.09 \times 10^{-3}$) and $2.50 \times 10^{-3}$ cm$^2$ s$^{-1}$ (SD $\pm 0.10 \times 10^{-3}$) respectively. The time effect on D was
significant for both red oak (p < 0.001) and white pine (p = 0.030), while the effect of core position was significant only for red oak (p = 0.018). Seasonal trends in D are generally inverse of the observed MC trends (Fig. 1.9), as D of white pine at DBH decreases slightly, though significantly (p = 0.043), from a value of 2.54×10⁻³ in June to 2.45×10⁻³ cm² s⁻¹ in the month of October. Similarly, D within red oak was determined to be significantly different only for the month of July. We report a strong inverse correlation between ρd and MC across species and core positions (Fig. 1.10). This relationship appears to be consistent across a wide range sapwood densities based on data available from 28 softwood and 35 hardwood tree species reported by Ross (2010), in which the slope of a quadratic fit differs from this study by a value of 0.001.

**Fig. 1.10** Sapwood water content (MC) as a function of sapwood density (ρ). Circles and squares denote white pine and red oak respectively, open and closed shapes denote measurements obtained from the breast height and stump position respectively. Shaded region shows the 95% confidence interval of the second-order polynomial fit.
3.3.3 Sap flow sensitivity analysis

At greater rates of sap flux density, the influence of the differences between MC, ρd, and D sampled across the four discrete time periods of this study resulted in a higher degree of uncertainty (error) relative to the mean value for each species. These differences are apparent when observing diurnal patterns of Js, such that the variability between time periods at which core samples were obtained is greatest at times of peak flow between the hours 12:00-16:00 (Fig.1.11). Conversely, at periods of low flow rates, the variability induced by the sampling period of sapwood properties has very little influence on Js.

**Fig. 1.11** Estimates of sap flux density (Js) in red oak (A) and white pine (B) for three days in early June. Each color indicates the substitution of sapwood properties measured on a single sampling date and applied to the raw data.
Due to the nature in which these sapwood parameters scale with $V_h$ to estimate $J_s$ (i.e. greater density and MC will produce greater $J_s$), we note that the increasing deviation at larger flow rates is expected. We found large differences in $J_{max}$ between the two species throughout the study period, such that red oak exhibits a mean peak of 11.6 cm$^3$ cm$^{-2}$ h$^{-1}$ and white pine exhibited a mean peak of 30.9 cm$^3$ cm$^{-2}$ h$^{-1}$. The highest estimated flow rates for red oak and white pine were 23.2 and 44.2 cm$^3$ cm$^{-2}$ h$^{-1}$ respectively. The variance among $J_D$ assuming sapwood properties sampled at different points in the growing season increased with $J_{max}$ for both red oak (Fig. 1.12) and white pine (Fig. 1.13). The overall variance in red oak was found to be considerably lower than variance estimates for white pine, largely due to lower values of $J_{max}$.

The compounded error is somewhat muted as MC and density are inversely related (Fig. 1.10), such that at lower sapwood densities will typically retain higher MC. We report error associated with assumption of MC, $\rho_d$, and D for a single sampling period relative to a seasonal mean resulted in a range of -3.5 to 2.1% in red oak, and a range of -2.5 to 6.1% in white pine across core positions considered over the entire sampling period for sap flow measurements (Fig. 1.14). Error rates between core positions were generally similar, however it is worth noting a difference of 3.8% between DBH and stump within white pine for estimates based on July measurements. This can be attributed to a deviation of 13.3% in MC measured between the two core positions for the month of July. While this difference was not found to be significant via a t-test between the positions ($p = 0.163$), it is still considerably greater than the difference in MC measured in the months of June, August, and October, with differences of 2.7, 1.1, and 4.3% respectively. Despite rejecting the null hypothesis of the effect of core position on MC and D for red oak (Table 1.4), we report minimal differences in the relative error between core positions.
for each month, such that differences between DBH and stump range from 1.4, 1.5, 0.2, and 1.4% in the months of June, July, August, and October respectively.

**Fig. 1.12** Variance in $J_D$ estimated from substituting the sapwood properties derived from the four collection dates as a function of $J_{\text{max}}$ for northern red oak. Each shape represents a different tree replicate ($n=3$) and the DBH/stump position is denoted by open and closed fill respectively.

**Fig. 1.13** Variance in $J_D$ estimated from substituting the sapwood properties derived from the four collection dates as a function of $J_{\text{max}}$ for eastern white pine. Each shape represents a different tree replicate ($n=3$) and the DBH/stump position is denoted by open and closed fill respectively.
Fig. 1.14 The relative error associated with assumptions of constant MC, $\rho_d$, and D sampled for a given month by species and location of sapwood core extraction (DBH, stump). Error rates are relative to setting the mean value of MC, $\rho_d$, and D across all months, reported in Table 1.2 and Table 1.3. Data includes 95 and 79 days for red oak and white pine respectively.

1.4 Discussion

This study found substantial variability within the geometry of stem sections of eastern white pine, such that models that scale to basal area and total sapwood area assuming a circular shape induced significant error. On average, circular models used to predict Asw tended to overestimate AAsw by 10% (Fig. 1.6). This finding is consistent with those of Biging and Wensel (1988), which found that the circular model resulted in an over estimation of 9.5% in estimates of basal area of Douglas-fir (*Pseudotsuga mensiesii* Mirb.). When scaling sap flow measurements to estimates of whole tree water use, given as the product of sap velocity and Asw, this error rate is directly propagated in addition to any potential error resulting from point
measurements of sapflow. An error rate on the order of 10% is significant when dealing with transpiration estimates over extended time periods or scaled to larger spatial orders (Hernandez-Santana et al., 2015; Kume et al., 2010). Throughout this study, radii measured on disks are assumed to be analogous of increment core samples that are typically collected in the field for determination of sapwood thickness. To this end, it was found that increasing core replication will result in more accurate estimate of Asw. The average of two core samples should be considered an absolute minimum for estimating sapwood thickness and scaling to Asw. If possible, using a combination of core sample from the major axis will provide an estimate of Asw with a high degree of accuracy. Locating the major axis of a tree can be accomplished in the field by rotating graduated tree calipers about the stem until the maximum diameter is found. This field determination of the major axis depends somewhat on a uniform bark thickness, which was shown to be more variable as DBH increases (Fig 1.2A). If four or more core samples are obtained over the course of a study period, two additional core samples from the minor axis would allow for the use of more accurate model estimates, such as the geometric mean diameter.

Mean stem eccentricity for the 30 eastern white pine disks measured in this study was found to be 0.93 cm cm$^{-1}$, identical to the value reported for western hemlock (Tsuga heterophylla (Raf.) Sarg.) based on 87 disk samples (Kellogg and Barber, 1981). In general, it would be advisable to avoid eccentric trees, since it was found that variability of the IBR tends to increase (Fig. 1.3). Sampled trees with greater eccentricity also tended to retain piths at a greater distance from the true geometric center. This is relevant in cases where locating the pith matters, such as obtaining a complete timeseries for dendrochronology studies. This study found no obvious pattern relating to the location of the pith within eccentric samples. Similarly, a study of pith eccentricity in teak (Tectona grandis L.F.) reported high variability within trees, but could
not relate this to aspects of ring width, tree form factor, or crown depth (Akachuku and Abolarin, 1989). Additionally, average eccentricity of western hemlock was found to be independent of DBH, age, and stem height (Kellogg and Barber, 1981). In eastern white pine, it is somewhat common for stems to exhibit multiple branches of the main trunk below the live crown due to damage induced by white pine weevil (Pissoides strobi Peck), especially in stands established on abandoned agricultural land (Major et al., 2009; Ostry et al., 2010; Taylor and Cozens, 1994). This will occur when the leading terminal shoot is damaged, in which case a secondary shoot will take over for the primary growth. This complex branching was observed at the study site where disks were collected, as many of the sampled trees were noted to have two to four distinct stems occurring above DBH. This may have contributed to the eccentricity of individuals, particularly when the branching occurred just above the point where disks were collected. Since the secondary branches are not often the same size, partitioning of sapwood and structural components may have been offset to support the resulting multi-stem architecture. The influence of slope has also been shown to significantly alter stem eccentricity through the mechanical stress induced by the requirement to support standing biomass on uneven terrain (Malik and Wistuba, 2012). When considering estimates of Asw by means of core sample, replication is key, both for within tree accuracy as well as among trees when developing allometric relationships to be used for hierarchical scaling objectives.

This study also reports significant variability in the physical properties of the sapwood over the course of a single growing season. The mean D value of $2.65 \times 10^{-3}$ cm$^2$ s$^{-1}$ for red oak and $2.28 \times 10^{-3}$ cm$^2$ s$^{-1}$ for white pine reported in this study are each close to the nominal value of $2.5 \times 10^{-3}$ cm$^2$ s$^{-1}$ first presented by Marshall (1958). While we found significant differences in D in the months of July and October in red oak and white pine respectively, these differences
equate to a deviation less than 0.0001 cm\(^2\) s\(^{-1}\) at both core positions. Because we did not detect significant differences in sapwood density between core position or sampling dates, the influence on calculated D, and therefore J\(s\), was largely driven by the temporal changes measured in MC for each species. Despite this variability, fluctuation in MC overall was quite small, with only a single month determined to be significantly different for each species (Fig. 1.7). It should be noted that determination of MC using bulk core samples is a relatively coarse measurement, as it has been shown that MC can fluctuate on a diurnal timescale and radially within the sapwood (Trcala et al., 2015). Several methods have been developed for obtaining in-situ estimates of MC in conjunction with sapflow measurements to better refine diel variability, as MC generally reduces while transpiration is occurring during daylight hours (Steppe et al., 2015). Among these include time-domain reflectometry (Irvine and Grace, 1997; Wullschleger et al., 1996), frequency domain reflectometry (Hao et al., 2013), gamma radiation attenuation (Edwards and Jarvis, 1983), and nuclear magnetic resonance (Byrne et al., 1986; van As et al., 2009). While each of these methods have the inherent advantage over the core sampling method in that destructive sampling can be reduced and the temporal resolution is greatly increased, there are other tradeoffs with data acquisition that must be considered. For instance, both time- and frequency-domain reflectometry require a gravimetric species-specific calibration to empirically calculate MC, which can be time intensive to determine. These sensors are also costly ($200-300 USD / sensor) and thus may be prohibitively expensive for large-scale sap flow studies. The two later methods, while proven to be effective, are currently limited in their applicability due to health risk involved with working with gamma radiation, and the expense of MRI techniques and its difficulty of applying in the field. There have been recent methods developed for estimating MC in conjunction with sap flow measurements using the CHP and Sapflow\(^*\) methods (López-
Bernal et al., 2012; Vandegehuchte and Steppe, 2012), which can be fully automated and do not require addition instrumentation. While there are currently few studies utilizing or validating these techniques in the field among other woody species, the potential exists to further refine transpiration measurement by taking into consideration subtle changes in seasonal and even daily variability in MC. However, determination of MC continues to be most commonly conducted using the core technique described herein.

Within this study we fail to reject the null hypothesis regarding the effect of core position on the sapwood properties of white pine. For red oak, we found only a marginally significant effect of core position for \( \rho_d \) (\( p = 0.048 \)), but a larger effect when calculating \( D \), based in part of our measurements of MC and \( \rho_d \). However, when evaluating the relative error in \( J_s \) between core positions over time, we found differences to be < 1.5% between measurement obtained at DBH and stump. This finding is encouraging when considering the potential reduction in destructive sampling near the installation point of sap flow probes, which is most often located at DBH. While we did not obtain cores directly from the trees monitored for sap flow in this study, Looker et al. (2016) reports that obtaining cores from locations other than the individual tree used for the transpiration measurement (i.e. from a neighboring tree, plot, or stand) resulted in an average relative error of 0.115 across spatial scales. An acceptable error rate should be considered prior to implementation of a specific sap flow technique and in determining the methodology used for parametrizing calculations of transpiration based in part on properties of the sapwood. If the goal is to generate a water budget at a large spatial scale, for example: estimating total transpiration of a watershed for developing an economic valuation of ecosystem services, then a high degree of accuracy is critical for ensuring fair payment and water security (Ford et al., 2007; Hernandez-Santana et al., 2011). Under other circumstances, such as
evaluating a relative treatment effect on transpiration via a field experiment, a higher level of measurement uncertainty may be tolerated if the goal is to simply compare the effects of two or more treatments, so long as the error rate is assumed to be nearly the same across treatments. As with any measurement that includes an element of uncertainty, increasing the replication can aid in refining error rates. Some attention has been given to the topic of within- and among-tree variability of sap flow measurements, and it has been suggested that it is better to replicate more trees than focus on multiple measurements within a single tree (Chelcy R Ford et al., 2007; Ford et al., 2004; Kume et al., 2010). While sap flow studies are not known for being particularly thrifty, advancements have been made to reduce costs, such as in-house construction of high-quality sensors in lieu of expensive commercial sensors (Davis et al., 2012).

1.5 Conclusion

One aim of this chapter was to refine aspects of heat-pulse based sap flow measurements as they relate to the physical properties of the sapwood. The sapwood area was found to vary considerably among stems of eastern white pine depending on the model used to estimate $A_{sw}$. While the circular model was found to consistently overestimate $A_{sw}$, it is unlikely that researchers will adopt a different geometric model, in large part because of its ease of use compared to other geometric models presented throughout. In light of this, to obtain better estimates of $A_{sw}$ using the circular model, it is recommended to use two or more core samples for estimates of sapwood thickness within study trees, and if possible, using the average of two cores extracted from the major axis of the stem. In general, eccentric stems should be avoided to reduce potential error in $A_{sw}$, due to variability in sapwood thickness but also for practical purposes relating to the installation of sap flow probes that could potentially overshoot the
sapwood and extend into the heartwood. For the sapwood parameters used in calculating $V_h$ and $J_s$ (MC, $\rho$, and D), the variability throughout the growing season is minimal, but can be significant when measurements are integrated over long time periods. Using a mean value for MC and D obtained from two or more core samples collected at the either end of a sampling period would potentially mute error rates induced by assuming a single constant value. Thus, based on trends observed in this study, we recommend obtaining at least two core samples for estimating the relevant sapwood properties, ideally at the beginning and end of the study period defined for sapflow measurements. To limit damage on the main stem, our data indicates that measurements can be obtained from the stump location without significantly influencing estimates of $J_s$. 
CHAPTER 2

IMPACTS OF WHITE PINE NEEDLE DAMAGE ON SEASONAL LITTERFALL
DYNAMICS AND WOOD GROWTH OF EASTERN WHITE PINE IN NORTHERN NEW ENGLAND

Abstract

White Pine Needle Damage (WPND) is a complex of foliar fungal pathogens that have established as a chronic disease impacting eastern white pine (Pinus strobus L) stands in the northeastern United States. With long-term ecological and economic impacts in mind, it is critical to quantify the negative effects of this disease on tree and forest health to make informed management decisions. We measured litterfall to determine the timing and magnitude of WPND-induced defoliation across four study sites in the northeastern US between 2014-2016. We measured N concentrations of needles cast throughout the 2014 growing season to estimate total litter N flux resulting from WPND. Additionally, to quantify growth declines we measured annual basal area increment (BAI) from six symptomatic study sites in the infected region. We found that WPND-induced defoliation in the months of June and July accounted for 47% of the total annual litterfall across the study sites, often exceeding normal needle senescence in October. Foliar %N in June and July was 0.78 and 0.84% respectively, significantly higher than October concentrations of 0.40%, suggesting incomplete resorption of N during the summer months. Untimely summer defoliations resulted in a mean estimated N loss of 0.92 g N m\(^{-2}\) yr\(^{-1}\), representing 63% of the total growing season N input from foliage. Growth of symptomatic trees at all sites was reduced following outbreaks of WPND initiating between 2007-2009. Severely
infected trees reduced BAI 25-73% compared to pre-outbreak years. Our results show that
WPND-induced defoliation significantly alters litterfall and N dynamics of affected stands, and
suggest that subsequent N limitation in addition to reduced foliar area greatly reduces annual
wood growth within infected stands.

2.1 Introduction

White Pine Needle Damage (WPND) is a disease complex caused by several pathogenic
ascomycete fungi presumed to be native to the northeastern United States (Broders et al., 2015;
acicola* Thümen, *Lophophacidium dooksii* Corlett and Shoemaker, *Bifusella linearis* Peck, and
*Septorioides strobi* (Wyka and Broders, 2016), are now commonly found on the mature needles
of eastern white pine (*Pinus strobus* L) within the northeastern part of its range. These fungi are
known to occur independently or concurrently on trees within infected stands (Wyka et al.,
2017a). In recent years, outbreaks of these foliar pathogens have caused untimely needle casting
during the summer months, resulting in thinned crowns and the dieback of lower branches within
the crown. Changes in the regional climate in recent decades, specifically a trend of warmer and
wetter springs, may be a driver of contemporary fungal disease outbreaks and are strong
predictors of annual WPND outbreak severity (Wyka et al., 2017a, Wkya et al. 2018). The
dispersal and growth of primary pathogens such as those associated with WPND are thought to
be enhanced during wet periods due to the increased availability of water and humidity
conducive to fungal growth and reproduction (Kolb et al., 2016). Projections from atmosphere-
ocean general circulation models of the northeastern US suggest that both annual temperature
and precipitation will continue to increase through the end of the century (Hayhoe et al., 2007).
The development of fruiting bodies and spore dispersal of WPND fungi are most sensitive to early growing season precipitation in the months of May, June, and July (Wyka et al., 2017a). Within the past decade the northeast US has experienced the wettest (2006) and second wettest (2009) summer on record, while 2011 was the wettest year (annually) since 1895 (Kunkel et al., 2013). Given the recent trends in regional climate and the unprecedented outbreaks of fungal pathogens associated with this disease complex, it is critical to quantify the negative effects of this disease on tree and forest health in order to make informed management decisions.

Needle blight diseases on white pine have been observed and studied since 1908 (Clinton, 1908; Faull, 1920), however exact causal agents went unidentified until much later and in some cases pathogenic symptoms may have been attributed to ozone damage (Dreisbach, 1989). The white pine needle pathogen *L. dooksii* has been previously reported as being widespread throughout the northeastern US, but with low incidence rates on infected trees (Baldwin, 1954; Merrill et al., 1996). It is possible that several or all the fungal pathogens associated with WPND have been historically present in the region, but recent favorable weather has facilitated the rapid spread of the current epidemic. While outbreaks of WPND were first reported in Maine in 2006 (Munck et al., 2012), the year 2010 is often cited as the first time widespread defoliation was observed throughout the northeastern US (Broders et al., 2015; Munck et al., 2012). Presently, chronic outbreaks continue to occur in the region as WPND fungi are established and active throughout white pine stands. Among New England pine stands sampled for needle pathogens between 2011-2014, only 7% were found to be asymptomatic for fungi associated with WPND (Wyka et al., 2017a). To date, mortality associated with WPND is low and typically observed in conjunction with other biotic and abiotic stressors. Infected trees that are in intermediate or overtopped crown positions tend to succumb more rapidly than dominant and emergent
individuals, in part due to the rain-splash dispersed nature of the pathogens. The asexual spores carried by rain droplets disperse predominantly downward through crowns (Wyka et al., 2017b), thus the lower portions of crowns and presumably understory trees receive the highest amounts of inoculum.

Despite the low levels of observed mortality, pest and pathogen defoliations occurring mid-growing season are known to have significant impacts on tree growth and physiological processes among conifer species. Marked reductions in wood growth resulting from defoliation have been documented in several other Pinus species including P. pinaster Aiton in response to processionary moth (Thaumetopoea pityocampa Denis and Schifferüller) herbivory (J. S. Jacquet et al., 2013; Jacquet et al., 2012; Puri et al., 2015), Scleroderris canker (Gremmeniella abietina M. Sars) on P. silvestris L. (Oliva et al., 2016; Sudachkova et al., 2015), P. taeda L. (Lashomb et al., 1978; Weise et al., 2016) attacked by Nantucket pine tip moth (Rhyacionia frustrana Scudder in Comstock), and P. radiata D. Don defoliated by ascomycete fungal pathogens Mycosphaerella cryptica (Cooke) Hansf. and M. nubilosa (Cooke) Hansf. (Carnegie and Ades, 2003; May and Carlyle, 2003). Unlike many broadleaf tree species, conifers are not able to produce a new flush of foliage following a mid-season defoliation event. Additionally, white pine fascicles (needle clusters) of the current year elongate over several months between May and August, thus are only partially developed upon WPND-induced defoliations of previous-year needles. As a result, infected trees generally bear thinned crowns with greatly diminished amounts of mature foliage. Early leaf drop due to pests and pathogens is also known to significantly alter the recycling of nitrogen (N) within trees, such that infected stands redistribute a greater proportion of N to the forest floor in lieu of resorption into storage tissues ahead of natural senescence (Lovett et al., 2002). As N is closely linked to photosynthetic capacity
(Evans, 1989), a large loss of organic N from foliage mid-growing season can modify carbon assimilation rates and N uptake demand. Forest managers in the region are concerned about the impacts of WPND on wood growth and the potential for tree mortality in diseased stands. Therefore, information regarding the impact WPND is having on tree health and physiology will aid us in understanding the consequences of this emerging disease complex and allow us to make better predictions concerning the future of WPND-infected white pine stands. The first purpose of this study was to quantify the magnitude, temporal distribution, and foliar N concentration of litter cast due to defoliation by WPND across sites in New Hampshire and Maine. In turn, due to significant losses of mature foliage during the growing season, we hypothesize that gross seasonal carbon assimilation rates will be reduced, resulting in a reduction of carbon allocated to wood growth throughout infected stands in the northeastern US.

### 2.2 Methods

#### 2.2.1 Study sites

We studied tree growth rings at six white pine dominated stands located in Maine, New Hampshire, Vermont, and Massachusetts. Litter fall was sampled on a subset of three sites in Maine and New Hampshire, plus an additional location in Durham, New Hampshire where only litterfall collection was conducted because the stand was initially identified as asymptomatic for WPND during an initial survey in 2012 (Fig. 2.1, Table 2.1). The study area includes several biophysical regions (Krohn et al., 1999; Sperduto and Nichols, 2004; Thompson, 2002) and spans four USDA cold-hardiness zones (USDA 2012). The climate within this region is characterized by a humid continental climate, with cold winters, moderately warm summers, and a relatively even distribution of rainfall throughout the year. These sites also include a range of
silvicultural management strategies and land use histories. The plots at Mohawk Trail Reserve in Charlemont, MA (MHT) are within an unmanaged white pine stand regarded as old-growth forest, with several of the largest known pines in the state. The study area within the St. Johnsbury Municipal Forest, VT (STJ) is an even-aged white pine stand planted in 1925 following agricultural abandonment, with timber harvests taking place in 1967, 1978, and 2004. The study area in Richmond, VT (RMD) is located at the south end of Richmond Pond on private land dominated by mature white pine which is actively managed for timber and wildlife. The sampling area at the Fox State Forest located in Hillsborough, NH (FOX) is within a mixed white pine and eastern hemlock (*Tsuga canadensis* (L.) Carrière) stand that naturally regenerated following a stand-replacing hurricane in 1938 and is actively managed for timber, wildlife, recreational, and research purposes (Allen and Seaboyer, 2017). The litterfall plots in Durham, NH (DUR) are located within a white pine stand mixed with red pine (*Pinus resinosa* Aiton) and northern hardwood species that regenerated naturally following agricultural abandonment in the early 1900s on land owned and managed by the University of New Hampshire. The Massabesic Experimental Forest is federally owned land in Lyman, ME (MEF). Natural regeneration of white pine and northern hardwood species occurred after stand replacing fires in 1947 and the WPND plots are in close proximity to three clearcuts that were conducted in 2007. The study area in Bethel, ME (BTH) is located on private land owned and managed for industrial scale timber harvesting of white pine. All study sites except DUR were established in 2012 as long-term WPND monitoring plots and have been observed annually by U.S. Forest Service and State Forest Health Cooperators for crown condition (Broders et al., 2015; S. A. Wyka et al., 2017a). Within the long-term monitoring sites, trees were initially tagged in pairs of low- and high-severity WPND symptomology. The symptoms of WPND are estimated via ocular
measurements of chlorosis in late June and needle retention within the crown following the untimely defoliation event (Broders et al., 2015). Trees are rated on a numerical scale (0-3), where 0 corresponds to a healthy crown free of signs and symptoms of WPND, 1 infers that ≤ 1/3 of the crown is affected, 2 infers that 1/3 > 2/3 of the crown is affected, and a score of 3 infers that > 2/3 of the crown exhibits infection. Throughout this paper, trees referred to as low-severity received a rating of 0 and 1 upon initial surveys in 2012, while high-severity trees received ratings of 2 and 3.

Fig. 2.1 Map of the New England states showing the location of the eight US Forest Service White Pine Needle Damage (WPND) monitoring sites assessed in this study. All sites except for DUR were sampled for tree ring analysis. Sites DUR, FOX, MEF, and BTH were sampled for litterfall during the 2014-2016 growing seasons.

2.2.2 Litterfall and foliar nitrogen

Foliar litterfall was measured during the 2014, 2015, and 2016 growing seasons at study sites DUR, FOX, MEF, and BTH. Due to the time-intensive nature of monthly litter collection
and sorting, we selected this subset of sites based on ease of access. Additionally, these sites are all within WPND-symptomatic white pine dominated stands and representative of the regional epidemic. At each location, three 900 m² square plots were established where at least 60% of the total basal area consisted of *P. strobus*. Litter traps were constructed of 0.17 m² rectangular plastic baskets lined with a fine vinyl mesh. Five litter traps were randomly located within each plot using a tessellated random sampling design and fixed to the ground using lawn staples along the long edge of the trap. Traps were cleaned of debris in late April and remained fixed in the same location within plots each year of the study to best capture variability in litterfall from year to year, rather than the within-plot variability. Litter was collected on the last 1-2 days of each month starting May 1 and ending October 31. Upon collection, foliar material was stored in paper bags and oven dried at 70°C for 48 hr. Dried litter was sorted by species, and white pine litter weighed to 0.01 g. A stem inventory of all litterfall plots was conducted during the summer of 2014 in which DBH (diameter 1.3 m above ground level) was measured for all stems >10 cm to determine the total plot basal area (m²) composition of white pine. Litter fall measurements are expressed as dry weight unit per area (g m⁻²) and normalized for the relative basal area of white pine to account for differences in stem density between plots (g m⁻² m⁻² BA). A *t*-test was used to identify significant differences in litterfall abundance between months within each study site (α = 0.05).

Foliar percent N was measured on a subset of dried needles from four litterfall traps per study site for each month of the 2014 growing season. To reduce the effect of foliar leaching through precipitation, litter traps were outfitted with drainage holes so water would not accumulate between collections. Chapin and Kedrowski (1983) suggest that foliar leaching is much less important that retranslocation of N in senescing leaves, reporting that leaching
removes < 0.6% of total foliar N. Furthermore, re-immobilization of N within conifer litter has been measured to occur on a time scale of 1.1 to 2.6 years in coniferous and deciduous forests respectively (Parton et al., 2007), thus N loss from foliage upon litter trapping on a monthly time scale is assumed to be non-significant in this study. Dried needle samples were ground to a fine powder using a ball mill and approximately 4.0 mg used to determine %N by EA-IRMS (IsoPrim100 IRMS, IsoPrime Ltd., Stockport, UK) at the University of New Hampshire Stable Isotope Laboratory. Data from each site were pooled to calculate a mean nutrient concentration for each month and a t-test was used to identify significant differences between months (α = 0.05). The monthly foliar N flux, the product of litterfall and mean foliar N concentration, was estimated at the four field sites for the 2014 growing season. The total amount of nitrogen lost due to early needle drop from mature trees was estimated by calculating the difference between the actual monthly N flux and the monthly N flux at foliar N concentrations equivalent to the month of October during the months of WPND-induced litterfall, thus assuming a more complete N resorption by infected trees. This metric is derived to provide an estimate of the total amount of N redistributed to the forest floor that would otherwise be retained in healthy white pine stands through normal/healthy N resorption.

2.2.3 Dendrochronology sampling and processing

Increment cores were extracted from between 10-20 dominant eastern white pine per site collected in 2013, 2014, and 2015 (Table 2.1). A minimum of two cores per tree were collected at opposite locations on the bole, perpendicular to the slope at either breast height or near stump height (40-60 cm above ground level) to minimize damage in commercially valued trees or on individuals that were recently harvested. Cores were dried, mounted, and sanded (120-1000 grit)
using standard dendrochronological procedures (Stokes and Smiley 1996). Annual ring widths were measured to 0.001 mm precision from the last year of growth towards the pith using a manual micrometer (Velmex UniSlide, Velmex Inc., Bloomfield, NY) in conjunction with J2X® tree ring measurement software. Crossdating accuracy was validated using the COFECHA software program to identify possible missing and false rings throughout the chronology of each individual core sample (Grissino-Mayer, 2001; Holmes, 1983). All trees within a site were crossdated against each other.

Two master chronologies were created per stand, one for high severity trees and one for trees with low or no symptoms of WPND as recorded during plot establishment in 2012. High severity trees were observed to have chlorosis and/or significant needle loss extending into at least one-third of the total crown area. The mean DBH of trees exhibiting high-severity WPND infection was consistently lower than the low-severity trees at all study sites except RHD. Differences in DBH between severity class were found to be statistically significant at sites BTH (p < 0.001) and STJ (p = 0.018) using a paired t-test (α=0.05). Site inventory data, mean tree age, diameter, and interseries correlations are reported in Table 2.1.

2.2.4 Growth decline analysis

Raw ring widths were converted to basal area increment (BAI), the radial growth metric used to quantify changes in growth in response to the contemporary WPND outbreaks. Conversion to BAI normalizes age-related allometric growth across DBH sizes (Biondi and Qeadan, 2008; Johnson and Abrams, 2009) and has been used in similar studies with the objective of detecting and assessing growth declines (Jump et al., 2006; Livingston et al., 2017; Voelker et al., 2008). BAI was derived from the last year of growth towards the pith using
measured tree diameter, ring width increments, and estimates of inside bark diameter derived from allometric relationships for eastern white pine provided in Li and Weiskittel (2011).

Detection of the initial onset years of growth decline and quantification of post-WPND reductions in BAI was calculated using the decline-score (D-score) method proposed by Livingston et al. (2017). In this analysis approach, the D-score is analogous to an independent two-sample t-test between the 3-year mean BAI before and after each sample year, incorporating the pooled variance of the sample years to account for natural year-to-year variability within a chronology that are less likely to be the product of a stress-induced decline. The year identified with the highest D-score (D$_{max}$) is regarded as the onset of decline. The percent BAI decline (BAI$_{decl}$, Eqn. 3) is then calculated from the lowest 3-year mean BAI (BAI$_{min}$) following the year of D$_{max}$ and the 3-year mean BAI prior to the onset of decline (BAI$_{prior}$).

$$BAI_{decl} = \frac{(BAI_{prior} - BAI_{min})}{BAI_{prior}} \times 100$$ (3)

Considering that WPND outbreaks are a recent issue in the region, this analysis was applied to a truncated portion (post-1960) of the high-severity chronologies and ending two years prior to the last year of measured growth.

A two-factor repeated measures analysis was applied separately for each of the six study sites to quantify the effects of time (pre- and post-outbreak) and observed WPND severity between the high-severity and reference low-severity chronologies with a single degree of freedom and least squares means using JMP Pro 13 (SAS Institute Inc., Carry, NC). To focus on recent growth trends associated with contemporary WPND outbreaks, the period analyzed is between the year 2000 and the last measured year of growth for each site chronology. The pre-outbreak period is defined as the year 2000 leading up to the year prior to D$_{max}$ and the post-
outbreak period includes the year of $D_{\text{max}}$ through the last measured year of growth. Effects are considered significant if $p \leq 0.05$.

To estimate an average growth decline from resulting from WPND outbreaks, we analyzed all 12 chronologies, truncated to year 2000 as described above, from the 6 study sites. A restricted maximum likelihood analysis was used to evaluate the interactive effects of WPND severity class, timing of disease outbreak, study sites, and year on BAI using the following model:

$$BAI = \text{severity class} + \text{time} + \text{severity class} \times \text{time} + \text{year} (R) + \text{site} (R)$$

Where BAI is the measured wood growth for a given year, severity class differentiates the low- and high severity chronology within each site, time corresponds to the period before or after WPND outbreak based on the year of $D_{\text{max}}$, year is the individual measurement years (2000 – last year of growth), and site refers to the six sampling locations. Both year and site are treated as random effects within the model.

2.3 Results

2.3.1 Seasonal changes in litterfall abundance

Litter trapping revealed variability in the abundance of foliar biomass cast throughout the six months of the growing season in each year of the study and between the four study sites (Fig. 2.2). The month of June alone accounted for 32.8 (± 3.7), 28.0 (± 5.4), and 34.8% (± 2.6) of the total seasonal litterfall averaged across sites in the years 2014, 2015, and 2016 respectively. Integrating June and July, predominantly WPND-induced litterfall accounted for 50.0 (± 6.9), 37.7 (± 7.8), and 54.4% (± 6.6) of the total seasonal litterfall for each respective year. The months of May, August, and September were consistently measured to have relatively low levels
of needle drop with a compiled annual mean across sites of 3.7 (± 0.7), 6.2 (± 0.6), and 7.3% (± 2.7) for each month, respectively. The timing of the WPND-induced defoliation in June and July coincides with observations of signs and symptoms of fungi associated with WPND. Broders et al. (2015) report that L. acicola, the most commonly observed WPND pathogen, is consistently associated with needle chlorosis and defoliation in late June. Since there is no re-flushing of foliage following defoliation, this study observed an inverse relationship between the total WPND induced litterfall (June + July) and the amount of litterfall cast in the month of October proportional to the total seasonal litterfall. At one site (BTH), the total amount of litterfall collected in June and July exceeded the amount of litter collected during the natural needle abscission in October in all three years.

Table 2.1 Study site location, basal area (BA), trees per hectare (TPHA), and dendrochronological sampling statistics.

<table>
<thead>
<tr>
<th>Study site (code)</th>
<th>BA (m² ha⁻¹)</th>
<th>TPHA (ha⁻¹)</th>
<th>% BA P. strobus</th>
<th>No. of trees (cores)</th>
<th>Age (±SE) (years)</th>
<th>DBH (±SE) (cm)</th>
<th>Interseries correlation (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohawk Trail Reserve Charlemont, MA (MHT)</td>
<td>47.1</td>
<td>290.3</td>
<td>83.6</td>
<td>20 (40)</td>
<td>85 (4.5)</td>
<td>68.4 (4.7)</td>
<td>0.518</td>
</tr>
<tr>
<td>Fox State Forest Hillsborough, NH (FOX)</td>
<td>39.9</td>
<td>623.9</td>
<td>76.0</td>
<td>18 (54)</td>
<td>65 (1.5)</td>
<td>54.4 (3.9)</td>
<td>0.557</td>
</tr>
<tr>
<td>Thompson Farm, Durham, NH (TOM)</td>
<td>42.0</td>
<td>662.7</td>
<td>55.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Massabesic Exp. Forest, Lyman, ME (MEF)</td>
<td>26.7</td>
<td>628.9</td>
<td>62.8</td>
<td>16 (64)</td>
<td>73 (3.2)</td>
<td>56.8 (3.6)</td>
<td>0.680</td>
</tr>
<tr>
<td>Chadbourne Tree Farm, Bethel, ME (BTH)</td>
<td>28.7</td>
<td>340.8</td>
<td>97.0</td>
<td>16 (64)</td>
<td>66 (2.2)</td>
<td>53.6 (2.7)</td>
<td>0.528</td>
</tr>
<tr>
<td>St. Johnsbury Municipal, St. Johnsbury, VT (STJ)</td>
<td>33.1</td>
<td>568.1</td>
<td>80.2</td>
<td>16 (32)</td>
<td>73 (2.3)</td>
<td>54.1 (1.3)</td>
<td>0.501</td>
</tr>
<tr>
<td>Richmond Pond, Richmond, VT (RHD)</td>
<td>20.0</td>
<td>224.2</td>
<td>89.7</td>
<td>10 (20)</td>
<td>51 (1.5)</td>
<td>41.0 (2.7)</td>
<td>0.570</td>
</tr>
</tbody>
</table>
Fig. 2.2 Mean monthly litterfall totals of eastern white pine during the 2014-2016 growing seasons ± 1SE. These data are normalized using the total basal area of white pine for each plot. Values with the same letter are not significantly different between months within the measured year ($\alpha = 0.05$).
2.3.2 Foliar litter nutrient dynamics

Foliar nitrogen was significantly lower in the month of October compared to any other month of the growing season (Fig. 2.3). The mean foliar percent N for needles collected in May through September was 0.87 (±0.05) and 0.40% (±0.03) in the month of October. Monthly values of percent C ranged from a minimum 49.57% (±0.01) in the month of May, to a maximum of 50.52% (±0.09) in the month of October. The ratio of foliar carbon to nitrogen (C:N) was variable in the months of May through September, ranging from 49.85 to 65.79; while the mean C:N value for October was 126.21 and significantly higher than all other months.

Fig 2.3 Foliar N content (%) measured in litter samples collected throughout the 2014 growing season. The horizontal line within the box indicates the median, boundaries of the box indicate the upper and lower quartile, and the whiskers indicate the highest and lowest values of the results. Values with the same letter are not significantly different (α = 0.05).
Fig. 2.4 The estimated nitrogen flux (g N m$^{-2}$) as white pine litter for each study site throughout the 2014 growing season. Error bars show the propagated standard error derived from monthly litterfall and foliar N measurements.

Scaling the foliar N data with gross litterfall measured in the 2014 growing season, we estimated the amount of N per unit area (g N m$^{-2}$) that was deposited by mature needles cast during the six-month study period at each study site (Fig. 2.4). Large fluxes of N from foliage occurred in June and July, proportional to the WPND-induced defoliation and exacerbated by relatively high foliar N concentrations during that time. The high amount of foliar N in needles shed during the summer months in contrast with lower N concentration in October resulted in the largest proportion of foliar N flux to occur in June and July, rather than during natural needle senescence in October. The mean flux of N across the four study sites is estimated to be 0.58 (± 0.14), 0.34 (± 0.12), and 0.30 (± 0.04) g N m$^{-2}$ for the months of June, July, and October respectively, with a mean annual foliar N flux of 1.46 (± 0.52) g N m$^{-2}$. Thus, the pulse of litter induced by WPND generates 63.0% of the total N flux from foliage throughout the growing
season, while October only accounted for only 20.1% of the total. The total estimated growing season \( N \) flux assuming foliar \( N \) concentrations in June and July are equivalent to the October \( N \) content is \( 1.0 \) (± 0.27) \( g \) \( N \) \( m^{-2} \), thus early needle drop caused by WPND represents a loss of tree available \( N \) of 0.46 \( g \) \( N \) \( m^{-2} \) over the course of the six-month growing season. This is a net increase in \( N \) input via pre-mature needle drop of 45.8% that is deposited on the forest floor ahead of natural needle abscission.

2.3.2 WPND-induced growth declines

At all study sites, high-severity white pine chronologies were found to be in recent decline, consistent with the onset of observed WPND outbreaks in the region (Fig. 2.5). The year of \( D_{\text{max}} \) ranged from 2007 to 2009 and was higher than any D-score calculated between 1960 and the last year of measured growth for all sites (Table 2.2). The high-severity chronology had a lower BAI than the low-severity chronology during the post-outbreak period for all sites. At all sites except for STJ, the high-severity trees exhibited lower growth rates than the low-severity reference trees throughout the period in which the BAI \(_{\text{decl}}\) was calculated. At STJ, the high-severity trees declined sharply after the 2007 WPND outbreak, while the low-severity trees appeared to be experiencing a period of sustained positive growth. A similar trend was observed at FOX, where there was relatively strong correlation between the low- and high-severity chronology from 1960 to 2008 prior to \( D_{\text{max}} \) (\( R^2 = 0.691 \)), followed by a large divergence in BAI trends post-outbreak. The year of \( D_{\text{max}} \) was observed to be a year of marked divergence between high- and low-severity reference chronologies, most notably at sites BTH and MEF. At MHT, the magnitude of growth decline attributed to WPND was greater than any other period in the
126-year tree ring record at this site and exceeded all other sites measured in this study. The mean BAI\textsubscript{decl} across all sites in this study was 41.3% and ranged from 25.8 to 72.8% (Table 2.2).

Changes in BAI during the pre- and post-outbreak period were found to vary based on WPND severity and by site (Table 2.3). The distinction between high- and low-severity trees was significant (p < 0.05) at all sites except for STJ. The time factor (BAI before and after D\textsubscript{max}) was significant at sites MEF, MHT, STJ, and RHD; while the interaction between observed WPND severity and time was significant for sites FOX, MEF, and MHT.

Table 2.2 Results of the D-score analysis on the high-severity tree ring chronologies.

<table>
<thead>
<tr>
<th>Site</th>
<th>D\textsubscript{max} year</th>
<th>D\textsubscript{max}</th>
<th>BAI\textsubscript{prior} ±SE (cm\textsuperscript{2} yr\textsuperscript{-1})</th>
<th>BAI\textsubscript{min} ±SE (cm\textsuperscript{2} yr\textsuperscript{-1})</th>
<th>BAI decline (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOX</td>
<td>2009</td>
<td>5.8</td>
<td>25.9 (0.4)</td>
<td>17.0 (1.5)</td>
<td>34.7</td>
</tr>
<tr>
<td>MEF</td>
<td>2009</td>
<td>2.6</td>
<td>27.4 (2.5)</td>
<td>20.3 (2.2)</td>
<td>25.8</td>
</tr>
<tr>
<td>BTH</td>
<td>2008</td>
<td>3.1</td>
<td>61.0 (2.6)</td>
<td>42.8 (5.6)</td>
<td>29.8</td>
</tr>
<tr>
<td>MHT</td>
<td>2009</td>
<td>8.0</td>
<td>59.1 (4.6)</td>
<td>16.1 (1.4)</td>
<td>72.8</td>
</tr>
<tr>
<td>STJ</td>
<td>2007</td>
<td>4.5</td>
<td>45.3 (3.3)</td>
<td>33.8 (0.5)</td>
<td>25.4</td>
</tr>
<tr>
<td>RHD</td>
<td>2008</td>
<td>5.2</td>
<td>32.0 (4.2)</td>
<td>13.1 (0.8)</td>
<td>59.0</td>
</tr>
</tbody>
</table>
Fig. 2.5 Master chronologies for low-severity (black) and high-severity (red) WPND-infected eastern white pines by site from 1960-2015. Shaded area around each time series shows ± 1 SE. Arrows within each panel indicate the year of $D_{\text{max}}$ in the high severity chronologies.
Table 2.3 Test results from the two-factor crossed repeated measure ANOVA for the six sites. The bottom portion of the table showing all sites gives the results for the maximum likelihood model.

<table>
<thead>
<tr>
<th>Site</th>
<th>Source</th>
<th>SS</th>
<th>F Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOX</td>
<td>Severity</td>
<td>1450.34</td>
<td>63.64</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>17.74</td>
<td>0.78</td>
<td>0.3872</td>
</tr>
<tr>
<td></td>
<td>Severity x Time</td>
<td>202.82</td>
<td>8.90</td>
<td>0.0069</td>
</tr>
<tr>
<td>MEF</td>
<td>Severity</td>
<td>255.17</td>
<td>11.50</td>
<td>0.0024</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>111.57</td>
<td>5.03</td>
<td>0.0344</td>
</tr>
<tr>
<td></td>
<td>Severity x Time</td>
<td>200.48</td>
<td>9.03</td>
<td>0.0061</td>
</tr>
<tr>
<td>BTH</td>
<td>Severity</td>
<td>1829.72</td>
<td>26.07</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>170.40</td>
<td>2.43</td>
<td>0.1323</td>
</tr>
<tr>
<td></td>
<td>Severity x Time</td>
<td>19.87</td>
<td>0.28</td>
<td>0.5995</td>
</tr>
<tr>
<td>MHT</td>
<td>Severity</td>
<td>1077.14</td>
<td>31.51</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>5860.92</td>
<td>171.45</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Severity x Time</td>
<td>307.74</td>
<td>9.00</td>
<td>0.0056</td>
</tr>
<tr>
<td>STJ</td>
<td>Severity</td>
<td>16.32</td>
<td>0.23</td>
<td>0.6357</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>362.61</td>
<td>5.11</td>
<td>0.0331</td>
</tr>
<tr>
<td></td>
<td>Severity x Time</td>
<td>187.99</td>
<td>2.65</td>
<td>0.1165</td>
</tr>
<tr>
<td>RHD</td>
<td>Severity</td>
<td>400.17</td>
<td>12.58</td>
<td>0.0014</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>961.76</td>
<td>30.24</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Severity x Time</td>
<td>24.96</td>
<td>0.78</td>
<td>0.3832</td>
</tr>
<tr>
<td>All sites</td>
<td>Severity</td>
<td>46.07</td>
<td></td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>12.80</td>
<td></td>
<td>0.0018</td>
</tr>
<tr>
<td></td>
<td>Severity x Time</td>
<td>8.43</td>
<td></td>
<td>0.0042</td>
</tr>
</tbody>
</table>

In the four years since establishment of monitoring plots, many of the trees initially exhibiting low-severity of infection have progressed in severity classification as the mean defoliation severity rating has increased by 43% for the six sites measured in this study. Compiling the site chronologies to analyze the mean WPND impact using maximum likelihood analysis revealed a significant effect from WPND severity class, time, and the interaction between severity class and time (Table 2.3). The resulting decline in BAI of high severity trees was estimated at 11.2 cm² following the WPND outbreak, accounting for differences in severity class and eliminating the time effect (Fig. 2.6).
Fig. 2.6 Pre-outbreak (2000 to year prior to $D_{\text{max}}$) and post-outbreak (year of $D_{\text{max}}$ to last year of growth) basal area increment (cm$^2$) in low severity (closed circles) and high severity (open circles) chronologies across the six study sites $\pm$1SE.

2.4 Discussion

This study reports a large deviation in the temporal distribution of white pine foliar biomass throughout the May-October growing season as a result of WPND-induced defoliation. We assert that litterfall in the months of June and July is largely due to defoliation by fungal pathogens, accounting for 47% of the total litterfall across sites and years. This large pulse of needle drop in June and July is not typical of white pine stands, where needle senescence from April through September has been reported to account for only 30% of the annual total (Wang and Chen, 2012). While this study did not measure litterfall in the month of April, we found that the sum of May through September litterfall accounted for 64% of the annual total, a two-fold increase in litterfall reported by Wang and Chen (2012) over a shorter time scale. This finding, along with observations of the timing of signs and symptoms associated with WPND fungi (Broders et al., 2015), support our claim that the large pulse of litterfall in June and July is
predominately due to defoliation by fungal pathogens. A small proportion of mature needles were measured in litter traps in the month of May of each year representing 2.8% of the annual total. While symptoms related to WPND can be present during the month of May, defoliation due to WPND has not been observed to occur until later in June. The litterfall measured in the month of May can be considered a background level of needle drop that is a product of wind gusts and crown abrasion. The same is likely true for the month of August, in which litterfall totals did not differ significantly from the month of May across all sites and years of this study, during which time WPND-infected needles have been cast and natural needle abscission has not yet begun.

We found that foliar N content of cast needles was significantly higher in the summer months than in foliage collected during the natural October senescence (Fig. 2.2). Needles that were shed during the WPND-induced defoliation in June and July had a mean foliar N content of 0.78 and 0.84% respectively, while the month of October exhibited a significantly lower foliar N content of 0.40%. The mean foliar N content of green foliage for eastern white pine in Maine and New Hampshire has been reported at 1.14% (Hallett and Hornbeck, 1997), indicating incomplete resorption of N in needles defoliated prior to the natural abscission. While healthy white pine typically retain 2-3 years of foliage, it has been verified that there is no relationship between leaf longevity and nitrogen resorption efficiency (Reich, 1998; Reich et al., 1992), therefore, any distinction between nutrient concentration of the 2\textsuperscript{nd} and 3\textsuperscript{rd} year needles was not considered in this study. The high foliar N measured during the summer months of the growing season in conjunction with increased litterfall biomass cast in June and July represent a large pulse of N to the forest floor that would otherwise be resorbed prior to natural needle drop. Scaling the N content of foliage measured for each month with total litterfall during the 6-month growing
season corresponds to a N flux from foliage of 0.96-2.17 g N m\(^{-2}\) among the four sites measured in this study. When considering N flux during WPND-induced defoliation to have the same foliar %N as during the natural October needle drop, the total N lost from foliage that would otherwise be recycled by infected trees is estimated to be 0.25-0.84 g N m\(^{-2}\) y\(^{-1}\). This loss of N through premature defoliation, presumed to be in part due to a low N resorption efficiency, represents a substantial change in the N uptake demands of WPND-infected stands, as mature temperate forest stands have been estimated to have an annual N requirement of about 10 g N m\(^{-2}\) y\(^{-1}\) (Butterbach-Bahl et al., 2011; Rennenberg and Dannenmann, 2015) and white pine stands in southern Wisconsin were measured to have an annual uptake of 7.7 g N m\(^{-2}\) y\(^{-1}\) (Nadelhoffer et al., 1983). Given this estimate of N uptake in white pine stands, the N deficit induced by WPND represents 3.2-10.9% of total N required by infected trees following defoliation, which may also be compounded over multiple years if that additional uptake requirement is not satisfied in the successive year.

In northeastern temperate forests subject to insect defoliation, trees lose N primarily as premature litterfall, frass, and insect biomass at the expense of resorption, such that the internal cycling of N within trees is compromised and N leaching is assumed to increase marginally (Lovett et al., 2002). In contrast to insect defoliators, a discrete fungal pathogen is not likely to retain an appreciable amount of N from the system, therefore, the majority of redistributed N is expected to remain within the cast foliage in WPND-infected stands. Studies addressing the fate of nitrate in different forests types have consistently determined coniferous forests to have higher rates of N leaching compared to deciduous forests (Schrijver et al., 2007; Wuyts et al., 2011), and forests composed primarily of trees with the genus *Pinus* have been found to be exceptionally high (Herrmann et al., 2005). The lower C:N ratio of white pine litter deposited in
the summer months, as reported in this study, may also exacerbate nitrate leaching (Gundersen et al., 1998). In N-limited forests, this change in organic N distribution could contribute to growth decline if N from premature needle defoliation is not rapidly mineralized for uptake, leached, or is more readily acquired by competing species. Suppressed understory hardwoods species associated with white pine, red oak (*Quercus rubra* L.) for example, may thrive under pine-dominated stands impacted by WPND in response to both increased light availability via reduction in canopy leaf area and large pulses of N from the decomposition of fresh, high-N pine litter in the summer months. It is well-established that N uptake in forests is directly proportional to fine root mass and N availability, such that when soil N pools are high the relative carbon allocation to fine roots is low (Aber et al., 1991; Nadelhoffer et al., 1985). Furthermore, N uptake is positively correlated with foliar litter, which is in surplus within WPND impacted stands because foliar litter is presumed a major source of N recycling. As carbon allocation to foliage is physically constrained by N uptake, diseased white pines are at a distinct disadvantage both in terms of their reduced foliage and internal N deficit. Thus, diseased stands suffering from chronic WPND defoliation may allocate a greater proportion of available carbohydrates to maintain adequate levels of foliage and to bolster fine root production for recouping internal N losses. This redirection of photoassimilates to foliage and roots likely comes at the expense of stem growth (Dybzinski et al., 2011). While WPND impacted sites may not necessarily be N limited, infected pines within the stand are at a substantial nitrogen deficit due to untimely mid-season defoliations. Although we did not directly measure belowground carbon allocation, this is supported in part by the declines in wood growth associated with WPND outbreaks that were measured across all white pine stands in this study.
Since initial outbreaks of WPND in the northeast region, stem wood growth measured in our heavily-affected white pine study stands has declined 28% since the year 2000 leading up to pre-outbreak growth rates (Fig. 2.6), and 41% across all sites when considering the growth difference based on the D-score analysis of high-severity chronologies (Table 2.2). Aside from the complex interactions between N uptake/availability and carbon allocation to various tissues cascading from WPND induced defoliation, a more straightforward pathway explaining these growth declines may be accounted for by the large reduction in functional foliar area during the most productive portion of the growing season. The premature loss of mature 2\textsuperscript{nd} and 3\textsuperscript{rd} year needles several months ahead of natural needle abscission greatly reduces the photosynthetic capacity of diseased trees. Declining growth rates have been found to occur following needle loss due to other defoliating pathogens of conifer species, such as Swiss needle cast (\textit{Phaeocryptopus gaeumanni}i (T. Rhode) Petrak) on Douglas-fir (\textit{Pseudotsuga menziesii} (Mirb.) Franco) (Black et al., 2010; Hansen et al., 2000) and Dothistroma needle blight (\textit{Dothistroma septosporum} Dorg.) on the species within the genus \textit{Pinus} (Bradshaw, 2004). Similar to \textit{D. septosporum}, defoliation caused by WPND does not typically result in mortality.

It is also important to note that there may be predisposing factors that facilitate high rates of infection within stands or among individual trees. Smaller tree size (DBH) and reduced growth rates have been found to be significant factors of predisposition for drought-induced mortality of eastern white pine in Maine (Livingston et al. 2018). We found that trees identified as being highly symptomatic for WPND were on average smaller in diameter within five of the six study sites used for dendrochronology analysis. High-severity chronologies generated from sites FOX, BTH, RHD, and MHT were found to have lower growth rates than low-severity counterparts prior to 2000, but symptoms and BAI declines also occurred in 2 locations without
an obvious difference in DBH between severity classes (Fig. 2.5). Also, we found that mean BAI from the year 2000 leading up to WPND outbreaks was similar between high and low-severity trees on all sites (Fig. 2.6), but after the outbreak there were lower growth rates in high-severity trees across all sites (Fig. 2.5). Therefore, lower growth rates prior to WPND outbreak weren’t a consistent factor in trees that developed high-severity symptoms.

Declines in wood growth commonly precede tree mortality, but high mortality has not yet been reported for WPND. Likelihood of tree death on trees with declining growth depends on the interaction of several abiotic or biotic agents. Drought-induced mortality has been linked to declines in both wood growth and non-structural carbohydrates (NSCs) stored within the sapwood of conifer tree species, resulting in the colonization of secondary pathogens and mortality (Camarero et al., 2015). It is hypothesized that severe reductions in stored NSCs can lead to stress-related mortality as trees struggle to induce defense compounds while maintaining basic metabolic processes (Mcdowell et al., 2011; Wiley and Helliker, 2012). Although tree mortality via carbon starvation is thought to occur at critically low levels of NSC concentration, trees stressed by defoliation have been shown to exhibit relatively high NSC stores, presumably sacrificing stem growth at the expense of NSC storage (Saffell et al., 2014). However, the role of NSCs leading up to tree mortality is highly complex is still debated (Sala et al., 2012), and other studies regarding defoliation of conifer species reject the NSC-growth trade-off (Deslauriers et al., 2015). In order to understand the likelihood of mortality resulting from WPND, additional research is required to disentangle the complex interactions between WPND, wood growth, and carbohydrate concentration/allocation within various tree storage pools.
2.5 Conclusion

This study analyzed the effect of WPND on litterfall timing, abundance, and foliar N concentration in addition to stem diameter growth in response to the current outbreak. We found that WPND results in a large pulse of foliar litter and organic N in the months of June and July. In turn, N resorption by infected trees is thought to be compromised and the natural cycling of organic N via foliar litter is fundamentally altered. Foliar N resorption prior to autumn senescence supplies a significant proportion of annual N demand in temperate tree species, thus diseased white pine stands will require additional N uptake following WPND-induced defoliation. Additionally, defoliation resulted in woody growth declines across all six white pine stands measured in this study. In many cases WPND induced declines in high-severity trees appeared to be preceded by lower growth rates compared to healthy/low-severity trees; though it is not a requisite, as we also observed significant declines in high-severity trees that exhibited very similar growth rates compared with low-severity trees prior to outbreak. Reductions in wood growth are of great concern for timber producers in the region, as eastern white pine is an economically important tree species in the northeastern US. Mortality attributed to WPND is currently low and isolated to exceptionally stressed individuals despite successive years of defoliation since ca. 2009. The long-term impacts of WPND are still unknown, thus this emerging disease complex warrants continued monitoring and additional study.
CHAPTER 3

IMPACTS OF WHITE PINE NEEDLE DAMAGE ON WATER USE, GAS EXCHANGE, AND THE ALLOCATION OF NON-STRUCTURAL CARBOHYDRATES

Abstract

Pathogen induced defoliations of eastern white pine fundamentally alter the canopy of diseased individuals through a reduction in functional foliar area. The impact of this mid-growing season loss of mature foliage has a direct influence on gross carbon assimilation. However, the physiological function of residual needles and how diseased trees are allocating photosynthate is largely unknown. This chapter addresses the effects of defoliation severity on leaf level gas exchange and whole tree water use using heat-pulse based sap flow measurements. Non-structural carbohydrates (NSCs) were measured across plant tissues at three periods in the 2015 growing season at two sites of varying disease severity to quantify potential differences in allocation over time due to WPND-induced defoliation. This study found no difference in the rates of photosynthesis, stomatal conductance, or intrinsic water use efficiency between trees of different defoliation severity. However, significant differences in leaf gas exchange are reported between the current-year and second-year foliage during the early portion of the growing season. Current-year needles were found to be only partially elongated at the time defoliation occurs in mid-June, and residual foliage does not appear to be compensating for the premature loss mature foliage. Rates of transpiration within infected trees was 17-29% lower than that of healthy reference trees throughout the 2014 growing season and significantly declined after defoliation occurred. The allocation of NSCs differed between tissue types depending on the time of year,
however, no significant difference was detected as a function of defoliation severity. This finding suggests that diseased trees are not carbon limited, as structural growth is being compromised at the expense storing mobile sugar and starch.

3.1 Introduction

The impacts of White Pine Needle Damage (WPND) on the water use and carbon dynamics of *P. strobus* is presently unknown. Water used through transpiration is necessary for physiological processes such as photosynthesis and nutrient uptake that are essential for maintaining plant life. The chronic summer defoliations induced by WPND in recent years have significantly reduced total leaf area of infected white pine and have been observed to coincide with declines in wood growth and the premature dieback of branches within the lower crown (McIntire et al., 2018). As tree water transport is primarily controlled via stomata on the leaf surface, diseased individuals may be required to upregulate gas exchange rates relative to heathy trees to maintain a similar level of productivity. Trees defoliated by WPND pathogens in June are lacking a significant proportion of mature second and third year foliage for the remainder of the growing season. Due to this loss of functional foliar area it is presumed that diseased individuals will experience a reduction in water use and gross carbon assimilate corresponding to the relative severity of defoliation. Such a response has been measured in *Tamarix* species defoliated by the saltcedar beetle (*Diorhabda carinulata*), in which episodic partial-defoliation resulted in a 16% reduction in mean annual sap flux density (Hultine, 2010). However, the relationship between foliar area, leaf gas exchange, and sap flux are complex; such that leaf-level compensatory response and changes in the canopy microclimate have shown to increase stomatal conductance and transpiration in some cases (Meinzer and Grantz, 1991; Parker, 1949; Reich et
al., 1993). This upregulation of gas exchange and transpiration is not consistent across tree species or among different classes of defoliators (Pataki et al., 1998), and therefore warrants additional research in order to better understand how reductions in leaf area may influence tree water use and productivity.

Reduced foliar area hinders the productive capacity of diseased individuals and may in turn alter the allocation of photosynthate throughout the tree to pools where it is needed most. Several studies pertaining to tree defoliation have reported reduced or altered allocation of non-structural carbohydrates (NSCs) to various storage tissues (Quentin et al., 2011; Schutz et al., 2011), particularly of the roots (Jacquet et al., 2014; Landhausser and Lieffers, 2012). However, other studies of tree defoliation have reported results to the contrary, showing little or no evidence of NSC decline as a direct impact of defoliation (Deslauriers et al., 2015). Measurements of plant NSCs (soluble sugars and starch) can be a direct and quantitative measurement of how trees are storing and allocating photosynthate (Quentin et al. 2015). In eastern white pine, NSCs have been found to have relatively fast rates of turnover and actively mix with new photosynthate (Richardson et al., 2015), and thus may be allocated rapidly in response to defoliation if needed.

One potential use of NSCs is the synthesis of secondary metabolites to be used for constitutive and induced defenses, though this comes at a cost. To generate secondary defense compounds and specialized tissues, trees must expend photosynthate that could be otherwise utilized for growth, development, and/or storage. This dilemma is addressed in the growth-differentiation balance (GDB) hypothesis (Herms and Mattson, 1992; Loomis, 1932; Lorio, 1986). The GDB states that a physiological trade-off exists between plant growth and the secondary metabolisms which varies across different environmental conditions. Plants must not
only be able to effectively defend themselves from herbivory and pathogen attack, but also grow fast enough to be competitive among neighboring trees. Plant growth is dictated by primary metabolites, those which are necessary for cell division, enlargement, and reproduction. Growth is highly resource demanding (light, water, minerals, carbohydrate, etc.), however, increased growth typically correlates to an increased photosynthetic potential. Cell differentiation refers to the process of morphological changes that lead to varying cell types and specialization such as phenolic parenchyma, resin ducts, and thickening of the cell wall (Herms and Mattson, 1992). Cell differentiation also leads to secondary metabolism, in which simple sugars are converted to more complex products such as terpenoids, alkaloids, and other chemical compounds used for induced defense through the process of biosynthesis (Bourgaud et al., 2001; Herms and Mattson, 1992). Studies have shown that the plant demand for secondary metabolites is substantial and have confirmed the aspects of the GDB hypothesis by measuring the pressure plants are under to simultaneously support physiological processes of growth, maintenance, storage, reproduction, and defense (Glynn et al., 2003; Koricheva et al., 1998; Neilson et al., 2013). Within the framework of the GDB hypothesis, the relative rate of secondary metabolism expense and plant growth rates are thought to have a non-linear relationship with resource availability, such that in resource rich environments growth processes are given photosynthate allocation priority for resources while decreasing the relative availability of secondary metabolites, and, at low levels of resource availability net assimilation, growth, and secondary metabolites are positively correlated (Herms and Mattson, 1992). Hence, there exists a trade-off at the resource sink-limiting range where sink-limiting plants will be more resistant to disease and herbivory than a plant experiencing no environmental constraints on growth (Herms and Mattson, 1992).
Based on the work presented in chapter two of this dissertation it is known that WPND has induced significant declines in wood growth since initial outbreaks occurred in the Northeastern US. Growth reductions in basal area increment on the order to 30% are thought to be a direct impact of defoliation. There are two established hypotheses relating to the cause of growth limitation in trees. The first is known as sink limitation, where a sink is regarded as the end-use for mobile photosynthates, such as for structural growth (e.g. buds, foliage, reproductive tissue) and respiratory metabolism. Sink limitation if thought to occur within trees growing under environmental stress (e.g. nutrient limitation, extreme cold, drought) where carbon priority is given to storage of NSCs due to constraints relating to a trees ability to utilize carbon (Körner, 2003). The alternative hypothesis relating to reductions in growth is known as source limitation, in which a trees ability to assimilate carbon is compromised, hence the leaves/needles are regarded as the source of photosynthate. Within white pine stands subject of WPND defoliation it is more probable that carbon source limitation is at play, since substantial reductions in leaf area are present for a four to five-month period of the growing season. The source of carbon can be manipulated in two ways, either through altering the atmospheric concentration of available carbon dioxide, or by removing foliage from the plant. To this end, source limitation has been tested within controlled experiments through free-air enrichment of CO₂ and via stepwise artificial defoliation (Arp, 1991; Handa et al., 2005; Li et al., 2002). While there is a general consensus that the source-limitation hypothesis can be confirmed, there is still much debate as to the role of NSC storage under different levels of defoliation stress. Research relating to the dynamics of NSC concentration in various storage tissue following defoliation have reported conflicting findings, both within laboratory and field studies. As defoliation severity increases, carbon allocation to growth unequivocally declines, however, response of NSC have shown to
vary. In some cases, NSC has been found to decline rapidly in conjunction with wood growth (Aguade et al., 2015; J. S. Jacquet et al., 2013; Wiley et al., 2013), while separate studies report the opposite funding, where storage of NSC were found to increase among defoliated individuals (Palacio et al., 2008). Additionally, studies have also found no significant difference (increase or decrease) in NSC concentrations among defoliated individuals (Wiley et al., 2013). These three different patterns of NSC concentration (increase, decrease, or no change) in response to defoliation indicate that the loss of source material may influence trees in a variety of ways, depending in part on species, stressor, and the severity (time and intensity) of the defoliation. There are a multitude of compounding factors that can influence NSC concentration and storage. Since NSCs are mobile it is not uncommon to observe changes within only a single tissue type or significantly different concentrations within tissue types over the course of a growing season. For a disease complex like WPND, which has defoliated stands for consecutive years, it is possible that the compounding stress of chronic needle loss could alter NSC dynamics over a time span greater than just a single growing season.

This chapter address three components of tree water and carbon dynamics as they relate to WPND-induced defoliation, thus the specific objectives of this research are: (1) monitor and assess the impact of untimely summer defoliation on tree sap flux density over the course of a growing season and in relation to atmospheric drivers of transpiration; (2) evaluate leaf level gas exchange (photosynthesis, stomatal conductance, and water use efficiency) as a function of needle age, time of year, and relative defoliation severity (3) quantify changes in NSC concentration as a function of tissue type (stem, root, and foliage) to address carbohydrate storage over time and between trees of varying defoliation severity.
3.2 Methods

3.2.1. Study sites

Four study sites throughout Maine and New Hampshire were used for the physiological measurements herein. These include Bethel, ME (BTH), Lyman, ME (MEF), Hillsborough, NH (FOX), and Durham, NH (TOM). As these are the same sites utilized for litterfall measurements in chapter two of this dissertation, detailed site descriptions can be referenced in Table 2.1. Sap flow measurements were conducted at MEF and FOX from May through August 2014, while leaf gas exchange was conducted at MEF, FOX, and BTH over the same period. An on-site weather station (HOBO RX3000, Onset Computer Corporation, Bourne, MA) was deployed at FOX to provide concurrent measurements of temperature, relative humidity (RH), wind speed, and solar radiation on a 15-minute time step. At MEF, a temperature-RH sensor (HOBO U23 Pro v2, Onset Computer Corporation, Bourne, MA) was fixed within the canopy of a dominant white pine within a housing shielding it from direct solar radiation. Additional meteorological data, including daily rainfall totals, for MEF was acquired from the nearby weather station in Sanford, ME and accessed using the xmACIS2 climate data product tool (available at: https://xmacis.rcc-acis.org). Sampling of NSCs occurred at MEF and TOM between June and October of 2015. Although each site is symptomatic for WPND, the site in Durham has shown the lowest levels of severity and it is possible to locate healthy individuals, thus it is considered as a control site within the NSC study.

3.2.2 Needle phenology

Foliar samples were collected on a weekly interval beginning in early May and continuing through needle maturity in late August of 2017 for a total of 12 collection dates at site TOM. A
single shoot segment exposed to full sun conditions from each of five mature, co-dominant, eastern white pine was excised using a pole pruner from a height of 4-6 m and immediately placed into a sealed plastic bag containing a moist paper towel and stored in an iced container for transport to the lab. Length and projected area of shoot and needle segments were measured to 0.1 mm using ImageJ software analyses (Schneider et al., 2012).

3.2.3 Sap flow measurements

Tree transpiration was estimated using the heat ratio method (HRM), a heat-pulse based sap flow technique that is excellent at resolving low flows and sap velocities up to approximately 50 cm h⁻¹ (Burgess et al., 2001; Steppe et al., 2010). Sensors were constructed in the Asbjornsen lab at the University of New Hampshire adapted from the protocol of Davis et al. (2012). Probes consist of three independent thermocouples along the length of a steel needle that allow for temperature measurements at depths of 1.0, 2.2, and 3.5 cm within the sapwood. Trees were outfitted with sap flow sensors at the FOX, and MEF sites in early of May 2014. At each site, a total of eight trees were selected for measurement, of which included four of each WPND severity class based on ocular measurements. Low-severity trees are defined as those that exhibited symptoms of disease is less than or equal to one-third of the live crown, whereas high-severity individuals exhibit symptoms in greater than one-third of the live crown. Trees diameter at breast height (DBH) ranged 21.9-55.1 cm (mean 38.0 ± 11.8) at MEF and 27.8-43.3 cm (mean 36.0 ± 5.1) at FOX. Two probe sets were installed at breast height on opposite sides of the tree in the north-south orientation. Prior to installation, bark and cambial tissue was remove from the measurement point to ensure probes were in direct contact with the xylem. A metal drill guide was placed onto the exposed area allowing for accurate spacing between sensor probes and
vatical alignment. Thermocouple probes were coated in petroleum jelly and positioned at a
distance 0.6 cm up- and downstream of a 3.7 cm nichrome line heater (17–20 Ω), installed
perpendicular to the sapwood. A reflective radiant barrier was fixed around the sensors to inhibit
potential heating via direct sunlight. Sensors were connected to a datalogger and multiplexor
(CR1000 and AM16/32; Campbell Scientific Inc., Logan, UT, USA) powered by an external 12
V battery and housed within a water-proof container. A heat pulse of 2.5 s duration was sent to
the central heating probe on a 15-min interval and the change in temperature 60 s following the
heat pulse was recorded for the up- and downstream thermocouple. Measurements were
conducted from mid-May through the end of August. The four-month time span allows for a
diagnostic approach in observing how the June defoliation directly impacts flow rates by
measuring tree water use before, during, and after the WPND needle cast event. Increment cores
were obtained at DBH at the start and end of the measurement period using a 5.0 mm increment
borer to estimate total cross-sectional sapwood area, water content (MC), wood density (ρd), and
thermal diffusivity of each tree according the methodology outlined in chapter one of this
dissertation. Data collection, battery replacement, and repairs if necessary were conducted on a
weekly interval throughout the measurement period.

3.2.4. Leaf gas exchange measurements

Leaf gas exchange measurements were collected at the FOX, MEF, and BTH field sites
using a Li-Cor Portable Photosynthesis System (LI-6400XT, Li Cor, Inc., Lincoln, NE).
Measurements were conducted during the months of June, July, and August on warm, relatively
cloud free days between the hours of 10:00 and 14:00. Shoot segments containing intact fascicles
were acquired from the upper canopy exposed to full light of four co-dominant trees from each
WPND severity class using a 12-gauge shotgun with #3-4 steel birdshot. Only shoots harboring both current-year and second-year foliage were selected for measurement so that potential variability across stem segments was minimized for comparisons of needle age class. Excised branch samples were immediately submerged and cut under water in an attempt to reduce the presence of embolism within the xylem (Venturas et al., 2015). Two complete fascicles consisting of ten intact needles were placed parallel to the long side of the 6.0 cm² cuvette for gas exchange measurement. Samples were allowed to stabilize within the chamber for approximately two minutes while holding constant the environmental parameters influencing photosynthesis.

Table 3.1 Dates of the gas exchange sampling for each study site. Dates in June marked with * indicate sampling periods in which current year foliage was not measured due to insufficient needle length.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>MEF</th>
<th>FOX</th>
<th>BTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>8/21/2014</td>
<td>8/14/2014</td>
<td>8/29/2014</td>
</tr>
</tbody>
</table>

Preliminary light response curves were conducted to determine a light saturation point of 1000 µmol m² s⁻¹ which was used for all subsequent measurements. Other parameters held constant included ambient CO₂ concentration (400 ppm), temperature (26-28°C), relative humidity (30-40%), chamber flow rate (400 mm³), and the chamber fan speed simulating wind currents. Once photosynthesis and conductance stabilized for a given sample, five instantaneous measurements were taken over a 30 second period and the average of those measurements are reported throughout. Since the LI-6400XT cuvette assumes a uniform leaf area and needles are irregular within the chamber, leaf area was adjusted by calculating surface area of each fascicle assuming a cylindrical shape (Hultine and Marshall, 2001; Johnson, 1984). Sampling dates at each site are reported in Table 3.1.
3.2.5 Non-structural carbohydrate sampling and processing

Sampling of NSCs occurred in the months of June, August, and October at each study site. The Durham, NH (TOM) site was regarded as a healthy/low-severity site based on multiple years of observations of crown condition for symptoms of WPND. The site in Lyman, ME (MEF) has experienced repeated defoliation due to WPND since at least 2012, and trees selected for NSC sampling are the same as those used in annual monitoring efforts by the US Forest Service. Six mature eastern white pine per site were selected and tagged for sampling, with a mean DBH of 50.3 cm (SD ± 13.8) and 53.4 (SD ± 14.5) at MEF and FOX respectively. Mean live crown ratio was also similar among sample trees between sites, reported at 40.1% (SD ± 4.7) and 43.6% (SD ± 9.6) at MEF and FOX respectively. These values are on the high end for white pine, in part due to the relatively open grown habit of the individuals, where trees at MEF are generally established on the edge of a recent clear-cut, and trees at TOM are on the boundary of a wide recreational trail exposed to high light conditions. Since NSCs are reallocated to different storage pools over time, it was crucial to assess sugar and starch content across root, stem, and foliar tissues to derive a robust understanding of NSCs throughout the tree. Carbon supply within various tissue types vary depending on the time of year that sampling occurs, such that higher NSC concentration is typically found within foliage and stems of the upper canopy early in the growing season (i.e. near the source location) and moves to storage pools within the bole and root system later in the growing season (Landhausser and Lieffers, 2012). Core samples were obtained at two locations using a 5.15 mm increment borer, from DBH on the main stem, and from a primary coarse root excavated < 1.0 m away from the trunk. Additionally, current-year upper stem (CYSU), current-year lower stem (CYSL), multiple-year (2-4 year) lower stem segments (MYSL), multiple year upper stem segments (MYSU), current-year needle of the lower
canopy (CYNL), as well as second-year needles from the lower canopy (Y14L) were collected. All samples acquired from the canopy were obtained using a 12-ga shotgun with #3-4 steel birdshot. Upon collection, samples were immediately placed within plastic bags and put into a container with dry ice. Once returned from the field, tissue samples were relocated to a -20°C freezer until carbohydrate extractions could be performed.

Sugar and starch assays were conducted in the lab of Dr. Brett Huggett at Bates College in Lewiston, ME. Tissue samples were oven dried at 100°C for 1 h, then at 70°C for 2-3 days. Dried samples were then ground to 40-mesh using a ball mill and stored in 20 mL scintillation vials with airtight seals. Determination of total soluble sugar concentrations was conducted according to the protocol of Chow and Landhäusser (2004). Using this method, a ground and homogenized tissue sample is subjected to heated ethanol extraction followed by colorimetric analysis with phenol-sulfuric acid. The final extract was read at an absorbance of 490 nm using a Spectronic 20 microplate reader (Thermo Fischer Scientific, Madison, WI) with sugar concentration calculated from a standard curve of 1:1:1 glucose-fructose-galactose. For starch determination, residual tissue was subsequently solubilized by sodium hydroxide, hydrolyzed to glucose by an enzyme mixture of α-amylase and amyloglucosidase, then measured colorimetrically using a peroxidase-glucose oxidase-o-dianisidine solution. Solution absorbance was read at 525 nm and starch concentration calculated based on a glucose standard.

3.2.6 Processing and analysis of sap flow data

Raw temperature data obtained before and after the heat-pulse was used to compute a mean difference averaged between 60-100 s for both the down-stream and up-stream sensor probes (ΔT). The ratio of ΔT is used to estimate the heat-pulse velocity:
\[ V_h = \frac{D}{x} \ln \left( \frac{\Delta T_{down}}{\Delta T_{up}} \right) \times 3600 \]

Where \( V_h \) is the heat pulse velocity (cm h\(^{-1}\)), \( D \) is the thermal diffusivity of the sapwood (cm s\(^{-1}\)), \( \Delta T_{down} \) and \( \Delta T_{up} \) is the change in temperature in the down-stream and up-stream probes respectively, and \( x \) is the distance between the heating element and thermocouple probes. A correction factor is applied to \( V_h \) that accounts for wounding created by the sapflow probes using a third order polynomial derived by Burgess et al. (2001):

\[ V_c = bV_h + cV_h^2 + dV_h^3 \]

Where \( V_c \) is the wound-corrected heat pulse velocity (cm h\(^{-1}\)), while the coefficients \( b, c, \) and \( d \) are constants determined for a wound diameter of 0.20 cm in the 0.6 cm sensor configuration used for this study. From \( V_c \) the sap flux density at the 15-minute timestep is calculated as:

\[ J_s = \frac{\rho_d}{\rho_s} \left( MC + \frac{c_{dw}}{c_s} \right) V_c \]

Where \( J_s \) is the sap flux density (cm\(^3\) cm\(^{-2}\) h\(^{-1}\)), \( MC \) is the sapwood water content (kg kg\(^{-1}\)), \( c_{dw} \) is the specific heat capacity of the wood matrix (1200 J kg\(^{-1}\) K\(^{-1}\) at 20°C), \( \rho_d \) is the dry density of the sapwood (kg m\(^{-3}\)), \( \rho_w \) is the density of the sap (assumed to be equivalent to water, 1000 kg m\(^{-3}\)), and \( c_s \) is the specific heat capacity of water (4186 J kg\(^{-1}\) K\(^{-1}\) at 20°C). As \( J_s \) is known to decline from the outer xylem towards the sapwood, the radial profile must be accounted for to ensure robust estimates of total sap flux (Alvarado-Barrientos et al., 2013; Nadezhdina et al., 2002a). Calculated \( J_s \) at each measurement depth was computed to a total \( J_s \) according to the radial fractions of the sapwood represented by each thermocouple and applying the assumed area in concentric circles bounded by the mid-point at each thermocouple extending toward the heartwood interface. Zero-flow conditions were calibrated using a combination of
night-time and meteorological data (Ambrose et al., 2010; Gotsch et al., 2014), for which we found a flat-line period for each tree between the hours of 22:00-05:00 which was equivalent to flow rates measured during periods of 100% relative humidity during the day. Thus, the full dataset of \( J_s \) was adjusted at each sensor depth to ensure accurate night-time rates of zero flow.

Gap-filling of data was conducted on a sensor-by-sensor and tree-by-tree basis through generating simple linear regressions with the most parsimonious available data. Hence, data gaps from a single depth within an individual was amended by creating a regression equation with an adjacent depth on the sample probe set. If data gaps existed in all depths within a sensor, due to failure of the heating element, then gap-filling was conducted via a regression equation with the probe-set situated on the opposite side of the bole. Finally, in the case that both probe-sets within an individual incur an overlapping data gap, data from an adjacent tree within the plot is used for generating the regression equation. All gap-fill regression equations used no less than 2000 data points (equivalent to 20.8 days of point measurements) and a \( R^2 > 0.9 \) for all linear fits. Overall, data from MEF and FOX was exceptionally clean and gap-filling was required for < 5% of the final data set. The average \( J_s \) of the two probe sets for each tree is reported throughout. The final data set includes 98 days between May 22 – August 27 at MEF, and 104 days between May 13 – August 24 at FOX.

Comparisons of sap flux across severity classes was conducted at the daily scale, thus \( J_s \) was integrated over a 24 h period to estimate total daily sap flux density \( J_D \) \( (\text{cm}^3 \text{ cm}^{-2} \text{ day}^{-1}) \). Prior to statistical analysis of sap flow data, a filter was applied to remove days in which atmospheric drivers of transpiration are known to inhibit sap flow. Any day in which the total rainfall exceeded 5 mm, the mean daily solar radiation was less than 100 W m\(^2\), or mean daily VPD was less than 0.2 kPa did not meet the threshold criteria and were excluded for analysis. Under the
atmospheric conditions stated above, transpiration approaches zero, and thus mutes potential differences between the independent variables of interest. A non-linear regression was fit to \( J_D \) using measured climate parameters to explore potential relationships among WPND severity class. At FOX, daily reference evapotranspiration (ETo) was calculated from the on-site weather station using measured temperature (°C), RH (%), wind speed (m s\(^{-1}\)), and solar radiation (W m\(^2\)) via the Penman-Monteith method (Zotarelli and Dukes, 2010). Since the MEF site lacked wind speed and solar radiation data, curve fits to \( J_D \) were conducted using the canopy-level VPD measurements averaged to the daily scale.

Low-severity trees within each site were regarded as a reference, thus high-severity individuals were assessed relative to the reference trees by computing the \( J_D \) ratio over the course of the growing season. This ratio was examined over three discrete time periods relative to the observed WPND-induced defoliation. These time periods are defined as pre-defoliation (mid-May through June 13), defoliation (June 14 – July 14), and post-defoliation (July 15 through end of August). This approach allows for testing the proportional effect of defoliation of the mature needles on \( J_D \) between the two observed severity classes. Differences in the ratio of \( J_D \) between high- and low -severity trees at each time step was tested using the non-parametric Wilcoxon method due to a difference in sample size between the defined time periods.

### 3.2.7 Analysis of leaf gas exchange data

Three response variables were considered for analysis of gas exchange measurements: photosynthesis (\( A, \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-2} \)), stomatal conductance (\( g_s, \text{mmol m}^{-2} \text{ s}^{-1} \)), and instantaneous water use efficiency (iWUE). Values of iWUE were calculated for each sample as the ratio of \( A \) to transpiration (mol H\(_2\)O m\(^{-2}\) s\(^{-1}\)) measured by the LI-6400XT. Mixed models were constructed
using a full-factorial design of the three independent variables considered across all sites: needle age, WPND severity class, and time. A separate model was run for each response variable of interest. Data from all study sites were pooled within the model and site was treated as a random variable. Since gas exchange measurements could not be conducted on the same dates for all study sites (Table 3.1), measurements were grouped into two discrete time periods for analysis such that the model considers time periods of July and August. Measurements obtained in June were omitted from the model due to a lack of current year needles to be paired with second year needles, thus the sampling dates on June 11 and June 18 at FOX and MEF were excluded. The data set used for analysis consists of 116 unique measurements conducted across all sites in the months of July (n = 50) and August (n = 66) of 2014. A post-hoc analysis was conducted using a paired t-test to test to determine sample means that were significantly different (α = 0.05) between needle age classes within a given severity class for each location.

3.2.8 Analysis of non-structural carbohydrate data

Measured concentrations of soluble sugar and starch were scaled to the whole-tree level using established allometric relationships of roots, foliage, and stem as a function of DBH (Jenkins et al., 2003). To assess the effects of time, tissue type, and WPND-severity on NSC content a repeated measures MANOVA using a full factorial (three-way) design was conducted in JMP Pro 13 (SAS Institute Inc., Carry, NC) using log-transformed values of NSCs. This analysis allows for a test of significant change in sugar or starch concentration over time (June-Aug-Oct), between WPND-severity class and tissue type, or whether there is an effect of WPND on NSCs. Thus, a within-subject factor of time is considered, while two conditions (WPND severity, tissue type) are considered between-subjects on the dependent variables of sugar and
starch. Additionally, allometrically scaled NSCs were assessed relative to the current year and previous year basal area increment of each tree. This ratio of carbohydrate concentration to woody growth rate (kg NSC cm\(^{-2}\) BAI) may provide insight to a potential trade-off between growth rates and carbon allocation among tissue types. In this analysis, data from all dates was pooled and a t-test was conducted within each tissue type to elucidate significant differences (\(\alpha = 0.05\)) between disease severity at the two study locations.

3.3 Results

3.2.1 Impacts of WPND on sap flux density

Total daily sap flux density (J\(_D\)) was found to be consistently lower in the high-severity trees throughout the duration of the study period at both sites (Fig. 3.1). Values of J\(_D\) were similar between sites, reaching a maximum of 402 and 399 cm\(^3\) cm\(^{-2}\) day\(^{-1}\) at MEF and FOX respectively. Average values of J\(_d\) across severity class excluding days in which atmospheric conditions greatly reduced J\(_s\) was 234 (SD ± 64.4) and 243 cm\(^3\) cm\(^{-2}\) day\(^{-1}\) (SD ± 57.0) at MEF and FOX respectively.
Fig. 3.1 Daily total sap flux density at FOX (A) and MEF (B) between May and August 2014. Black and red timeseries denote mean values (n =4) of low-severity and high-severity trees respectively. Ribbons about each timeseries show the standard deviation. Dashed lines indicate the limits of the discrete time periods relative the WPND-induced defoliation event beginning in mid-June.

On a daily scale, sap flux was found to be highly responsive to atmospheric drivers of transpiration, such that non-linear curve fits to ETo and VPD were strong predictors of $J_D$ at both FOX (Fig. 3.2A) and MEF (Fig. 3.2B). Second-order polynomial regressions resulted in a coefficient of determination of $R^2 = 0.73$ for the ETo-$J_D$ relationship at FOX ($J_D = -10.4x^2 + 128.3x - 90.1$) and $R^2 = 0.82$ for the VPD-$J_D$ relationship at MEF ($J_D = -201.4x^2 + 482.7x +27.2$). However, these correlations yielded no obvious patterns relating to the relative
timing of the WPND-induced defoliation event based on observation of potential outliers along the curve.

**Fig. 3.2** Total daily sap flux density as a function of ETo and VPD and sites FOX (A) and MEF (B) respectively. Point shapes are coded according to the relative timing relating to the WPND-induced defoliation event. Open and filled points represent the high- and low-severity trees respectively. Shaded area along the fitted curve indicates the 95% confidence interval.

The sample size (number of days) included within each discrete timestep after removing days with low J_D that were highly influenced by atmospheric variables was n = 16, 22, and 32 for the pre-, during-, and post-defoliation period at FOX and n = 13, 27, and 37 at MEF.

Analysis of the ratio of J_D pooled across the discrete time periods revealed a subtle, but significant reduction in J_D within the high-severity trees compared to low-severity reference trees via the Wilcoxon test across all pairs. At MEF, this reduction was noted in the post-defoliation period (p = 0.002), while at FOX J_D appeared to respond more rapidly as a significant reduction was found both during (p = 0.004) and after defoliation (p = 0.002) compared to the pre-defoliation period in May and early June (Fig. 3.3). This equates to a relative change in the J_D ratio of -7.2 and -8.4% in the defoliation and post-defoliation period compared to the pre-
defoliation \( J_D \) ratio at FOX. At MEF, the relative reduction in the \( J_D \) ratio between the pre- and post-defoliation period was 5.2%.

**Fig. 3.3** The ratio of daily sap flux density (\( J_d \) high-severity: \( J_d \) low-severity) for each discrete time period relating to the relative timing of WPND-induced defoliation at study sites FOX (A) and MEF (B). A change in connecting letters indicates a significant difference (\( \alpha = 0.05 \)) determined using the Wilcoxon method across each pair.

### 3.3.2 Leaf gas exchange and phenology

A mixed model analysis was conducted for each dependent response variable of interest (\( A, g_s, \) and iWUE) to test the interactions of time, needle age, and WPND-severity class while treating the three study sites as a random effect (**Table 3.2**). The effect of disease severity was not found to be significant except for its interaction over time (July-August) on measurements of iWUE, \( F(1, 106.0) = 5.531, p = 0.0205 \). Time was a highly significant factor in response of both \( g_s, F(1, 106.2) = 54.986, p < 0.0001, \) and iWUE, \( F(1, 106.1) = 153.930, p < 0.0001 \). Effects of needle age was also deemed significant for \( A, F(1, 106.1) = 18.524, p < 0.0001, \) and iWUE, \( F(1, 106.0) = 7.508, p = 0.0077, \) while the interaction of needle age with time was significant for \( A, F(1, 106.1) = 29.598, p < 0.0001, \) and \( g_s, F(1, 106.0) = 0.341, p = 0.0062. \) For each response
variable, the random effect of site was not determined to be a significant factor (Wald p-value > 0.32).

Table 3.2 Results of the three-factor mixed model run for photosynthesis, stomatal conductance, and intrinsic water use efficiency. Time indicates the month in which measurements were collected (July or August), severity corresponds to the distinction between observed low- and high-WPND severity, and needle age differentiates between current-year and second year foliage. Effects are considered significant at p < 0.05.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Source</th>
<th>NumDF</th>
<th>DenDF</th>
<th>F</th>
<th>p-value</th>
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</thead>
<tbody>
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</tr>
<tr>
<td></td>
<td>Time x Needle age</td>
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<td>29.598</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Severity x Needle age</td>
<td>1</td>
<td>106.1</td>
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<td>Water use efficiency (iwUE)</td>
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<td>153.930</td>
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<tr>
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<td>1</td>
<td>106.0</td>
<td>0.018</td>
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</table>

Taking a closer look at differences in physiological function between needle age class using a t-test with each sampling date, it was found that second year needles exhibited significantly higher rates of photosynthesis (p < 0.05) across severity classes during the July1 sampling at BTH (Fig. 3.4). On this same date at BTH, gs was found the be marginally significantly within low-severity trees (p = 0.048), while iWUE was significantly higher in second year needles of the high-
severity trees (p = 0.011). Effects at the other two sites were minimal, though it is worth noting significantly higher rates of A within the second-year needles of high-severity trees at MEF on July 8 (p = 0.033), and a significantly higher iWUE within the low-severity trees at FOX on July 21 (p = 0.037).

![Graphs](image)

**Fig. 3.4** Photosynthesis (A), stomatal conductance (gs), and intrinsic water use efficiency (iWUE) over time for current year (light-green) and second year (dark-green) needles. “NM” indicates dates in which the current-year needles could not be measured due to insufficient needle length in the month of June. Boxes marked with * indicate a significant difference between needle age class within each severity class at the corresponding date via a pooled t-test (α = 0.05).

Though measurements could not be conducted on current-year needles at MEF and FOX in the months of June, the data collected in early July at BTH are suggestive of a greatly reduced photosynthetic capacity during the early portion of the growing season. Needle elongation in eastern white pine occurred over a three-month period beginning in May and culminating in early August (**Fig. 3.5**). There was not a significant difference in needle length between the five collection dates ranging from August 3 throughout August 31. Needle elongation began approximately four weeks after the current year shoot growth initiated. Mean total first-order shoot length of the current year was 7.5 cm and mean total needle length in the month of August was 8.9 cm. Needle elongation measured on a weekly interval at TOM shows that the current-
year foliage is only at 30% of its total length by mid-June, the approximate time when WPND-induced defoliation begins. Between the dates June 2 and August 3, needles are growing at an average rate of 1.2 mm day\(^{-1}\).

**Fig. 3.5** Length of the current year needles over time. Sigmoidal curve fit using a 4-parameter probit model, \(R^2 = 0.937\).

### 3.3.3 Non-structural carbohydrate allocation

Repeated measures MANOVA analysis was conducted to test disease severity and tissue type effect on sugar and starch content over time (Table 3.3). Univariate tests showed there was a significant effect between tissue types \(F(4, 156) = 0.483, p < 0.0001\), but not relative disease severity on the concentration of NSCs., \(F(4, 156) = 0.483, p = 0.488\). The results showed there was also a significant interaction within subjects of tissue type over time \(F(4, 156) = 21.572, p < 0.0001\), though the interaction of disease severity over time did not meet the criteria for rejecting the null hypothesis nor did the interaction of disease severity and tissue type over time.
Table 3.3 Between-subject and within-subject results of the 3-factor repeated measures MANOVA for sugar and starch concentrations. Effects are considered significant at p < 0.001.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>F</th>
<th>NumDF</th>
<th>DenDF</th>
<th>p-value</th>
</tr>
</thead>
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<td>Between subjects</td>
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</tr>
<tr>
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<td>0.003</td>
<td>0.483</td>
<td>4</td>
<td>0.4880</td>
</tr>
<tr>
<td></td>
<td>Tissue x Severity</td>
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<td>2.317</td>
<td>4</td>
<td>0.0596</td>
</tr>
<tr>
<td>Within subjects</td>
<td>All</td>
<td>0.635</td>
<td>11.004</td>
<td>9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
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<td>90.961</td>
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<td>Time x Tissue x Severity</td>
<td>0.017</td>
<td>0.670</td>
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</tr>
</tbody>
</table>

A significant linear relationship was noted between the log-transformed sugar and starch content throughout the study period pooled across all tissue types (p = 0.014, \( R^2 = 0.239 \)), indicating that these carbohydrates are positively correlated with one another. Within each tissue type, NSCs between disease severity class were generally well correlated with each other (Fig. 3.6), though significant differences were noted in starch CYNL in August (p = 0.0163) and Y14NL in October (p = 0.0355).
Fig. 3.6 Mean (n = 6) starch and sugar content for the low- (open circle) and high-WPND-severity (filled circle) trees across the five tissue types and three sampling periods. Error bars show standard deviation and * represents a significant difference between WPND-severity class within a given sampling date. (CYNL = current-year needle lower canopy, MYSL = multi-year stem lower canopy, root = primary root, stem = main trunk at DBH, Y14NL = year 2014 needle lower canopy).

Examination of allometric-scaled NSCs to relative BAI of the current year (2015) and previous year (2014) revealed several significant differences between severity class of the five different tissues (Fig. 3.7). The ratio of sugar to BAI (kg cm\(^{-2}\)) was significantly higher (p < 0.05) within the WPND site for CYNL, MYSL, and Y14NL tissues when considering both years of BAI. Similarly, the starch to BAI ratio was significantly higher within CYNL, MYSL, and Y14NL across both years, while root and stem was only considered significant when comparing BAI relative to 2014.
Fig. 3.7 Ratio of allometrically-scaled sugar and starch content (kg) to basal area increment (BAI at DBH, cm$^2$) of the current (2015) and previous year (2014) wood growth. Letters indicate the results of a t-test ($\alpha = 0.05$) performed between WPND-severity class, where a change in lowercase letters denotes a significant difference when applying 2014 relative growth rate and uppercase letter corresponds to the 2015 relative growth rate within each tissue type. (CYNL = current-year needle lower canopy, MYSL = multi-year stem lower canopy, root = primary root, stem = main trunk at DBH, Y14NL = year 2014 needle lower canopy).

3.4 Discussion

This study did not measure large deviations from the ETo and VPD curve fits before, during, or after the time of WPND-induced needle cast, as daily sap flux rates were not significantly reduced under conditions that facilitated high transpiration rates. However, trees identified as high-severity individuals exhibited lower rates of $J_s$ throughout the entire period in which sap flux measurements occurred compared to the references healthy/low-severity trees at each site. There was a clear distinction between the two severity classes along the ETo and
VPD curves, though these conditions generally precede the defoliation event. This suggests that these trees inherently have lower rates of Js, which may be attributed to the multiple years of defoliation leading up to when the sap flow measurements were conducted. Alternatively, these initial differences in Js may also be attributed to variation in tree size (Berry et al., 2017; Oren et al., 1999), as high-severity trees were found to have smaller diameter of low-severity trees at both sites, with a mean difference in DBH of 9.6 and 5.3 cm at MEF and FOX respectively. All sampled trees were in the co-dominant canopy strata, thus it is unlikely that overstory shading played a significant role in the low sap flux rates measured in the high-severity trees. It is plausible that the impacts of defoliation are muted due to the concurrent elongation of the current year foliage during the time at which mature second and third year needles are being cast. In the month of June, the current-year foliage is still elongating and this not likely to be functioning at the sample level as fully mature foliage.

To this point, analyses of instantaneous leaf gas exchange response variables (A, gs, iWUE) revealed significant effects of needle age over time within both low- and high-severity individuals. Conifers are unique in the aspect of foliar morphology in that development of needles typically occurs over the time span of several months (Cuny et al., 2012; Rossi et al., 2009; Sampson et al., 2003). Rates of photosynthesis and transpiration have been shown to be closely linked with needle phenology in pine, such that carbon assimilation and Js generally increase as needles approach their maximum length (Maseyk et al., 2008). In contrast, temperate broadleaf tree species such as sugar maple (Acer saccharum March.) and American beech (Fagus grandifolia Ehrh.) will reach full leaf expansion on the order of 3-4 weeks and are at full photosynthetic capacity by the first week of June in the northeastern US (Gill et al., 1998). Another important distinction between conifer and broadleaf tree species is the ability of
broadleaf species to induce a second flush of foliage following a defoliation-type disturbance early in the growing season. For example, frost-defoliated quaking aspen (*Populus tremuloides* Michx.) have demonstrated the ability to induce a second flushing following leaf drop due to low temperatures in late May, though aspects of morphology and physiology of the second cohort can be significantly different from foliage of the primary flush (St. Clair et al., 2009). Defoliation via herbivory can also induce a second flush of foliage in many broadleaf species (Dury et al., 1998; Hrabar et al., 2009). However, this is not the case within species of *Pinus*, thus the reduction in leaf area caused by WPND will impact infected trees over the duration of the growing season. For some instances of defoliation-type disturbance, a certain degree of compensation response may be expected. A compensatory response suggests that plants subject to defoliation will stimulate vegetative tissue growth through increased photosynthetic rates and reallocation of assimilates (McNaughton, 1983). In the case of eastern hemlock (*Tsuga canadensis* (L.) Car.) defoliated by hemlock woolly adelgid (*Adelges tsugae* Annand), Williams et al. (2016) found a significant increase in chlorophyll abundance within infested trees, suggesting a compensatory response that may allow diseased individuals to photosynthesize at higher rates per unit leaf area. Experiments conducted in red pine (*Pinus resinosa* Sol. ex Aiton) and Japanese larch (*Larix leptolepis*) subject to artificial defoliation also confirmed compensatory response at the leaf level (Reich et al., 1992; Vanderklein and Reich, 2000). However, no such response is indicated in this study, as high-severity white pines appear to have rates of $A$ and $g_s$ similar to the low-severity reference trees. The only significant effect detected between severity class was in response of iWUE over the two sapling periods considered, indicating that diseased individuals may be using more water relative to carbon gain compared to reference trees. Since defoliated trees exhibit thinned crowns, it may be possible that increased
light exposure is influencing microclimate factors such as relative humidity which in turn alters stomatal regulation. A higher degree of temporal resolution and a more quantitative determination of disease severity may be required to better ascertain the subtle differences noted in leaf gas exchange.

Despite this apparent lack of compensation at the leaf level, diseased trees are storing NSCs at nearly the same concentration across tissue types as reference control trees (Fig. 3.6). No difference was found to be significant when considering the effect of disease severity, nor the interaction of severity with tissue type and over time (Table 3.3). This finding is interesting in light of what is known concerning the growth rates of diseased trees, which has unequivocally shown declines in basal area increment as a function of WPND disease severity since the onset of outbreaks in the northeastern US (McIntire et al., 2018). However, several studies show that stored NSCs and growth is not always tightly linked. In a disease similar to the WPND complex, Douglas-fir (*Pseudotsuga menziessi* (Mirb.) Franco) defoliation by Swiss needle-cast (*Phaeocryptopus gaeumannii* T. Rhode) was found to significantly reduce wood growth ahead of observed declines in NSC reserves (Saffell et al., 2014), suggesting that trees will store NSCs in times of defoliation stress to allocate towards new foliar material or defense compounds at the expense of radial growth. It is plausible that the impacts of WPND have yet to reach a critical threshold of reduced gross carbon assimilation, at which point it may be expected to observe declines in stored NSC. This assertion is supported in part by the low levels of observed mortality attributed to WPND. As depletion of NSCs is hypothesized as a pathway of plant mortality (McDowell et al., 2008; McDowell, 2011), it is reasonable to expect low soluble sugar and starch stores prior to tree death. This pathway, termed carbon starvation, is often discussed in the context of drought-induced mortality, though the subject is controversial due in part to its
difficulty to test in the field and lack of research to support the overarching hypothesis independent of other biotic and abiotic factors (Sala et al., 2010). There are in fact several studies relating to NSC dynamics within defoliated trees that contradict the findings of the Saffell et al. (2014) study on Swiss needle-cast. Defoliation intensity of processionary moth (*Thaumetopoea pityocampa* Dennis & Schiff) on maritime pine (*Pinus pinaster* Aiton) was found to have a negative linear relationship with soluble sugar concentrations within the sapwood of trees > 15 years old, but no impact on the sugar concentration with phloem nor starch content of the sapwood or phloem (J. Jacquet et al., 2013). A study of defoliated Scots pine (*Pinus silvestris* L.) found consistently higher total NSC within leaf, branch, trunk, and root tissues over the course of a growing season compared to non-defoliated trees coupled with drought stress (Aguade et al., 2015). Additionally, Wiley et al. (2013) reported significant reductions in both the belowground and above ground starch content of fully defoliated black oak (*Quercus velutina* Lam.), but no difference between control and half-defoliated trees. This last finding implies that severe levels of defoliation must occur in order to induce rapid changes in NSC content. In the context of WPND, seasonal litterfall data shows that trees are casting nearly half of their total annual litterfall in the months of June and July (McIntire et al., 2018), though this is not a direct estimate of total foliar area. Under the most extreme conditions in which all mature second-year needles are presumed to cast and assuming current year needle production is the same as the previous year, this would equate to approximately a 50% loss of total foliar area due to WPND. Furthermore, current year needles are not yet fully elongated/functional when defoliation occurs in mid-June. Despite this potential reduction in leaf area, this study found no significant reductions in NSCs as a function of defoliation severity except for subtle differences in starch
content within the current-year and second-year needle tissues in the months of August and October respectively.

Overall, the resulting impacts of defoliation on NSC concentration in trees within the available literature is mixed; there are many examples in which a decline or increase in NSCs is found to occur in some tissues types and not others, only at certain times in the growing season, in conjunction with other stressors, and at variable levels of relative defoliation intensity (Deslauriers et al., 2015; Handa et al., 2005; Li et al., 2002; Palacio et al., 2008; Piper et al., 2015; Puri et al., 2015). Each of these studies support the finding that the relative importance of stem growth is a lower priority than the carbon requirement for other physiological processes within trees, though the order hierarchy of such processes is complex. When considering the relative growth rates of each tree via the ratio of sugar to current year and previous stem basal area increment (kg NSC cm\(^{-2}\) BAI) we found a significant difference between the WPND site and the control site (Fig. 3.7).

Results from this study appear to support the finding that reductions in wood growth do not imply carbon limitation. However, Wiley et al. (2013) asserts that a lack of NSC decline does not necessarily indicate that reduced tree growth is caused by sink limitation. Several explanations are offered to support this claim, such as an internal sink limitation imposed by plant architecture or phenology, delays in growth due to competitions among tissues for carbohydrate, and the alteration of hormonal growth regulators as a result of defoliation. Despite an abundance of research in the past decade focused on topic of source-sink dynamics, considerable disagreement still exists regarding the interpretation of NSCs as evidence for sink limitation (Mcdowell and Sevanto, 2010; Ryan, 2011; Sala et al., 2012; Wiley and Helliker, 2012). Furthermore, only recently has a synthesis of laboratory techniques for measuring plant
NSCs been evaluated, making it difficult if not impossible to compare NSCs across studies except those conducted within the same lab group (Quentin et al., 2015).

In trees subjected to multiple years of defoliation due to the foliar pathogens associated with WPND, it appears that total NSC content across storage tissues is conserved, while any excess carbon after this baseline storage demand is met is allocated towards structural growth. It is worth noting that across all trees in which growth rings were measured, only two trees of high defoliation severity were determined to have missing rings in the post-outbreak period, indicating that though growth is reduced is has not yet ceased completely. If tree mortality due to carbon starvation was to occur within WPND-infected individuals it could be expected to observe multiple years of near-zero growth rates followed by a decline in total NSC storage. Presently, mortality due to WPND is low and isolated to smaller trees within intermediate crown positions, thus prolonged or more intense defoliation stress may be required before widespread mortality could be expected to be observed.

3.5 Conclusion

In this chapter several aspects of tree physiology were evaluated as a function of the relative disease severity induced by WPND. Measurements of sap flux revealed a subtle, yet significant decrease in whole-tree transpiration rates following defoliation in mid-June, and trees of high infection severity were found to have consistently lower rates of $J_t$ on the order of ~60% that of reference trees throughout the entirety of the study period. WPND severity was not a significant factor influencing leaf-level photosynthesis, stomatal conductance, nor iWUE. However, needle age was found to influence these parameters, as current year needles exhibit lower functionality in the early growing season while expansion is still occurring. As residual
foliage does not appear to be compensating for the reduction in leaf area, gross carbon assimilation is must be lower in high-severity trees. While woody growth declines have been well documented in stands impacted by WPND, this study found that carbohydrate stores are unaffected, maintaining similar concentrations across tissue types comparable to healthy trees. This latter finding may be indicative of a growth-defense trade-off within high-severity trees, though additional study would be required to evaluate the underlying physiological, phenological, and morphological traits that are known to drive the complex dynamics of carbon allocation.
CHAPTER 4

THINNING TREATMENTS REDUCE SEVERITY OF FOLIAR PATHOGENS IN EASTERN WHITE PINE

Abstract

The foliar fungal pathogens associated with the disease complex known as White Pine Needle Damage (WPND) are causing widespread defoliation of eastern white pine (*Pinus strobus* L.) in the northeastern United States and Canada. Presently, there are no specific management recommendations for addressing declining stand health relating to WPND induced defoliations. This study aims to test the effects of thinning at two different residual stocking densities (14 and 25 m$^2$ ha$^{-1}$) on mitigating the negative impacts of WPND within infected stands. To quantify the impacts of WPND on individual tree health, we generated a composite health index score using response variables measured in the field and weighted according to their association with observations of WPND severity. Post-thinning changes in disease severity were used to evaluate the effectiveness of stand thinning to reduce pathogen pressure and promote overall tree vigor. Results show that thinning had a rapid positive effect on overall tree health, with no significant difference between thinning treatment levels in the first two years following tree removal. Severity of WPND was reduced by 35% in low-density residual thinnings in the second year of the study. Our findings suggest that thinning as a silvicultural tool to reduce stocking densities within infected stands can effectively promote overall tree health and maintaining proper stocking densities is recommended for stands at risk of infection.
4.1 Introduction

Foliar pathogens of eastern white pine (*Pinus strobus* L.) in the northeastern US have been a recent cause of concern for land managers and timber producers in the region. Since ca. 2010, a complex of four native ascomycete fungi, termed White Pine Needle Damage (WPND), has induced widespread defoliation of mature needles in the summer months (Broders et al., 2015; Munck et al., 2012; Wyka and Broders, 2016). The incidence of WPND is ubiquitous throughout the northeastern US, with the presence of at least one of the associated pathogens confirmed at 93% of mature white pine stands sampled in a recent survey throughout Maine, New Hampshire, Vermont, and Massachusetts (Wyka et al., 2017a). Defoliation caused by WPND in the months of June and July have been shown to account for ~47% of the total annual foliar litterfall within infected stands, significantly altering the seasonal dynamics of litter deposition within diseased stands (McIntire et al. 2018). In turn, WPND has been shown to cause significant growth declines in stands subject to multiple years of defoliation stress, reducing mean annual basal area increment by 25-73% following the initial outbreaks in the region (McIntire et al. 2018).

Silvicultural practices have long been applied in white pine stands subject to insect and pathogen stress throughout its native range (Ostry et al., 2010). Historically, the greatest biotic threats to white pine health have been the native white pine weevil (*Pissodes strobi* Peck) and the introduced pathogen *Cronartium ribicola* Fisch, both of which have well established management recommendations (Major et al., 2009; Maloy, 1997; Ostrofsky, 1988; Stiell and Berry, 1985; Taylor and Cozens, 1994). Chemical control of foliar pathogens, while effective, is both expensive and time intensive, thus typically practiced in an urban forestry setting as opposed to landscape-level treatment. Presently, there are no specific silvicultural prescriptions
that address the impacts of defoliating pathogens affecting white pine. To curb the impacts of WPND across a diversity of land management regimes, it is crucial to develop a treatment that can be applied on a large scale and ideally in conjunction with previously established management guidelines for white pine (Anderson et al., 2002; Lancaster, 1984; Lancaster and Leak, 1978; Pinto, 1992; Robbins, 1984; Stiell, 1985). Therefore, the goal of this study was to test whether thinning treatments can be used to mitigate the negative impacts of WPND-induced defoliation in mature white pine stands. White pine has shown to respond well to thinning at virtually every age class, with increases in diameter increment on the order of 100%, 80% and 63% in age classes 19, 55, and 200+ years old respectively (Bebber et al., 2004; Burgess and Wetzel, 2000; Stiell, 1979; Stiell et al., 1994). A study involving thinning treatments in mature stands in Maine demonstrated that residual densities of 7.3 and 13.8 m$^2$ ha$^{-1}$ promoted growth, recovery, and regeneration of white pine seedlings in declining stands (Leak and Yamasaki 2013).

The rationale behind applying a thinning treatment to reduce stand densities in WPND-infected stands is in large part due to the nature of spore dispersal of associated fungi. Needle chlorosis and defoliation have been found to be most severe in the lower portions of infected crowns, consistent with rain-splash dispersed foliar diseases (Broders et al., 2015). In a study of spore dispersal of Lecanosticta acicola Thümen, the most prevalent pathogen associated with WPND, Wyka et al. (2017b) found that abundance of spores was greatly reduced at distances > 3 m from the outer edge of lower branches compared with the area directly below infected crowns. We hypothesized that reducing stand densities will result in a decrease of foliar pathogen pressure while promoting growth, crown development, and recovery. Through application of thinning prescriptions, the distance between crowns will be increased, thus potentially limiting
pathogen spread while exploiting the inherent benefits of increasing crown light exposure for
tree health and vigor (Pacala et al., 1996). In this paper, we (1) determine the impacts of WPND
on traditionally measured forest health traits, (2) generate a composite health index score used
for monitoring change over time, and (3) assess the response of WPND-infected trees to reduced
stand densities in the first two years following thinning treatment.

4.2 Methods

4.2.1 Study sites

We conducted thinning trials within two white pine dominated stands, separated by
approximately 100 km, located in central and southern New Hampshire, USA. The first stand is
located on the Bear Camp River property in the town of West Ossipee (OSP) and is owned and
maintained by the University of New Hampshire. This property is composed of 12.7 ha of
forested area, of which 63% is nearly pure white pine and 83% features white pine as a dominant
component. The white pine dominated compartments in which the thinnings were conducted are
approximately 8.1 ha. The symptoms and presence of WPND fungi were observed to be
ubiquitous throughout the stand during an initial survey in June of 2015. Other species present
within this site include eastern hemlock (Tsuga canadensis (L.) Carrière), northern red oak
(Quercus rubra L.), American beech (Fagus grandifolia Ehrh.), red maple (Acer rubrum L.),
sugar maple (Acer saccharum Marshall), and silver maple (Acer saccharinum L.). Soils at this
site are excessively drained and largely classified as Colton gravelly loamy fine sand. The
approximate date of establishment for the mature white pine is 1928. White pine saw timber and
pulpwood were harvested from OSP in 1976 and 1989, respectively totaling 236.8 and 353.1 m³
of volume removed. An additional 9.4 m³ of white pine was salvaged from blowdowns at this
site in 1980. A second stand is located within the 585 ha Caroline A. Fox Research and
Demonstration Forest in Hillsborough, NH (FOX) and is under the management of the state of
New Hampshire Department of Resources and Economic Development. FOX is included within
a permanent network of plots throughout the northeastern United States that have been
monitored for WPND incidence and severity since 2012 by the US Forest Service and state
forest health cooperators. Severity of symptoms associated with WPND have generally increased
annually at this site since monitoring began. The majority of mature trees at this site established
following a severe hurricane in 1938, which felled over 3,776 m$^3$ of standing timber (Allen and
Seaboyer, 2017). The compartment in which the thinning was conducted established later,
following abandonment of agricultural land in 1959. White pine and eastern hemlock are the
most abundant species at FOX, followed by northern red oak, red maple, and several other
northern hardwood species. Soils within the thinned stand are stony and generally classified as a
Marlow fine sandy loam. Pre-thinning stocking levels and additional site characteristics for OSP
and FOX are shown in Table 4.1.

Table 4.1. Initial conditions of the two naturally established eastern white pine stands in New
Hampshire measured in 2015.

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<th>FOX</th>
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<tbody>
<tr>
<td>Site description</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latitude</td>
<td>43.810</td>
<td>43.129</td>
</tr>
<tr>
<td>Longitude</td>
<td>-71.186</td>
<td>-71.923</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>160</td>
<td>240</td>
</tr>
<tr>
<td>Mean annual temperature (°C)</td>
<td>7.0</td>
<td>7.5</td>
</tr>
<tr>
<td>Mean maximum temperature (°C)</td>
<td>13.6</td>
<td>13.6</td>
</tr>
<tr>
<td>Mean minimum temperature (°C)</td>
<td>0.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Mean annual sum precipitation (mm)</td>
<td>1216</td>
<td>1205</td>
</tr>
<tr>
<td>Initial conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year of initiation</td>
<td>1928</td>
<td>1960</td>
</tr>
<tr>
<td>Stand age (yr)</td>
<td>89</td>
<td>57</td>
</tr>
<tr>
<td>Basal area (m$^2$ ha$^{-1}$) $^*$</td>
<td>40.3 ± 2.4</td>
<td>42.3 ± 1.9</td>
</tr>
<tr>
<td>Stem density (ha$^{-1}$) $^*$</td>
<td>447 ± 49</td>
<td>646 ± 18</td>
</tr>
<tr>
<td>Quadratic mean diameter (cm) $^*$</td>
<td>33.9</td>
<td>28.9</td>
</tr>
<tr>
<td>Stand density index $^a$</td>
<td>710.1</td>
<td>794.2</td>
</tr>
<tr>
<td>Relative density $^b$</td>
<td>0.457</td>
<td>0.791</td>
</tr>
<tr>
<td>Site index $^c$</td>
<td>71.1</td>
<td>83.5</td>
</tr>
<tr>
<td>Percent P. strobus (%BA)</td>
<td>91.8</td>
<td>84.0</td>
</tr>
</tbody>
</table>

* Mean and SE; $^a$(VanderSchaaf, 2013); $^b$(Ducey and Knapp, 2010); $^c$(Parresol and Vissage, 1998)
4.2.2 Experimental design

We implemented a blocked experimental design on a 2.4 ha area within each study site. Each block (n=4) consisted of three 4047 m² (1 acre) treatment plots, composed of two thinning variants and a single control plot. To accommodate the constraints of the white pine dominated portions of each stand the dimensions and subsampling of the plots differed between sites, but total area was conserved (Fig. 4.1). We conducted two thinning trials at each field site that aimed for a residual basal area of 25 and 14 m² ha⁻¹, henceforth referred to as the high-density residual (HD) and low-density residual (LD) thinning treatments, respectively. The high-density stocking level was selected to simulate a traditional white pine precommercial thinning, targeting the area between the managed B- and C-line according to the Leak and Lamson (1999) stocking guide for eastern white pine. The low-density thinning treatment was a more intensive tree removal, simulating what would be most commonly referred to as a crop tree release, placing these plots well below the managed C-line of the stocking chart. Low-density thinnings have previously been advocated for rapid growth of individual trees in white pine (Seymour, 2007). Individual trees were selected for trait measurements using variable radius (prism) subplots. A preliminary cruise of each stand was conducted to determine the mean diameter at breast height (DBH, 1.3 m above ground level) in order to estimate the mean expected inclusion zone radius for spacing prism points. Within each treatment plot, three permanent prism points (BAF 4.6 m² ha⁻¹) were established, oriented to maximize distance from each other to avoid double-sampling large trees while also allowing for a buffer of at least 18 m from the subplot center point to the edge of the plot (Fig. 4.1). Block and plot boundaries were adjoined at each site. A pre-thinning inventory was conducted for each stand in June of 2015, during which each tree selected for measurements was given an aluminum tag with a unique identification number and the distance and azimuth
from the subplot center was noted to ensure re-measurement of the same individuals in the years following the thinning treatments. A total of 158 (145 *P. strobus*) and 166 (130 *P. strobus*) live trees were tallied at OSP and FOX respectively during the 2015 pre-treatment inventory.

![Diagram](image)

**Fig. 4.1** Diagram of the blocked experimental design at the white pine stands in southern NH. Black circles show the orientation of the three replicated prism subplots within the treatment plots used to select trees for trait measurements.

Trees were selected for removal by the land managers of the respective study sites without a priori knowledge of individual tree health from data collected by the authors of this study during the pre-treatment inventory. Mechanical removal of trees was conducted in the winter of 2015-16 and re-measurement of the stands took place in June-July of 2016 and 2017, the months when symptoms associated with WPND are most prevalent. During tree removal at FOX, 5.3 m² of mature tree BA was removed from the control plot in order to accommodate a skid trail, resulting in a 13.6% reduction in BA post-treatment. Post-thinning stocking levels for each stand are reported in **Table 4.2**.
Table 4.2 Post-treatment metrics of stand level stocking for control, high-density (HD), and low-density (LD) thinning plots. Means and standard error are calculated from n=6 prism points per treatment per site.

<table>
<thead>
<tr>
<th></th>
<th>OSP</th>
<th>FOX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>HD</td>
</tr>
<tr>
<td>Basal area (m$^2$ ha$^{-1}$)</td>
<td>36.7 ± 5.6</td>
<td>25.3 ± 2.8</td>
</tr>
<tr>
<td>Stem density (ha$^{-1}$)</td>
<td>407 ± 95</td>
<td>142 ± 31</td>
</tr>
<tr>
<td>Stand density index</td>
<td>647.0</td>
<td>388.8</td>
</tr>
<tr>
<td>Relative density</td>
<td>0.564</td>
<td>0.358</td>
</tr>
</tbody>
</table>

4.2.3 Response variables

For each eastern white pine tree, measurements generally followed the crown-condition classification protocols given in Schomaker et al. (2007). Our study included seven traits that are regarded to be indicative of overall tree health and potentially relevant to WPND, especially those relating to the live crown. All ocular measurements were conducted by the same individual for each year of the study to reduce measurement variability. Each trait measurement is described in detail below.

4.2.3.1 Diameter at breast height

Diameter at breast height (DBH) was measured to 0.1 cm for all trees > 10 cm on the upslope side of the tree. Breast height was marked with paint pen in 2015 to ensure accurate measurement of DBH in subsequent years of the study.

4.2.3.2 WPND severity

Severity of WPND infection was measured in four classes on a continuous scale using a protocol developed for WPND monitoring by the US Forest Service (Broders et al., 2015). The
total crown area is visually segmented into thirds, then chlorosis and needle retention is assessed within each segment. Trees are rated on a 0-3 scale, where 0 corresponds to a healthy crown free of signs and symptoms of WPND, 1 infers that $\leq 1/3$ of the crown is affected, 2 infers that $1/3 > 2/3$ of the crown is affected, and 3 infers that $> 2/3$ of the crown exhibits infection. This 0-3 classification is also used throughout this paper as a discrete variable to quantify the relationship of WPND severity with all other response variables, where measurements are grouped as healthy (0), mild (1), moderate (2), and severe (3) infection severity classes respectively.

4.2.3.3 Crown light exposure

Light exposure refers to the direct sunlight that a crown receives when the sun is directly overhead (Schomaker et al. 2007). This measurement requires the crown to be visually divided into five parts; a top section and four equal vertical quarters. The light exposure is then rated on a 0-5 scale, where a 0 indicates trees receiving no direct sunlight, e.g. trees in the understory, overtopped, and often intermediate crown positions. A rating of 1 indicates that the top of the tree is receiving direct sunlight, the typical rating for co-dominant trees in a mature stand. For a tree to receive a rating of 2 there must be an additional 25% of the crown from one side experiencing direct sunlight. Trees receiving 50%, 75%, and 100% sunlight from the sides of the live crown receive a rating of 3, 4, and 5 respectively.

4.2.3.4 Crown transparency

Crown transparency is a measurement of the absence of foliage in the crown where foliage would normally be expected to occur and is often indicative of declining tree health (Schomaker et al. 2007). Crown transparency estimates the amount of light passing through the
observable live crown while excluding dieback and exposed branches. Transparency is measured in 5% intervals where a rating of 0% indicates no light passing through the crown (dense foliage) and 95% represents a tree largely void of foliage. This estimate is obtained by observing the crown from a distance with a clear view, typically in the same place where WPND severity and height measurements are taken. A rating is determined by first creating a visual outline of the uncompacted live crown, then ocularly estimating the percent of light passing through the observable area. Infected trees experiencing WPND defoliations were expected to have significantly reduced second and third year foliage, especially through the middle to lower portions of the crown.

4.2.3.5 Crown dieback

The crown die-back parameter is an estimate of recent branch mortality and is often associated with early signs of stress. Traditional measurements of die-back only include branches positioned within the continuous live crown while excluding those beneath (Schomaker et al. 2007). However, due to the nature of WPND defoliation and observations of branch mortality resulting from severe and chronic needle cast we were primarily interested in lower branch death. Dead branches at the lower end of the live crown were included in the overall assessment if there was visible evidence of branch death caused by defoliation, identified by the presence of fine branch tips and/or branches that retain only dead needles. Although white pine is known to lose branches from the bottom of the crown naturally, we assert that trees continuously defoliated by needle pathogens will exhibit advanced dieback that is exacerbated by defoliation and is proportional to observed WPND severity. Crown die-back was quantified on a percent scale similar to transparency in 5% increments used to compare the observed branch death to the
total live crown, where 0% is a healthy crown with no apparent die-back and 95% is a crown near total death.

4.2.3.6 *Live crown ratio*

The uncompacted live crown ratio (LCR) is the length of the tree that supports live foliage relative to the total height of the tree (Schomaker et al., 2007). The LCR has been used as a reliable predictor variable of early to mid-range tree stress (Pontius and Hallett, 2014). Height measurements were obtained using a Vertex IV hypsometer (Haglöf, Lensele, Sweden). Branches supporting foliage at a distance > 2 m below the main crown were excluded from measurements.

4.2.3.7 *Crown diameter*

Crown diameter is a measurement of the horizontal distance from branch tip to branch tip of the observable lower crown. Our method differs from that recommended by Schomaker et al. (2007) in that we did not implement the dripline method at the widest diameter. Instead, to ensure replicating this measurement accurately in subsequent years, we measured from the north-south and east-west cardinal directions for each crown through the center of the stem. This procedure is consistent with recommendations by Kershaw et al. (2016, Chapter 5). The mean crown diameter (m) from these two measurements is reported throughout.

4.2.4 *Pre-treatment analyses*

For analysis of pre-treatment data, we were primarily interested in determining the impact of WPND on the traditionally measured tree attributes described above. Mixed effects
models were constructed using WPND severity as a fixed effect predictor variable for each of the six response variables of interest: DBH, light exposure, transparency, dieback, LCR, and crown diameter. Additionally, the two study sites and their interaction with WPND severity were treated as fixed effects, with treatment blocks and prism plots treated as random effects in which the response variables of individual trees are nested. Although there are theoretical reasons for departure from normality for each response variable (e.g. DBH cannot be less than zero), visual examination of quantile and residual plots revealed no substantial departures from either normality or homoscedasticity assumptions. For variables recorded as a set of discrete levels (light exposure, transparency, dieback), exact normality cannot be achieved. However, examination of the original distributions and of the pure and conditional residuals revealed no outliers and no obvious homoscedasticity. Hypothesis tests for these variables (p-values) should be regarded as approximate. Post hoc comparisons were conducted using a Tukey honestly significant difference (HSD) test ($\alpha=0.05$). Additionally, principal component analysis (PCA) was conducted on all measured response variables and coded according to WPND severity class to visualize potential groupings within the dataset and evaluate the relative weights of each response variable to WPND severity. All response variables used in the PCA were relativized using a z-score based on the means and standard deviations of all eastern white pine measured in the pretreatment year ($n=275$), calculated as:

$$z = \frac{(X_i - \bar{X})}{\sigma} \times \pm 1$$

Where $X_i$ is a single measurement value, $\bar{X}$ is the sample mean, and $\sigma$ is the sample standard deviation. An adjustment factor ($\pm 1$) is applied to the z-score based on the relationship with the response variable to WPND severity, such that a negative z-score will correspond to a tree that is healthier than the mean, while a positive value indicates more diseased. The z-score has been
demonstrated to be well suited for generating a composite index value that can be used to quantify overall tree health and is especially useful for monitoring changes in tree health over time (Hanavan et al., 2015; Pontius and Hallett, 2014). The mean z-score of all response variables was plotted as a supplemental variable within the PCA, but not factored into the analysis. For assessing individual tree response to thinning we calculated an overall health index score \( z' \) using all six of the measured response variables and weighted each according to their respective eigenvector derived from the first PCA axis (PC1), explaining the largest proportion of variance within the dataset. Since the incidence and severity of WPND is of primary interest to this study, WPND severity was included as an unweighted component of \( z' \).

4.2.5 Analysis of thinning effects

To evaluate the effectiveness of the thinning trials on overall tree health in subsequent years of the treatment, we calculated the difference in \( z' \) in the two years following the thinnings \( (\Delta z') \). We ran a mixed effects model identifying treatments and time post-thinning as fixed effects while including site and blocks as random effects with the model, given as:

\[
\Delta z' = \text{Treatment} + \text{Time} + \text{Treatment} \times \text{Time} + \text{Site} \,(r) + \text{Block}(r)
\]

Where treatment refers to the HD, LD, and control plots, time indicates the response year post-treatment, site differentiates the two study stands (OSP, FOX), and block applies to the four treatment groupings between the two stands. All mixed model analyses and PCA were conducted in JMP Pro 13 (SAS Institute Inc., Carry, NC).

4.3 Results

4.3.1 Impacts of WPND on tree health
We found ocular ratings of WPND severity within infected crowns to be negatively correlated with all measured response variables based on the initial conditions within the stands (Table 4.3). Discrete differences between severity classes were also found to be significant in post hoc analysis of DBH, LCR, and crown diameter (Fig. 4.2). There were no significant differences among the measured response variables between sites, although DBH was found to be marginally significant (p = 0.052), in which the mean DBH at OSP was 11.6 cm (33%) greater than at FOX. The random effects of block and plot were not considered significant for any of the measured response variables, while the interaction between WPND severity and site was significant for light exposure and crown diameter (Table 4.3).

Table 4.3 Summary of mixed effects models testing sources of variation on the six tree response variables of interest prior to stand thinning. The severity source consists of four ocular classes of WPND symptom (chlorosis and defoliation) severity: healthy, mild, moderate, and severe.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SV</th>
<th>ndf</th>
<th>ddf</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBH</td>
<td>Severity</td>
<td>3</td>
<td>261.7</td>
<td>17.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Site</td>
<td>1</td>
<td>2.9</td>
<td>10.2</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Severity x Site</td>
<td>3</td>
<td>261.7</td>
<td>1.9</td>
<td>0.133</td>
<td></td>
</tr>
<tr>
<td>LCR</td>
<td>Severity</td>
<td>3</td>
<td>260.1</td>
<td>24.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Site</td>
<td>1</td>
<td>2.3</td>
<td>3.9</td>
<td>0.170</td>
<td></td>
</tr>
<tr>
<td>Severity x Site</td>
<td>3</td>
<td>260.1</td>
<td>2.0</td>
<td>0.109</td>
<td></td>
</tr>
<tr>
<td>Crown diameter</td>
<td>Severity</td>
<td>3</td>
<td>261.3</td>
<td>22.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Site</td>
<td>1</td>
<td>2.3</td>
<td>8.6</td>
<td>0.086</td>
<td></td>
</tr>
<tr>
<td>Severity x Site</td>
<td>3</td>
<td>261.3</td>
<td>5.4</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Light exposure</td>
<td>Severity</td>
<td>3</td>
<td>263.6</td>
<td>12.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Site</td>
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<td>2.6</td>
<td>0.3</td>
<td>0.656</td>
<td></td>
</tr>
<tr>
<td>Severity x Site</td>
<td>3</td>
<td>263.6</td>
<td>2.6</td>
<td>0.051</td>
<td></td>
</tr>
<tr>
<td>Transparency</td>
<td>Severity</td>
<td>3</td>
<td>259.3</td>
<td>120.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Site</td>
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<td>3.0</td>
<td>0.1</td>
<td>0.793</td>
<td></td>
</tr>
<tr>
<td>Severity x Site</td>
<td>3</td>
<td>259.3</td>
<td>0.5</td>
<td>0.667</td>
<td></td>
</tr>
<tr>
<td>Dieback</td>
<td>Severity</td>
<td>3</td>
<td>147.1</td>
<td>54.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Site</td>
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<td>23.8</td>
<td>1.0</td>
<td>0.335</td>
<td></td>
</tr>
<tr>
<td>Severity x Site</td>
<td>3</td>
<td>147.1</td>
<td>1.0</td>
<td>0.376</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4.2 Post hoc analysis (Tukey HSD) of LCR, crown diameter, and DBH as a function of WPND severity class. Values with the same letter are not significantly different between severity classes ($\alpha=0.05$).
Principal components analysis of z-score relativized traits explained 71.8% of the total variation in the dataset within the first two axes, with PC1 alone accounting for 49.7% of the total (Fig. 4.3). Eigenvectors corresponding to the response variables were found to be nearly bisected by the PC1 axis, such that loading of DBH, LCR, crown diameter, and light exposure were highly correlated with each other, while dieback and transparency were more correlated with the numerical ratings of crown health. Including the mean z-score of all parameters as a supplemental variable indicated a loading parallel with PC1, approximately bisecting the eigenvectors and indicating that the loading scores of PC1 are a strong predictor for an overall health index score. Trees within the PCA were also coded according to four observed WPND severity classes (healthy, mild, moderate, severe). This revealed discrete groupings between severity classes, with considerable overlap between trees of a mild and moderate degree of severity, but with no overlap between healthy trees and the most severely affected individuals. The separation of these groupings occurred primarily along PC1, further supporting the use of the corresponding eigenvectors for weighting the respective response variables. Parameters used for calculating trait z-scores and corresponding PC1 eigenvectors are shown in Table 4.4.
Fig. 4.3 Principal component analysis of the seven z-score relativized traits grouped according to WPND severity in two dimensions. The mean z-score eigenvector is plotted as a supplemental variable, but not factored into in the analysis.

Table 4.4 Variable mean, standard deviation (n=275), and adjustment used in calculating individual trees z-scores. PCA 1 indicates the factor loadings for each variable used in calculating $z'$, but was not applied to the WPND severity parameter.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Z-score adjustment</th>
<th>PCA 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBH (cm)</td>
<td>40.8</td>
<td>13.3</td>
<td>-1</td>
<td>0.408</td>
</tr>
<tr>
<td>Light exposure (1-5)</td>
<td>1.4</td>
<td>0.6</td>
<td>-1</td>
<td>0.263</td>
</tr>
<tr>
<td>WPND severity (0-3)</td>
<td>1.5</td>
<td>0.7</td>
<td>1</td>
<td>0.405</td>
</tr>
<tr>
<td>Dieback (%)</td>
<td>11.8</td>
<td>6.0</td>
<td>1</td>
<td>0.364</td>
</tr>
<tr>
<td>Transparency (%)</td>
<td>25.8</td>
<td>11.8</td>
<td>1</td>
<td>0.363</td>
</tr>
<tr>
<td>LCR (m m$^{-1}$)</td>
<td>0.24</td>
<td>0.08</td>
<td>-1</td>
<td>0.399</td>
</tr>
<tr>
<td>Crown diameter (m)</td>
<td>4.4</td>
<td>2.3</td>
<td>-1</td>
<td>0.420</td>
</tr>
</tbody>
</table>
The overall tree health index score, \( z' \), was found to be effective at differentiating between WPND severity class based on the pre-treatment data (Fig. 4.4). The difference in \( z' \) is significant between each severity class (\( p < 0.05 \)).

*Fig. 4.4* Mean health index score (\( z' \)) as a function of WPND severity class. Positive \( z' \) represents trees that are more diseased than the average, while negative values are healthier.

### 4.3.2 Response to thinning treatments

Thinning treatments were found to have a significant effect on \( \Delta z' \) in both years following tree removal (Fig. 4.5). The \( \Delta z' \) in the second year of the study was reduced by 12 and 50% for the high-density and low-density treatments respectively, while the \( \Delta z' \) of control plots increased over this period by 110% relative to pre-thinning measurements of \( z' \). We detected no significant difference in the \( \Delta z' \) between the HD and LD treatments for either of the post-thinning years (*Table 4.5*), however the mean \( \Delta z' \) of the LD treatment was 46 and 38% lower than the HD treatment in years 2016 and 2017, respectively. There were no significant
differences in mean DBH between treatment plots post-thinning in 2016, suggesting that there was no initial selection bias for larger (or smaller) trees in the HD and LD treatments that may have influenced size-related covariates associated with $z'$. Additionally, relative basal area increment did not differ between any treatments in the two years following thinning; therefore, changes in $z'$ do not appear to be driven by a rapid positive growth response to thinning. Crown light exposure was a component of $z'$ that experienced an immediate increase following thinning in the HD and LD treatments; however, this effect was somewhat muted in the calculation of $z'$ since it was the lowest weighted variable according to the PC1 eigenvector and therefore did not contribute as much as other variables that are more relevant to overall tree vigor (Table 4.4). Analysis of changes in WPND severity over time by treatment indicated a reduction in severity in the summer of 2017 for both LD and HD thinning treatments, however, differences in recovery from WPND were only significant when comparing trees in the LD treatment with control plots ($p = 0.006$, Fig. 4.6).

**Table 4.5** Summary statistics of the mixed effect model testing sources of variation on $\Delta z'$ in the years following thinning treatments.

<table>
<thead>
<tr>
<th>Source</th>
<th>ndf</th>
<th>ddf</th>
<th>F ratio</th>
<th>P value</th>
<th>Wald P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>338.3</td>
<td>34.8</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>336.9</td>
<td>0.3</td>
<td>0.560</td>
<td></td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>2</td>
<td>336.9</td>
<td>2.0</td>
<td>0.143</td>
<td></td>
</tr>
<tr>
<td>Site (r)</td>
<td></td>
<td></td>
<td></td>
<td>0.488</td>
<td></td>
</tr>
<tr>
<td>Block (r)</td>
<td></td>
<td></td>
<td></td>
<td>0.679</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 4.5 Changes in \( z' \) in the first two years of thinning treatments. Horizontal line within the box indicates the median, boundaries of the box indicate 25th and 75th percentile of the four treatment blocks. Dashed line at zero indicates no change from the pre-treatment year, positive values of \( \Delta z' \) indicate trees are more stressed on average, negative values are healthier.

Fig. 4.6 Differences in WPND severity between treatments for each year of the study. Values represent block means and standard error. Values followed by the same letter are not significantly different within a given year (\( \alpha=0.05 \)).
4.4 Discussion

In this study, our goals were to quantify the impacts of WPND on traits associated with tree health and to test whether thinning of infected mature stands can be implemented as a silvicultural practice to mitigate damage caused by foliar pathogens (WPND) of white pine. Differentiating WPND severity classifications using ocular ratings of crown condition, we found strong evidence that crown transparency, dieback, DBH, live crown ratio, and crown diameter were all negatively affected by repeated annual defoliations. Additionally, trees with lower initial light exposure and those in the intermediate and overtopped canopy strata were found to have the highest degree of WPND severity, likely both predisposing factors that facilitated infection via rain-splash dispersed spores. Measurements of DBH are often used as a dependent variable in allometric equations of traits such as LCR and crown diameter, therefore, we may expect some degree of correlation with DBH and other traits included in calculating \( z' \) in this study even though direct assessment, rather than allometric approaches, was used (Bechtold, 2003; Ducey, 2009; Rijal et al., 2012). For example, based on pretreatment inventory data of 275 trees we found significant simple linear relationships with DBH to LCR \((r^2 = 0.37, p < 0.001)\) and mean crown diameter \((r^2 = 0.68, p < 0.001)\). The mean uncompacted LCR of 0.24 measured in this study is markedly lower than the mean value of 0.43 reported from growth and yield plots in central and southern New Hampshire measured prior to WPND outbreaks in the region (Jordan and Ducey, 2007), and the mean value of 0.41 reported by Forest Inventory and Analysis Program for the state of New Hampshire between 2011-2016 (Forest Inventory and Analysis Database 2017). We argue that despite the potential redundancy of including multiple relativized tree size related metrics in \( z' \), LCR and crown diameter can be uniquely impacted by
defoliations, resulting in measurements that stray from allometric model predictions based in part on DBH. Specifically, defoliations of the lower portions of crowns can rapidly reduce LCR, and dieback of the lowermost (i.e. largest) branches can cause a significant reduction in crown diameter following multiple years of severe needle loss, during which time DBH will of course not get smaller, though annual increment will likely decline as increment growth has been shown to be linearly related to leaf area in eastern white pine (Innes et al., 2005). Consequently, tree DBH can be considered both a predisposing factor and a responsive variable, since smaller trees appear to be more susceptible to WPND infection and high-severity trees have been shown to exhibit growth declines in the years following infection (McIntire et al. 2018). Using z-scores based on the pre-treatment inventory data, growth response in the years following thinnings is relativized to the initial stand conditions. We used a total of seven variables in developing our composite health score index, \( z' \), which was found to be a strong indicator of WPND severity based on initial stand conditions (Fig. 4). Collecting each of the seven variables on trees within this study was found to be time intensive in the field, requiring 10-15 min per tree to obtain all measurements during the initial inventory. Thus, from a white pine management perspective, if the goal is to develop a large-scale network of WPND monitoring, it may be advisable to reduce the number of traits measured in the field to save time and cost as a trade-off for increasing the potential sampling area. Z-scores have been used previously in forest health applications, specifically in monitoring declining hemlock impacted by *Adelges tsugae* Annand, in which z-scores were shown to be useful for differentiating severity classifications using remote sensing data (Hanavan et al., 2015). The z-score developed in this study differs from previous studies in that the constituted variables are weighted according to PCA eigenvectors of the first component axis. Thus, we do not assume that all measured variables should contribute equally within the z-
score, such that variables that are thought to be more relevant to disease response are given priority. Similarly, correlation coefficients derived from the relationship with a single predictor variable, such as disease severity, could also be used to provide weight to individual variables within a z-score.

Not surprisingly, we did not detect a treatment effect on diameter growth increment in the first two years following thinning. Other thinning trials within mature white pine stands did not detect positive growth response to thinning until at least 3-years post-treatment (Bebber et al., 2004; Burgess and Wetzel, 2000). Since the stands selected for thinning in this study have been experiencing defoliation prior to silvicultural treatments, we expected to see an increase in tree vigor in response to reducing stocking densities. In thinning trials of oak (Quercus spp.) subject to defoliation by gypsy moth (Lymantria dispar L.), thinning was not found to have an immediate positive impact on volume growth because it was conducted simultaneously with the first year of an outbreak (Fajvan et al., 2008). As WPND has been present in the northeastern US since at least 2010 and most sampled white pine stands have been found to be symptomatic for associated fungal pathogens, we expect that overstocked stands would generally respond well to thinning within the first two years and should not induce any additional stress or growth decline.

We did measure significant reductions in WPND severity in the second year within the thinnings treatments, where mean severity was significantly lower in the LD plots relative to the control plots, which have increased since 2015 (Fig. 4.6). This one-year lag between treatment and a measurable reduction in WPND severity was expected, since associated fungal pathogens require one-year to mature after colonizing the partially elongated first-year needles and tree removal took place after spore dispersal in the 2015 growing season. We did not detect a difference between the HD and LD treatments in the first two years based on $\Delta z'$, suggesting that
a traditional pre-commercial thinning (stocking guide B-line) is adequate for reducing pathogen pressure while retaining a greater number of residual trees per hectare (Table 4.5, Fig. 4.5). However, response to WPND and growth may become more relevant to differences in stocking densities on an extended time scale. Longer term studies are needed to understand the impact of this emerging disease complex on forest growth, dynamics, and responses to thinning treatments.

4.5 Conclusion and recommendations

Thinning stands of eastern white pine impacted by defoliating pathogens has shown positive results based on early research. Increasing the distance between crowns and enhancing crown light exposure likely play a key role in reducing pathogen pressure through limiting dispersal of fungal inoculum (Wyka et al., 2017b). Infected trees within thinned plots were shown to have reduced WPND severity by the second year of the study, while individuals within control plots increased in severity over the same period. We also found that larger diameter trees exhibited a lower initial severity of WPND symptoms and were generally more vigorous (lower $z'$) overall, as determined by our composite health index. This finding is consistent with WPND-induced growth declines in the northeastern US, where smaller diameter trees tended to experience the greatest reduction in post-outbreak basal area increment (McIntire et al. 2018). Stand conditions which facilitate an improved growth rate may allow for individuals that are more resilient to fungal infection and defoliation. Thus, maintaining appropriate stocking densities for eastern white pine may also be viewed as a preventative measure that may benefit at-risk stands.


Dybzinski, R., Farror, C., Wolf, A., Reich, P.B., Pacala, S.W., 2011. Evolutionarily stable


134


Soil Inorganic N Leaching in Edges of Different Forest Types Subject to High N Deposition Loads. Ecosystems 14, 818–834.


