Climate Interactions Drive Tree Physiology and Growth in a Northeastern Forest Ecotone

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CLIMATE INTERACTIONS DRIVE TREE PHYSIOLOGY AND GROWTH IN A
NORTHEASTERN FOREST ECOTONE

By
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B.S. Biology and Psychology, Virginia Commonwealth University, 2019

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Climate change is a threat to global forest ecosystems. In the northeastern United States, forest trees are facing rising temperatures and increasingly inconsistent moisture regimes. In addition to long-term changes in climate conditions, there is concern about the potential for more frequent and intense climate extremes, which can have severe and rapid negative effects on tree physiology and growth. Further, climate extremes may co-occur to produce a greater magnitude of effect than the sum of their parts, with a prominent example being hot droughts, which are increasing in occurrence and severity. The impact of these and other extreme climate interactions may be especially large at range margins, where trees may already experience climate conditions near their tolerance limits. A region of note is the temperate-boreal ecotone, where temperate trees near the northern limit of their range interact with cold-adapted species with a more northerly range. Because of the rapidly changing climate conditions in this region, it is critical to understand the mechanisms of how these trees respond to current and future climate conditions to better predict how they will respond to future climate extremes.
In this study, we used a greenhouse experiment to examine the effects of climate extremes on physiology and growth of seedlings, as well as a field study to assess how daily climate conditions drive the probability of daily growth ($P_D$) for canopy trees. In the greenhouse experiment, we implemented a full factorial design of three levels of drought (short, long, and fully irrigated), two levels of temperature (2-week heat wave and ambient), and two levels of planting treatment (planted alone, and planted with an associate) on seedlings of red spruce ($Picea rubens$), northern red oak ($Quercus rubra$), and paper birch ($Betula papyrifera$). We measured growth and vigor in addition to metrics of physiological stress including midday leaf water potential and net photosynthesis on all seedlings. In the field study of forest trees, we installed point dendrometers on 10 mature trees of each species of red maple ($Acer rubrum$), red spruce, and eastern hemlock ($Tsuga canadensis$) at the Howland Research Forest in central Maine. We developed species level models using environmental conditions to predict $P_D$.

In the greenhouse experiment, we found that the long drought significantly reduced growth of red spruce and paper birch, while red oak growth was relatively resistant to drought stress. The moderate heat wave treatment and interaction of heat wave with drought did not have a significant effect on growth or vigor of any species, although photosynthesis declined more rapidly with increasing temperature under conditions of low moisture for red spruce and red oak. We found that total diameter growth was significantly lower in red spruce and paper birch seedlings that experienced turgor loss during the drought. In the observational field study, each species showed responses to unique combinations of climate conditions, with red maple being the least sensitive to daily climate conditions, red spruce showing a lower $P_D$ during days with high vapor pressure deficit (VPD) and long days with low soil moisture, and hemlock showing a lower $P_D$ on days of high VPD only when soil moisture was low. Combined, these studies
demonstrate the importance of climate interactions on physiological responses and growth, suggesting that longer term correlations between climate on growth do not capture the complexities of the physiological response to climate conditions. Predictions for tree responses to future climate change should continue to explore the important effects of interacting climate conditions that can drive physiology and growth in trees of this climate-sensitive region.
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<table>
<thead>
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<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>$A_{\text{net}}$, $A_{\text{max}}$</td>
<td>Net light-adapted photosynthesis, maximum photosynthesis ($\mu$mol CO$_2$ m$^{-2}$s$^{-1}$)</td>
</tr>
<tr>
<td>BAI</td>
<td>Basal area increment (cm$^2$)</td>
</tr>
<tr>
<td>DBH</td>
<td>Diameter at breast height (cm)</td>
</tr>
<tr>
<td>DOY</td>
<td>Day of Year</td>
</tr>
<tr>
<td>$F_v/F_m$</td>
<td>Ratio of variable fluorescence maximum fluorescence; measure of quantum efficiency of Photosystem II centers</td>
</tr>
<tr>
<td>EOS</td>
<td>End-of-season</td>
</tr>
<tr>
<td>EOT</td>
<td>End-of-treatment</td>
</tr>
<tr>
<td>$g_{\text{min}}$</td>
<td>Minimum epidermal (cuticular) conductance</td>
</tr>
<tr>
<td>$g_s$</td>
<td>Stomatal conductance to water vapor (mol H$_2$O m$^{-2}$s$^{-1}$)</td>
</tr>
<tr>
<td>$g_{s90}$</td>
<td>Leaf water potential at 90% stomatal closure</td>
</tr>
<tr>
<td>SOS</td>
<td>Start-of-season</td>
</tr>
<tr>
<td>$T_{\text{avg}}$</td>
<td>Average temperature ($^\circ$C)</td>
</tr>
<tr>
<td>TCP</td>
<td>Thermocouple psychrometer</td>
</tr>
<tr>
<td>TLP</td>
<td>Turgor loss point (MPa)</td>
</tr>
<tr>
<td>VPD, $VPD_{\text{max}}$</td>
<td>Vapor pressure deficit, maximum vapor pressure deficit (kPa)</td>
</tr>
<tr>
<td>VWC</td>
<td>Volumetric water content (% or proportion)</td>
</tr>
<tr>
<td>$\Psi$, $\Psi_{\text{leaf}}$</td>
<td>Water potential (MPa), leaf water potential</td>
</tr>
</tbody>
</table>
CHAPTER 1. DIFFERENTIAL SENSITIVITY OF THREE NORTHEASTERN TREE SPECIES TO COMPOUNDED CLIMATE EXTREMES

1.1 Abstract

Climate change is raising concerns about how forests in the northeastern United States will respond to extreme droughts, heatwaves, and their co-occurrence. Climate extremes are especially important in ecosystem transition areas, like central Maine, where tree species composition may change rapidly with climate change. In this study, we tested how carbon and water relations drive tree seedling growth during extreme drought, heat, and combined drought and heat. We focused on red spruce (Picea rubens Sarg.), a sub-boreal species, and how its response to these stressors differs from and depends on the presence of a common associate (paper birch, Betula papyrifera Marsh.) and a species expected to increase abundance in this region with climate change (northern red oak, Quercus rubra L.). We planted 216 two- to three-year-old seedlings of red spruce, northern red oak, and paper birch in containers either alone or with another red spruce (planting treatment). By controlling water availability, we tested three levels of drought (irrigated, 15-day “short”, and 30-day “long”) crossed with two levels of temperature (ambient and heat; +3.5 °C average daily maximum temperature with passive warming chambers). We measured growth and vigor (as percent brown foliage), gas exchange, and midday leaf water potential ($\Psi_{\text{leaf}}$) across the three-month study period. We also measured stomatal responses to drought and heat, minimum epidermal conductance, and the turgor loss point (TLP) of each species. Overall, we found dominant effects of the drought reducing both physiology and growth of spruce and birch, but that oak showed considerable resistance to the drought stress. Spruce and birch both experienced declines in growth when $\Psi_{\text{leaf}}$ exceeded their
TLP, but oak had a lower (more resistant) TLP and was able to avoid growth declines even when it did experience some turgor loss. Despite all species experiencing declines in photosynthesis with higher temperatures that were even stronger when compounded by drought, the moderate heatwaves did not impact growth. Further, in this first year of the study, planting treatment had a relatively minor effect on growth responses; however, that may change as the study continues into the recovery year. This study supports other work that suggests that native species to this region like red spruce and paper birch are susceptible to future climate extremes that may favor other species like northern red oak. Furthermore, this work suggests that more extreme and prolonged heatwaves in the northeastern US, particularly when combined with drought, may impact seedling growth and vigor to change tree community dynamics.

1.2 Introduction

Climate change extremes such as drought and heat waves are not only becoming more common, but increasingly co-occur to produce compounded effects that threaten forest ecosystems (Howarth et al., 2019; Trisurat et al., 2022). The compounded effects of these extreme “hot droughts,” during which above-average temperatures may increase evaporative demand and subsequent soil water deficit (Choat et al., 2018), often drive rapid declines in physiology and growth of forest trees (Rehschuh & Ruehr, 2022; Teskey et al., 2015). The effects of these compounded climate extremes on forest community composition may be strongest in transition zones (ecotones) where many species are already limited by climate conditions (Ettinger & HilleRisLambers, 2013; Heilman et al., 2021). For example, cold-adapted species at their southern range margin may be particularly vulnerable to hot drought, whereas temperate species at their northern range margin may see an increase in suitable habitat as temperatures warm (Reich et al., 2022). These shifts in habitat suitability and subsequent die-back or migration may
drive novel species assemblages and thus the need for trees to adapt to new associative neighborhoods (Hobbs et al., 2018; Janowiak et al., 2018). While much literature on climate change focuses on shifts in average conditions, there is a growing understanding that discrete climate extremes may be the largest drivers of change. Combined with a focus on individual drivers (i.e., only heat or drought), climate change predictions based on yearly or decadal averages may vastly underestimate the likelihood and rate of climate driven forest change (Jackson et al., 2009; Kitzberger et al., 2022). Therefore, there is a growing need to study the impacts of compounded climate extremes on current species assemblages in addition to novel community assemblages under potential habitat shifts.

Drought may be defined environmentally as a quantifiable decrease in precipitation and soil water availability (Trenberth et al., 2013), or physiologically in plants as water stress leading to increased tension in the water column of the xylem (Choat et al., 2018). Ultimately, the key factor that determines if a tree will survive a drought is the extent of embolism formation and spread in the xylem (Adams et al., 2017; Hammond et al., 2019). Therefore, many trees have adaptations that allow them to limit excessive water loss (and declines in water potential) during drought and even maintain cell turgor during moderate drought (Choat et al., 2018; Zhang et al., 2013). As drought progresses, the water potential progressively declines. To avoid excessively low water potentials, most trees close their stomata at water potentials above the turgor loss point (the water potential at which the leaves wilt) (Bartlett, Scoffoni, & Sack, 2012). In a moderate or short drought, stomatal closure may be enough to avoid turgor loss and allow for recovery after water becomes available again (Hammond et al., 2019; McGregor et al., 2021). However, if the drought continues, the degree to which the plant can limit water loss through the cuticle and leaky stomata may become an important factor determining the rate at which water potential
continues to decline eventually driving turgor loss and, in extreme cases, xylem embolism formation and spread (Barnard & Bauerle, 2013; Duursma et al., 2019).

The majority of experimental evidence suggests that most trees prioritize avoiding hydraulic failure and mortality through stomatal closure but at the expense of carbon gain (Choat et al., 2018; D’Orangeville et al., 2018; Kannenberg, Novick, & Phillips, 2019). This leads to two prevailing mechanisms by which a non-lethal drought can reduce tree growth (Körner, 2003). Long-term or sustained stomatal closure can reduce photosynthesis and may eventually lead to a reduction in carbon available for growth (often termed a ‘source’ limitation on growth). On shorter timescales, drought results in lower water potentials that may limit turgor-driven expansion of cells during growth despite no carbon limitation (‘sink’ limitation) (Körner, 2015). While carbon assimilation is necessary for tree growth, the deciding factors in growth of trees tend to be ‘sink’ mechanisms, by which water availability and temperature limit turgor pressure in developing cells (Cabon et al., 2022; Cabon & Anderegg, 2022; Peters et al., 2021). Although separating source and sink limitations can be challenging experimentally, it is important to consider how these different mechanisms may be driving the broadscale patterns in changing growth rates, particularly for seedlings and saplings of trees that can shift associative dynamics in forests.

In contrast to drought, extreme heat can have very different effects on trees. Increasing temperatures can increase metabolism (including respiration rates), lengthen growing seasons, stimulate growth processes, and reduce the likelihood of cold limiting factors (Huang et al., 2020; Saxe et al., 2001; Vaughn et al., 2021). However, as warming drives extreme heat waves during the growing season, the likelihood of surpassing critical physiological thresholds increases (Crous et al., 2022). The primary mechanism of extreme heat-driven mortality seems to
be heat damage to key structures involved in photochemistry (Guha et al., 2018; Rehschuh & Ruehr, 2022). However, the temperatures required to damage photochemical structures are often very high (>40 °C, Kunert et al., 2022). It is important to note that the physiological effects of heat on seedlings may vary with both the magnitude and the duration of temperature increase, and that a singular extreme heat wave, defined as a prolonged period of consecutive days with significantly above-normal temperatures (Teskey et al., 2015), may have more pronounced effects on seedling growth than increased baseline temperatures over the long term (Bauweraerts et al., 2013; Colombo & Timmer, 1992). Still, more moderate heat waves may impose secondary effects on physiology, with increasing temperatures and higher vapor pressure deficits driving stomatal closure and ultimately increasing the risk of embolism in the xylem and potential hydraulic failure (Day, 2000; Ruehr et al., 2016; Schönbeck et al., 2022). The complex and often conflicting responses of warm-adapted and cold-adapted seedlings to elevated temperature should be studied further in order to better predict how tree communities, especially those at sensitive range margins, will respond to impending climate extremes.

Regardless of the hypothesized mechanisms, at forest transition zones, moderate heat waves may favor temperature-limited species at their northern range margin but also drive small but sustained declines in species at their southern range margin. Evidence on the effects of extreme heat events on tree species in forest ecotones is abundant but inconclusive (Teskey et al., 2015). For instance, some cold-adapted species at southern range margins show increased seedling growth under experimental heat wave conditions, while others show growth declines; warm-adapted species at their northern ranges tend to more consistently benefit from increased temperatures (Reich et al., 2022; Vaughn et al., 2021; Wright et al., 2018). In some cases, these responses contradict projections of significant habitat decline under warming scenarios (Iverson
et al., 2008; Janowiak et al., 2018). Furthermore, the threat of drought and heat co-occurring more often in the future (Fernandez et al., 2020) drives the need for a better understanding of how water deficits will compound with extreme heat events to produce unique effects in tree seedlings (Adams et al., 2009).

A lot of work has focused on separate effects of drought and heat, despite many of the worst forest mortality events in recent years having resulted from co-occurring extreme drought and heat (Mitchell et al., 2014; Rennenberg et al., 2006; Teskey et al., 2015). Under conditions of extreme heat, well-watered plants are able to prevent tissue damage with evaporative cooling, by which the transpiration of water from open stomata lowers the temperature of the leaves (Ruehr et al., 2016). However, in times of hot drought, stomatal closure to prevent water loss also prevents cooling, and greater declines in physiological function may occur as a result of the compounded effects of low water potential and extreme heat (Rehschuh & Ruehr, 2022). Impacts of warming have been shown repeatedly to be more extreme under reduced water availability, particularly in species adapted to cool and moist climates (Bauweraerts et al., 2014; Reich et al., 2018; Ruehr et al., 2016; Vaughn et al., 2021). More recently, studies are beginning to recognize the importance of examining compounded stressors performed on seedlings planted together to simulate the community interactions that exist in forest ecosystems (Didion-Gency et al., 2022). Collectively, this work suggests that it is critical to examine the effects of compounded climate extremes on current and future species assemblages at sensitive range margins.

Compounded heat and drought stress is projected to increase in many forests of the world including the Acadian Forest of Maine, which contains a large number of species at both their southern and northern range margin (Krakauer et al., 2019). The Acadian Forest is historically cool and moist, and characterized by many northerly-distributed species including red spruce
(Picea rubens Sarg.) near the southern margin of its range, its common associate paper birch (Betula papyrifera Marsh.), and northern red oak (Quercus rubra L.) near the northern limit of its range. Climate change is expected to increase the likelihood that red spruce and red oak may co-occur as suitable habitats shift. Regional predictions for increasingly variable moisture availability and warmer temperatures in this historically cool and moist region warrant investigation of extreme climate effects on forests in this under-studied and rapidly changing environment (Fernandez et al., 2020).

In this study, we tested how the physiology and growth of red spruce, paper birch, and red oak seedlings respond to climate extremes of drought, heat, and compounded drought and heat. We used red spruce, a sub-boreal species that is a defining feature of Acadian Forests, as a focal species and examined its physiological and growth responses to climate extremes alongside paper birch, a common associate, and northern red oak, a species which may associate more commonly with red spruce in the future. Because the regeneration and spread of a species hinge on early life stages when trees are arguably most vulnerable, we assessed the effects of climate extremes on seedlings. In order to simulate current and novel species assemblages, we planted each species on its own as well as in pairs with red spruce. Our objective in this study was to determine how water use and carbon sequestration predict the growth of these seedlings during extreme drought, heat, and the combination of the two, under intra- and interspecific associations.
1.3 Materials and Methods

1.3.1 Experimental Design

In May 2022, we planted 216, 2–3-year-old bare-root seedlings of red spruce, northern red oak (hereafter “red oak”), and paper birch in an 11× 6 m high-tunnel hoop house (van Kampen et al., 2022) on the University of Maine campus in Orono, Maine (44° 54' 5.3" N, -68° 39' 37.2" W, Plant Hardiness Zone 5b). Seedlings (120 red spruce, 48 red oak, 48 paper birch) were obtained from the New Hampshire State Forest Nursery in Boscawen, New Hampshire and were 11 to 55 cm tall at time of planting (Table A.1). The roof of the hoop house was covered in a shade cloth rated for 30% attenuation of ambient sunlight and temperature and humidity were logged every 2 hours with dataloggers (DS1923-F5# Hygrochron, iButtonLink LLC, Whitewater, WI) following van Kampen et al. (2022). The datalogger was shielded inside an opaque plastic cup with holes to allow for airflow, with a second opaque shield mounted above it to reduce direct solar radiation.

To assess how the potential for intra- and interspecific associations may impact our focal species red spruce, 24 seedlings of each species were planted singly in 1-liter containers and another 24 seedlings of each species were planted with a red spruce seedling in 2-L containers (Fig. 1.1). This planting pattern resulted in six unique treatment levels for “planting treatment”: each of the three species planted individually and each species planted with a red spruce. Planting media was two parts growing mix composed of sphagnum peat, coarse perlite, and medium vermiculite (Jolly Gardener Pro-Line C/25, Oldcastle) to one part additional coarse perlite, with a medium application of fertilizer (six g L⁻¹ Osmocote® 5-6 mo. Patterned Release fertilizer, 15-9-12).
Prior to planting, tap roots of less than 30% of the red oak seedlings that did not fit into planting containers were clipped (removing <25% of the root mass).

Figure 1. Schematic representation of greenhouse experimental design, with four experimental blocks split into two temperature treatments: heat wave and ambient half-blocks. Within each half-block, we planted red spruce, paper birch, and red oak seedlings either singly in 1-L containers or with another red spruce ("double") in 2-L containers. There were three replicates of each species and planting treatment that were then assigned to either a short drought, long drought, or control treatment (see Methods for details). The treatments in the half-block are shown here as being arranged according to factorial design, but arrangement of drought treatments in the greenhouse was randomized within each half-block with placement alternating between single and double planting treatment containers (Fig. A.1A).

Seedlings were randomly sorted into four experimental blocks, each split into two identical half-blocks (Figure 1.1). Each half-block contained three replicates of each planting treatment (as
described above) that was assigned to one of three drought treatments: full irrigation, a short drought treatment, or a long drought treatment. Within each block, one half-block was randomly assigned to a heat wave treatment and the other an ambient temperature treatment (Fig. A.1A). Application of drought and heat treatments is described in more detail in the next section.

After planting, all seedlings were given an eight-week acclimation period (DOY 125-181; May 5 – June 30, 2022) during which all were kept watered to 10-30% volumetric water content (VWC, %) of planting media with an automated drip irrigation system and subjected to ambient temperatures within the greenhouse. We had initially planted eight experimental blocks; however, following leaf out, we discovered that 34 out of the 100 birch were actually gray birch (B. populifolia; n = 33) or yellow birch (B. alleghaniensis; n = 1). Combined with some mortality, excluding these trees limited us to four complete blocks with 34 extra seedlings across all species available for additional measurements without disturbing the treatment blocks. After the acclimation period, any seedlings determined to be dead or of poor vigor due to browning or foliage loss (~1% of trees) were removed from the experimental group and replaced with extra seedlings.

1.3.2 Drought and Heat Wave Treatments

Our treatment design included a full factorial design of the six planting treatments (described above) crossed with three levels of drought (irrigated, short, and long), also crossed with two levels of heat (ambient and heat wave). The drought treatments both started on the same day and extended for 15 (short drought) or 30 (long drought) days (Fig. 1.1). Short and long drought treatments were initiated on July 1, 2022 (DOY 182) by reducing irrigation from control levels (50% of container volume in water every two days) to 25% of their container volume in water
every four days for the 15-day duration of the short drought. At the conclusion of the short drought (DOY 196; July 15), seedlings assigned to the short drought treatment were returned to control conditions. Seedlings assigned to the long drought treatment continued their treatment for an additional 15 days, but only received 25% of their container volume in water once (DOY 206; July 25) during this second 15-day period before returning to control levels on DOY 214 (August 2). VWC of the planting media in all containers was measured at least once per week during the experimental period and at the end of each treatment, using a 12-cm probe inserted vertically into two locations at opposite sides of the container (HSII Handheld Soil Moisture Sensor, Campbell Scientific, Logan, UT).

On July 8, 2022 (DOY 189), halfway through the short drought period, a heat treatment was initiated on one half-block per block by installing rectangular passive radiation heat chambers made of PVC frames covered on all sides with 0.7 mm thick clear polyethylene sheeting (Fig. A.1A). In order to allow for some air circulation within the chamber and limit moisture accumulation, an approximately 0.6 m × 0.07 m rectangular slit was cut in the top panel of each chamber. Each half-block not assigned to the heat treatment was covered in an open-sided PVC frame with a polyethylene top panel identical to those on the heat chambers in order to control for light attenuation by the polyethylene. In order to measure climate conditions within the chambers, a temperature and humidity datalogger (DS1923-F5# Hygrochron, iButtonLink LLC, Whitewater, WI) was suspended in all eight half-blocks at foliage level (~0.43 m above the ground, Fig. A.1B) and programmed to record temperature and humidity conditions every two hours. The datalogger was contained in an opaque plastic shield with holes drilled at regular intervals to allow for airflow. At the end of the heat wave period (DOY 206; July 25), the polyethylene was removed from the sides of all heat chambers so that all seedlings were in
ambient greenhouse conditions. The top panels remained on all chambers until they were fully disassembled following final growth measurements on September 30, 2022.

1.3.3 Growth and Vigor

In order to assess the effects of planting, drought, and heat treatments on the growth and vigor of seedlings, we measured height, diameter, and seedling vigor at several points during the study period. Height, diameter, and vigor were all measured within a week after planting (DOY 125-130; May 5-10), just before the start of the drought treatments (DOY 179-181; June 28-30), as well as at the ends of the short drought treatment (DOY 196; July 15; height and vigor only), heat treatment (DOY 206; July 25), long drought treatment (DOY 213; August 1), and growing season (DOY 273; September 30).

Seedling height was measured as the length of the main stem. Diameter was recorded as the average of two perpendicular measurements approximately 3 cm above the media surface. Seedling vigor was rated using a percent brown foliage assessment (to the nearest 5%).

1.3.4 Carbon and Water Relations

To determine the magnitude of physiological drought imposed on seedlings, we measured midday (between 11:00 am and 2:00 pm) leaf water potential ($\Psi_{\text{leaf}}$) and paired container VWC on 10 seedlings per species at the end of each treatment (DOY 197, 205, 213-214) and on five to 10 seedlings per species at six other points throughout the study period (DOY 192, 200, 201, 204, 205, 236). To measure water potential, two leaf discs per seedling were sampled using a hole punch and both were immediately transferred to a thermocouple psychrometer (TCP) chamber following Wason et al. (2018). TCP chambers were kept in a closed cooler until being transported to the lab, connected to a datalogger, and equilibrated in a temperature-controlled
water bath for at least 6 hours at which point they were assessed for stability and $\Psi_{\text{leaf}}$ was recorded.

In order to quantify drought and temperature effects on photosynthesis and gas exchange, we measured net photosynthesis ($A_{\text{net}}$) and stomatal conductance ($g_s$) of our study species during six sampling campaigns covering a range of plant water potentials (-0.4 MPa to <-7.7 MPa) and temperatures between 20 °C and 46 °C. Temperatures were monitored and recorded using the LI-6400/XT used for photosynthesis and stomatal conductance measurements (LI-COR Biosciences, Lincoln, NE). During each sampling campaign, $A_{\text{net}}$ and $g_s$ were measured between 6:00 am and 4:00 pm on a single leaf of five to 10 seedlings per species using an LI-6400/XT fitted with either an opaque conifer chamber (6400-22) lit with an RGB light source (6400-18/A) for measurements on red spruce, or a standard chamber with 2×3 cm cuvette with an integrated LED light source (6400-02B) for measurements on paper birch and red oak. In order to standardize measurements by leaf area on the needle leaved red spruce without having to harvest shoots, we used 14 samples of current and previous year growth from extra trees to develop an equation that predicted projected leaf area ($A_\text{; cm}^2$) based on the number of needles ($N$) on the shoot ($R^2 = 0.95$, Fig. A.2).

We also quantified photosystem efficiency with end-of-treatment (EOT) measurements using dark-adapted quantum yield of photosystem II ($F'_v/F'_m$) (Maxwell & Johnson, 2000). Measurements were taken between 10:00 am and 4:00 pm on irrigated seedlings and seedlings in the long drought treatment in both heat wave and ambient temperature treatments at the end of the short drought period (DOY 195; July 14), periodically throughout the heat wave for monitoring purposes (DOY 201, 203, 205; July 20, 22, 24), at the end of the heat wave (DOY 206; July 25), and at the end of the long drought period (DOY 213-214; August 1-2).
Measurements were conducted by placing opaque clamps on two of the greenest available leaves on each plant and allowing the leaf tissue to dark adapt for at least 20 minutes, then taking a reading using a handheld fluorometer (FluorPen FP 110, Photon Systems Instruments, Drásov, Czech Republic). Due to limited time and resources, we measured only one leaf per seedling on DOY 195 and 201, but changed to two leaves per seedling beginning on DOY 203.

1.3.5 Species-Level Drought Resistance Traits

In order to establish baseline species-level water use and drought resistance traits, we sampled from control and extra seedlings of each species for water potential at 90% stomatal closure \((g_{s90})\), minimum epidermal conductance after stomatal closure \((g_{\text{min}})\), and the water potential at turgor loss (turgor loss point; TLP).

To determine species-level stomatal response to drought stress, we estimated the \(g_{s90}\) of each species. This metric was estimated by taking 22 to 31 paired measurements per species of stomatal conductance to water vapor \((g_s)\) and leaf water potential \((\Psi_{\text{leaf}})\), using the measurement protocols for \(g_s\) and \(\Psi_{\text{leaf}}\) described above. Because these \(\Psi_{\text{leaf}}\) measurements were paired with \(g_s\) readings and not being used to estimate minimum water potentials, we took measurements during a wider timeframe on sampling days, from 8:00 am to 4:00 pm. Once a measurement of \(g_s\) was completed on a leaf, a leaf disc was immediately sampled from the same leaf using a handheld single-hole punch and placed directly into a TCP chamber to begin equilibration.

To determine species-level leaf \(g_{\text{min}}\), we followed the protocol by Sack and Scoffoni (2011). Seven to 10 total leaves collected from five individuals of each species with the cut end of their petioles sealed with glue were gradually dried down and weighed at regular intervals to develop a curve of mass-loss over time. Leaves were suspended in the dark during dry-downs and with
sustained airflow to remove the leaf boundary layer. The slope of the linear portion of this curve as well as the $g_{\text{min}}$ based on time intervals were then calculated using the $g_{\text{min}}$ analysis spreadsheet tool, which standardizes for leaf area and controls for environmental conditions (Sack, 2011). Temperature and relative humidity were monitored inside the dark container and manually recorded at each time interval (HOBO Temperature/Relative Humidity Datalogger, Onset, Bourne, MA).

To estimate the TLP for each species, 10 control seedlings per species were sampled once in June (DOY 179; June 28) and again in July (DOY 195; July 14) for TLP estimation using the rapid osmometer method developed by Bartlett et al. (2012) and described in protocol by Bartlett and Sack (2012), substituting TCPs connected to a datalogger for the osmometer. Briefly, we sampled leaf discs in the method described above (see Carbon and Water Relations) from shoots hydrated overnight, then made small punctures in the discs before wrapping them in foil and submerging them in liquid nitrogen for two minutes to quickly freeze them. We then unwrapped the discs and placed them into TCP chambers for equilibration and measurement of $\Psi_{\text{leaf}}$, from which TLP may be determined using a regression equation (Bartlett, Scoffoni, Ardy, et al., 2012). Data from each sample period were averaged by sample period as well as by species across both sample periods.

1.3.6 Statistical Analysis

In order to quantify declines in VWC during the drought treatments, we conducted Analysis of Variance (ANOVA) on logit-transformed values of container VWC (as proportions of 1) using data from two time periods: prior to the beginning of the drought treatments and at the end of each treatment. To quantify the effect of the heat wave chambers on environmental conditions
relative to ambient half-blocks, we used a linear mixed effects model predicting daily maximum ($T_{\text{max}}$) and minimum ($T_{\text{min}}$) temperatures and VPD ($\text{VPD}_{\text{max}}$, $\text{VPD}_{\text{min}}$) as a function of heat treatment (categorical) both during and after the heatwave period model with random effect for DOY within block.

To quantify changes in relative height and relative diameter in response to our treatment conditions, we used linear mixed effects models with a random effect for half-block within each block. Vigor data (%) were logit transformed and analyzed with ANOVA for each species testing if vigor varied by treatment at end-of-season (EOS). To determine how the number of individuals in the long drought with at least 10% brown foliage changed over the course of the season, we used logistic regression for each species predicting the likelihood of an individual having at least 10% brown foliage as a function of DOY.

In order to estimate $g_{s90}$ values for each species, our paired measurements of $g_s$ and $\Psi_{\text{leaf}}$ were used to fit negative exponential models to capture the decline from maximum potential $g_s$ to very low $g_s$. To fit models through the region of maximum potential $g_s$ at high $\Psi_{\text{leaf}}$, $g_s$ measurements at water potentials above the vertical asymptote representing $g_s$ decline, that were below 0.05 mol m$^{-2}$s$^{-1}$ were presumably driven by limitations not related to $\Psi_{\text{leaf}}$ and thus excluded from the model (Day, 2000). The $\Psi_{\text{leaf}}$ at which each model reached 90% loss of the maximum $g_s$ as predicted by the model, was taken as the $g_{s90}$ for that species.

For each species, we used multiple regression to test how each species’ $A_{\text{net}}$ responded to $T_{\text{leaf}}$ and VWC. We ran species-level linear models with $A_{\text{net}}$ as a response variable and $T_{\text{leaf}}$ and VWC as interacting predictors, then determined significance of each predictor and their interaction with ANOVA using a threshold of $p < 0.05$. 
To test how EOT \( \Psi_{\text{leaf}} \) differed across drought treatments, we used ANOVA. To determine whether seedling water relations predict growth, we calculated a hydraulic safety margin (HSM) as the difference between \( \Psi_{\text{leaf}} \) at the end of each treatment and species TLP, based on concepts of safety margin from \( \Psi_{50} \) and \( \Psi_{88} \) in Anderegg et al. (2019) and from hydraulic safety margins in Wason et al. (2018). Next, we used ANOVA on log-transformed relative growth values to determine whether EOS relative height and diameter were significantly different within a species if that individual was above or below HSM = 0.

All analyses were run in the statistical software R ver. 4.2.3, using the environment RStudio (ver. 4.2.3, R Core Team 2023; RStudio Team 2023), using packages ‘nlme’ and ‘emmeans’ (Pinheiro et al., 2023; Lenth et al., 2023).

1.4 Results

1.4.1 Drought and Heat Treatments

We found that the drought treatments drove significant declines in container VWC for all three species and planting treatments (Fig. 1.2). Whereas all treatments started the drought period at high VWC (22.4 ± 0.26 %; mean ± SE), by the end of the short drought period (DOY 197) VWC in both drought treatments was significantly lower (1.35 ± 0.12 %) than controls (16.8 ± 0.67 %; \( p < 0.001 \)). The short-drought trees all returned to control levels by the next measurement round at 8 days after their treatment ending and long-drought trees continued at low VWC through DOY 214 before being rewatered. We found no consistent effect of an interaction with the heat wave treatments on patterns in VWC (ANOVA, interaction effect \( p > 0.05 \)). However, there was a slight effect of container size on patterns in VWC for the control trees only, with double
containers having 3.0% higher VWC than single containers \((p < 0.001)\). There was no consistent interaction on VWC between drought treatment and planting treatment \((p > 0.05)\).
We found that relative to control chambers, the heat wave chambers resulted in a 3.5 ± 0.9 °C average increase in $T_{\text{max}}$ ($p < 0.001$; Fig. 1.3A) and no significant increases in $\text{VPD}_{\text{max}}$ ($p = 0.14$; Fig. A.3) or $T_{\text{min}}$ ($p = 0.97$) during the heat treatment. As expected, after ending the heat wave
(DOY 206) we found no significant difference in VPD ($p = 0.42$; Fig. A.3) or $T_{\text{max}}$ or $T_{\text{min}}$ ($p_{\text{max}} = 0.87$; $p_{\text{min}} = 0.76$; Fig. 1.3) between heat wave and ambient chambers.

**Figure 1.3** Mean maximum (A; $T_{\text{max}}$) and minimum (B; $T_{\text{min}}$) daily temperatures in heat treatment and ambient control chambers throughout the study period (DOY 182-234). Horizontal bars along the bottom of each panel denote the period of time during which we implemented the heatwave and drought treatments. Error bars represent ± 1 standard error of the mean.

### 1.4.2 Growth and Vigor

Overall, seedlings showed more pronounced differences in relative diameter growth than relative height growth in response to the treatments. For all three species, we found no significant two- or three-way interactions among temperature, drought, and planting treatments on EOS relative
diameter or relative height growth of seedlings. There was also no significant effect of heat treatment on relative diameter or relative height of any species. Therefore, our results below focus on the main effects of planting treatment and drought treatment for each species.

For red spruce and paper birch, we found lower EOS relative diameter growth in the long-drought treatment compared to controls (25.1 ± 6.1% and 144.9 ± 28.2% lower, respectively) but no effect of the short drought on diameter growth (Fig. 1.4A, 1.4C). In contrast, the short drought had some effect on height growth with *P. rubens* height growth in the long- and short-drought both significantly lower than controls (9.1 ± 3.0% and 9.8 ± 3.0% lower, Fig. A.4A, A.4C) and, similar to diameter growth, only paper birch height growth in the long-drought was lower than controls (77.6 ± 24.8% lower, Fig. A.4B). Patterns of height and diameter growth were more variable for red oak and despite a pattern suggesting lower diameter growth in the long-drought relative to controls starting to develop, we detected no significant treatment effects by the end of the season for either diameter or height (Fig. 1.4B, A.4B).
Figure 1.4 Relative diameter growth (represented as a percentage of diameter at the beginning of the growing season) of each species: (A) red spruce (*Picea rubens*), (B) red oak (*Quercus rubra*), (C) paper birch (*Betula papyrifera*). Irrigated trees were kept above 10% VWC throughout the experiment, short and long drought trees received 25% of their container volume in water every four days for 15 days, and long drought trees continued to be droughted for another 15 days after the short drought, receiving 25% of their container volume of water once during this period. Single planted trees were planted alone in a 1-liter container; double planted trees were planted with an individual of *P. rubens* in a 2-L container. Horizontal bars along the top of each panel denote the period of time during which we implemented the heat wave and drought treatments. Error bars represent ± 1 standard error of the mean. Italicized *p* values represent main effects in a linear mixed effects model and are denoted with a (*) if significant.
We found a significant effect of planting treatment on EOS relative diameter growth for red spruce (lower when double planted; Fig. 1.4A) and paper birch (higher when double planted; Fig. 1.4C), but no significant effects of planting treatment on EOS height growth (Fig. A.4). For red spruce, the focal species, we were able to assess how being planted with different species impacted growth. We found that red spruce planted with paper birch had significantly lower EOS relative diameter than red spruce planted with a red oak ($p = 0.03$), or planted singly ($p = 0.002$; Fig. A.5). EOS relative diameter of paper birch seedlings planted with red spruce seedlings in double containers was 69% higher than relative diameter growth of paper birch planted alone (planting treatment main effect $p = 0.005$) regardless of drought or heat treatment (Fig. 1.4C).

At the end of the growing season, all three species had a significantly higher proportion of individuals with at least 10% brown foliage in the long-drought treatment than seedlings subjected to the short drought and irrigated treatments (Fig. 1.5). However, paper birch reached 50% of individuals with at least 10% brown foliage quickest (drought day 11; DOY 193) relative to red spruce (drought day 26; DOY 208) and red oak (26 days after the end of the 30-day long drought; DOY 240). There was no significant difference in percent brown foliage at the end of the growing season between seedlings in the short drought and seedlings that had been fully irrigated ($p > 0.05$, Fig. A.6). There was a moderate interaction effect between planting treatment and drought treatment on red spruce EOS percent brown foliage ($p = 0.04$). Red spruce had significantly more EOS brown foliage when planted with paper birch than when planted with another red spruce ($p = 0.02$) or planted singly ($p = 0.03$), but only when subjected to the long drought treatment.
Figure 1.5 Logistic regression curves of percent brown foliage for (A) red spruce (Picea rubens), (B) red oak (Quercus rubra), and (C) paper birch (Betula papyrifera) across the study period (DOY 136-266). Observational percent brown foliage measurements (0-100% scale) were sorted into categories of 0 (below 10% brown foliage) and 1 (above 10% brown foliage), plotted against day of year, and fit with a logistic regression curve. Points are jittered on the x-axis for visualization purposes. Red, orange, and pink lines on each plot indicate the duration of the long drought, short drought, and heat wave respectively within the study period. Data were logit transformed prior to analysis. Generalized linear model $p$ values are preceded by (*) if regression was significant.
1.4.3 Physiological Effects of Heat and Drought: Water Potential and Turgor Loss, Stomatal Responses, Photosynthesis

The TLP of paper birch was significantly higher (-1.5 ± 0.08 MPa; mean ± SE; p = 0.0002) than the TLPs of red spruce (-2.0 ± 0.04 MPa) and red oak (-2.1 ± 0.09 MPa), which did not differ from each other (horizontal lines in Fig. 1.6A-C). Seedlings of all species in the long drought had significantly lower EOT leaf midday water potential (Ψ_{leaf}) than seedlings in the irrigated or short drought treatments (Fig. 1.6). The short drought did not cause significant reductions in leaf midday water potential relative to controls for any species. Although red oak tended to have Ψ_{leaf} closer to its TLP in irrigated and short drought treatments relative to the other species, the long-drought treatments surpassed the TLP for all species.
Figure 1.6 Midday leaf water potential ($\Psi_{\text{leaf}}$, MPa) of each drought treatment on the final day of the treatment (Irrigated treatment includes data from irrigated trees from both the short and long drought end dates; end of 15-day short drought on DOY 197; end of 30-day long drought on DOY 214), for (A) red spruce (Picea rubens), (B) red oak (Quercus rubra), and (C) paper birch (Betula papyrifera). Dashed black line represents turgor loss point (TLP) for each species; dotted gray lines represent ±1 SE of the mean TLP based on 10 individuals per species. In some cases, $\Psi$ values went below the measurement threshold of our sensors (-7.7 MPa) and were reported as -7.7 MPa. Different lowercase letters in each panel indicate statistically significant differences between treatments for a species.

Red oak had significantly lower $g_{\text{min}}$ than red spruce and paper birch ($p = 0.02$), which did not differ from each other (Fig. A.7). Red spruce and paper birch each had $g_{s90}$ values that were higher (less negative) than their respective TLP ($g_{s90} = -1.09$ MPa and -1.22 MPa respectively, Fig. 1.7A, C; Table A.2), whereas red oak’s $g_{s90}$ value was 0.31 MPa lower (more negative) than its calculated TLP ($g_{s90} = -2.42$ MPa, Fig. 1.7B).
We found that photosynthesis ($A_{\text{net}}$, $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) declined with leaf temperature ($T_{\text{leaf}}$) for all three species, but this effect was dependent on VWC for red spruce and red oak (significant...
interactions; Fig. 1.8A and B). In red spruce, the negative effect of $T_{\text{leaf}}$ on $A_{\text{net}}$ was moderately stronger under conditions of low VWC ($VWC < 3.1\%$; $p_{\text{interaction}} = 0.03$, Fig. 1.8A). In red oak, the negative effect of $T_{\text{leaf}}$ on $A_{\text{net}}$ was much stronger under conditions of low VWC than moderate ($3.1\% \leq VWC < 10.9\%$) or high ($VWC \geq 10.9\%$) VWC ($p_{\text{interaction}} = 0.003$, Fig. 1.8B).

In paper birch, we found that $A_{\text{net}}$ was negatively associated with $T_{\text{leaf}}$ (slope = $-0.319$, SE = 0.075, $p < 0.0001$, Fig. 1.8C).

Heat treatment did not have any effect on EOT (DOY 213) $F_{v}/F_{m}$ values in any of the study species, while drought treatment significantly affected only $F_{v}/F_{m}$ of red oak and paper birch seedlings. Red oak and paper birch seedlings in the long drought group had significantly lower EOT $F_{v}/F_{m}$ values than seedlings in the irrigated group regardless of heat ($p < 0.05$), and red spruce seedlings did not show differences in EOT $F_{v}/F_{m}$ values between long drought and irrigated levels ($p = 0.88$).
Figure 1.8 Net photosynthesis $A_{\text{net}}$ as a function of leaf temperature ($T_{\text{leaf}}$) in each species (A) red spruce ($Picea rubens$), (B) red oak ($Quercus rubra$), (C) paper birch ($Betula papyrifera$); separated by container percent volumetric water content (VWC) class. Low VWC is classified as a percent volumetric water content (%VWC) below the first quartile (Q1) value ($VWC < 3.09\%$), moderate VWC as a %VWC between the Q1 and median values ($3.09\% \leq VWC < 10.88\%$), and high VWC as a %VWC above the median value ($VWC \geq 10.88\%$). Note that the y-axis scale changes across panels. Interaction $p$ values are indicated where interaction between temperature and VWC (media moisture) is significant; main effect $p$ values are indicated where interactions not significant.
1.4.4 Predicting Growth From Physiological Stress Metrics

Since the major impacts on growth were driven by drought treatments, we finally tested if the degree of physiological drought stress a plant experienced could predict growth. We found that red spruce and paper birch showed significant sensitivity to their hydraulic safety margins in relative diameter growth (Fig. 1.9). EOS relative diameter growth, but not relative height growth, declined significantly in red spruce and paper birch if the magnitude of EOT $\Psi_{\text{leaf}}$ exceeded the species TLP (diameter $p < 0.001$, height $p > 0.05$). Red oak did not show significant responses to EOT $\Psi_{\text{leaf}}$ in relative diameter growth or relative height growth ($p = 0.506$).
Figure 1.9 Hydraulic safety margin, the difference between end-of-treatment (EOT) midday leaf water potential ($\psi_{\text{leaf}}$) and species turgor loss point (TLP), plotted against (A) end of season (EOS) relative diameter growth (as a percentage of initial diameter) and (B) EOS relative height growth (as a percentage of initial height) of each species: red spruce ($Picea rubens$), red oak ($Quercus rubra$), and paper birch ($Betula papyrifera$). Panels to the right of (A) and (B) show the relative growth of each species (diameter top, height bottom), separated by whether their EOT safety margin was negative (i.e., $\psi_{\text{leaf}} < \text{TLP}$) or positive (i.e., $\psi_{\text{leaf}} > \text{TLP}$). Dotted line at x = 0 indicates point at which $\psi_{\text{leaf}}$ is equal to species TLP. Points that line up at the same x-value are driven by our $\psi_{\text{leaf}}$ cutoff of -7.7 MPa, which is the minimum value that can be measured by the thermocouple psychrometers. Significant differences between safety margin groups, tested with ANOVA on log-transformed relative growth values, are represented by *** ($p < 0.01$), ** ($p < 0.05$), * ($p < 0.1$), and n.s. ($p > 0.1$). Error bars represent ± 1 standard error of the mean.
1.5 Discussion

In this study, we tested how the growth of seedlings common to the Acadian Forest ecotone was related to their climate-driven physiological responses, under simulated extreme conditions. We generally found that drought stress was the dominant driver of growth responses and that species interactions and the moderate heat wave had only minor effects. Surprisingly, although we saw some clear indications of compounded climate extremes driving declines in physiology, the relatively moderate heat wave did not seem to stress trees enough (even when combined with drought) to reduce growth in the first year. We also found that the more northerly species red spruce and paper birch were more sensitive to environmental stressors than red oak in terms of both physiology (with the exception of photosynthesis) and growth. Red oak had lower TLP and $g_{\text{min}}$ than red spruce or paper birch, suggesting a higher level of drought tolerance. Photosynthesis declined with leaf temperature for all three species, and this effect was dependent on moisture availability for red spruce and red oak but not for paper birch. However, all species had reduced end-of-season vigor in response to the long drought. Because we noted relatively few interacting effects on growth in this study, our discussion will address each treatment effect separately first and then focus on the interactions of those effects.

1.5.1 Dominant Link Between Drought Resistance and Tree-Growth Responses to Drought Stress

Although both the long and short drought resulted in significantly lower VWC than the irrigated treatment, only midday water potentials of long drought trees differed significantly from controls at the end of the treatment, suggesting that short drought trees did not experience a high level of physiological stress. Our study used a continuous drought period similar to that in van Kampen
et al. (2022), which imposed six-week drought treatments; however, in the current study, seedlings were periodically given small amounts of water during drought in order to limit mortality and generate sustained stress conditions. Other longer-term drought studies impose thoroughfall exclusion that allows a reduced percentage of rainfall to reach the soil instead of irrigating, simulating a months- to years-long period of low precipitation (Asbjornsen et al., 2021) or reduce irrigation differently across their study species based on water use traits (Ruehr et al., 2016). Our results, and results from other drought study designs, highlight the diverse nature of droughts and their potential effects on trees and underscore a continued need to explore how these dimensions relate to natural droughts.

Diameter growth was only reduced by the long drought, and only in red spruce and paper birch. The absence of drought effects on diameter of red oak may be for a number of reasons. Ring-porous species like red oak tend to have completed the majority of their radial growth by early July (our treatments began July 1), making them more sensitive to spring rather than summer conditions (D’Orangeville et al., 2022). Further, the apparent resistance of red oak growth to drought in our study may be due to more pronounced changes in its root system, which were not measured in our study (Wang et al., 2023). On the other hand, radial growth of diffuse-porous species and conifers tends to continue further into the summer (D’Orangeville et al., 2022). Additionally, the less negative TLPs we found for red spruce and paper birch in this study compared to red oak suggest that drought stressed spruce and birch may have spent more time near or below their TLP, potentially limiting radial growth from occurring during periods of high water deficit (Zweifel et al., 2016, 2021).

Height growth of both red spruce and paper birch was reduced by the long drought, but was only reduced in the short drought for red spruce. Height growth of red spruce has been shown to
decline even at moisture levels higher than what spruce ultimately experienced in our study (Vaughn et al., 2021). Height growth of spruce species may be particularly sensitive to water restriction, especially under warm temperatures (Pau et al., 2022), and given that spruce height growth is determinate, may be significantly truncated by stress during its relatively short shoot growth period (Canham et al., 1999; van Kampen et al., 2022). For birch, early- to mid-summer drought has been shown to limit height growth; however, a moderate drought ending early enough in the year could allow for later compensatory growth of stressed seedlings (van Kampen et al., 2022). This growth response may explain the overall reduced relative height growth of paper birch in the long, extreme drought but not for the short, moderate drought in our study.

Red spruce and paper birch $g_{s90}$ estimates were both higher (less negative) than their respective TLPs, which is to be expected given that stomatal closure is typically adapted to avoid turgor loss. $g_{s90}$ for red oak, however, was lower than its TLP suggesting the leaves would lose turgor before closing stomata. This apparent contradiction may be driven by the potential for high plasticity in TLP for red oak (0.89 MPa) relative to spruce (0.28 MPa) and birch (0.08 MPa) that was not captured in our study design (Bartlett et al., 2014). Indeed, accounting for this plasticity would suggest that oak did not lose turgor before $g_{s90}$ and that spruce and birch may have closed stomata with slightly larger safety margins than we estimated. Our study aligns with claims that TLP is a useful indicator of species drought tolerance (Bartlett, Scoffoni, Ardy, et al., 2012; Choat et al., 2018) and highlights the additional value of incorporating direct estimates of seasonal plasticity in TLP (Bartlett et al., 2014).

Minimum epidermal conductance ($g_{min}$) is increasingly recognized as a useful component of drought resistance (Barnard & Bauerle, 2013; Choat et al., 2018; Duursma et al., 2019). Despite reports of wide variability among sites and high plasticity within species, we found that $g_{min}$ for
red spruce in our study is consistent with other estimates for spruce species (Anfodillo et al., 2002; Duursma et al., 2019). The relatively low $g_{min}$ for red oak may be explained by the tendency of *Quercus* species to have smaller stomata and higher leaf thickness relative to other broadleaved trees including paper birch (Abrams & Kubiske, 1990), allowing the red oak in our study tighter control over water loss after stomatal closure. The significantly lower $g_{min}$ of red oak compared to spruce and birch suggests that tight stomatal control may be another trait that allows red oak to avoid physiological drought for longer periods of reduced soil moisture and should be considered in future studies.

In this study, we linked physiology directly to growth by calculating a safety margin between midday leaf water potential ($\Psi_{leaf}$) and species TLP. Red spruce and paper birch showed significant growth sensitivity to this safety margin and were more likely to have reduced EOS diameter growth if they had lost turgor by the end of the long drought, while red oak was not sensitive to this safety margin. This suggests that red spruce and paper birch growth were strongly limited by this safety margin, potentially due to reduced cell turgor during periods critical for growth (sink limitation) (Peters et al., 2021; Zweifel et al., 2021). However, the absence of safety margin effect on red oak may be due to a number of reasons discussed above including plasticity in TLP (Bartlett et al., 2014), growth phenology (D’Orangeville et al., 2022), and higher investment in roots (Wang et al., 2023). The paradigm of “source” and “sink” limitations, respectively the environmental limitations on photosynthetic capacity and actual growth, may offer context for the connection between water potential and subsequent growth in seedlings (Campany et al., 2017). While environmental conditions such as moisture can affect source activity, i.e., stomatal closure to conserve water, it is the primary driver of sink activity, i.e., cell expansion and subsequent growth (Körner, 2015). Thus, the physiological water
limitation on growth observed in our study may be a result of limited sink activity, by which cell
turgor-driven expansion was reduced during the growth period, rather than a limitation on
assimilation of new carbon (Cabon & Anderegg, 2022). However, if there is a significant source
limitation that reduced non-structural carbohydrate production and storage, it may affect growth
in the recovery year (Kannenberg, Novick, Alexander, et al., 2019; Teets et al., 2022). Our
physiological measurements of red spruce and paper birch showed 90% stomatal closure at \( \Psi_{\text{leaf}} \)
approximately 0.3 MPa (paper birch) to 0.91 MPa (red spruce) higher than their TLPs. This
suggests that the seedlings in the long drought with \( \Psi_{\text{leaf}} \) more negative than their TLP had
already closed their stomata and reduced carbon assimilation at the point when turgor began to
limit growth, raising the question of whether we will see reductions in subsequent year growth
due to ‘source’ limitations (Cabon et al., 2022; Teets et al., 2022).

1.5.2 Limited Effect Of Moderate Heat Waves On Growth and Physiology

The 3.5 °C increase in average daily maximum temperature throughout the two-week heat wave
did not impact EOS growth or vigor of seedlings. This moderate warming for a short period of
time also did not drive night-time warming that could have increased respiration rates. However,
our physiological data did suggest that our trees are sensitive to increasing temperatures and
there were periods of time that approached critical threshold (Kunert et al., 2022). Therefore, we
suspect the primary reason for limited heat effects in this study are that we did not achieve
extreme levels of heat stress for long enough to accumulate into a total growth response.

Consistent with other studies, we saw a consistent reduction in net photosynthesis \( (A_{\text{net}}) \) with
increasing temperature for all species that also depended on soil moisture in spruce and oak
(Reich et al., 2018; Ruehr et al., 2016). Indirect effects of heat such as vapor pressure deficit
(VPD), which increases with temperature, can also put stress on the hydraulic system of the seedling and decrease water use efficiency (Ruehr et al., 2016). Therefore, the heat stress imposed on the seedlings in our study was sufficient to reduce photosynthesis (particularly when also experiencing drought), but likely not sustained enough to significantly reduce total growth.

### 1.5.3 Compounded Drought and Heat Stress

Despite the strong effects of drought and clear evidence that high temperatures reduce photosynthesis, we did not see a significant interaction effect on growth between the heat and drought treatments in our study, likely because of moderate nature of our heat wave treatment, as described above. A more extreme and prolonged period of heat may have resulted in higher and less recoverable levels of stress in our seedlings (Bauweraerts et al., 2013). However, prior studies have established the need to examine the compounded effects of drought and heat on tree growth (Didion-Gency et al., 2022; Rehschuh & Ruehr, 2022; Vaughn et al., 2021; Wright et al., 2018). While we did not see these compounded effects on growth and vigor in our study, we did see an interaction effect between temperature and moisture on $A_{\text{net}}$. The compounded effects of drought and heat on photosynthesis have been well-examined, to show that moderate warming and plentiful moisture often have positive effects on $A_{\text{net}}$, while the same amount of warming and reduced moisture has negative impacts on $A_{\text{net}}$ in spruce, red oak, and paper birch (Reich et al., 2018). A hypothesis for this compounded effect is stomatal limitations to photosynthesis under both elevated temperature and water stress (Haldimann et al., 2008). Stomatal closure due to low water potential coincides with higher ratios of internal to atmospheric carbon ($C_i/C_a$), indicating photochemical reductions potentially linked to heat stress (Ruehr et al., 2016; Teskey et al., 2015). The interaction we observed in this study between temperature and moisture in $A_{\text{net}}$
response may be a result of compounded physiological effects that represent both source and sink activity in the seedling.

1.5.4 Species Interactions During Individual and Compounded Climate Extremes

We observed moderate effects of planting treatment on diameter growth of red spruce in this study. Namely, red spruce planted with paper birch or another red spruce in any drought treatment showed significantly reduced EOS diameter growth compared to red spruce planted alone or with red oak. Previous studies have found changes in photosynthesis (Didion-Gency et al., 2022), stomatal responses (Zenes et al., 2020), and growth (Kerr et al., 2021) when planted with an interspecific versus an intraspecific companion; however, we did not observe these patterns in our study. Our data suggest that competition for water primarily drove reductions in growth of red spruce in our study. The relatively variable responses to interaction shown even in a limited sample of species underlines the need for further study of the complex effects of species interactions on growth and physiology, especially in the changing communities of the temperate-boreal ecotone.

1.5.5 Conclusions

In this study, we observed an overarching effect of water availability on tree growth and physiology. The effect of water availability varied across species depending on drought resistance traits and growth patterns. Based on drought resistance traits and growth responses to drought, we found that northern red oak is likely to be most tolerant of extreme drought, although its response to warmer temperatures may still compound with low moisture to reduce photosynthesis. On the other hand, red spruce and paper birch, two more northerly-distributed species, are much less resistant to extreme drought conditions than red oak but are not as
susceptible to moderate increases in temperature as we expected. Moderate drought and modest increases in temperature with climate change may not have significant short-term impacts on growth and vigor of seedlings in the Acadian Forest region; however, there is a significant risk of reduced growth and vigor under projections for more extreme droughts and heat waves. Our data on decreasing $A_{\text{net}}$ with increasing temperature, particularly under dry conditions, suggests a more critical role of hot drought in tree carbon assimilation than can be deduced from observed growth responses. Recovery year measurements will be important in discerning whether the limitation to photosynthesis under compounded drought and heat caused reductions in available carbohydrates for growth in the subsequent season. Future research should include further experimental and observational examinations of the role of species interactions in climate response, as well as heat and drought experiments using a wider range of conditions in order to separate the impacts of climate extremes on the characteristic species of this region.
CHAPTER 2. DAILY RADIAL INCREMENT OF TWO NORTHERN TEMPERATE CONIFERS IN CENTRAL MAINE IS SENSITIVE TO HIGH VAPOR PRESSURE DEFICITS

2.1 Abstract

Secondary growth of trees is driven by species traits and environmental factors including climate. Although dendrochronology (tree-ring) studies have been immensely valuable for identifying the relationships between climate and growth at annual scales, these correlations do not always clearly align with mechanisms by which climate drives growth at finer (e.g., daily) scales. In order to better understand how climate impacts the daily growth of trees, we tested how environmental variables related to daily radial increment of 30 trees in central Maine. We installed automatic point dendrometers on ten mature trees each of red maple (Acer rubrum), red spruce (Picea rubens), and eastern hemlock (Tsuga canadensis) for one growing season to monitor 15-minute changes in stem radial increment. We modeled the probability of radial increment on a given day as the function of local environmental variables including daylength, soil moisture, air temperature, and vapor pressure deficit using binomial generalized linear mixed effects models. We also derived species-level traits for maximum light-adapted photosynthesis, turgor loss point, and stem capacitance. We found that the environmental drivers of the probability of daily stem increment differed in important ways for each species and that interactions between climate variables were central to most responses. Vapor pressure deficit (VPD) emerged as a key predictor for red spruce in all conditions, and for eastern hemlock during soil drought. Although recent dendrochronology studies at this site have not identified VPD as an important predictor for annual growth, our results suggest that that the physiological
effects of daily climate on growth may differ in important ways and should be considered when linking fine-scale studies to longer-term tree growth.

2.2 Introduction

2.2.1 Patterns of Secondary Growth In Trees

When and how trees grow is determined by an array of interacting factors including species traits, climate, and daylength (Dulamsuren et al., 2023; Etzold et al., 2022; Huang et al., 2020). However, climate change is driving novel climate conditions in many places that are, or will be, beyond the conditions to which trees are adapted (Anderegg et al., 2019; Hollinger et al., 2021; Krakauer et al., 2019; Kunert et al., 2022). Therefore, it is becoming challenging to rely on previous tree-growth responses to climate in order to predict future responses. Furthermore, an emphasis on seasonal and monthly climate metrics and annual growth may miss how daily growth responds to important but rare extreme climate conditions that may become more common in the future (Duchesne & Houle, 2011; Etzold et al., 2022). Therefore, we need to better understand the mechanisms by which climate drives daily tree growth on shorter timescales to predict future tree responses.

In temperate and boreal regions, trees exhibit seasonal patterns of secondary growth, by which cells of the vascular cambium divide and differentiate, expand, and eventually lignify and undergo programmed death during a growing period in the spring and summer, resulting in an increment of radial expansion that is completed in late summer to early fall (Deslauriers et al., 2003; Rathgeber et al., 2016). These patterns are predictable in that they are stimulated in part by annually consistent fluctuations in daylength, in addition to regional variables such as temperature and moisture availability (Bauerle et al., 2012; Rohde et al., 2011). Within a forest
community, different species may also reach peaks of secondary growth at different times during the season based on their wood type and life history (Basler & Körner, 2014; D’Orangeville et al., 2022; Huang et al., 2020; Takahashi et al., 2013). Consequently, timing of environmental stressors such as drought, extreme heat, or high vapor pressure deficit (VPD) may coincide with a time of particular importance in a tree’s seasonal pattern of radial increment (D’Orangeville et al., 2018; van Kampen et al., 2022).

While a growing season is usually defined as a consecutive period during which a tree can grow, a mounting body of research shows that the most important determinant of annual radial growth is the number of individual days within a season that irreversible radial expansion occurs (Drew et al., 2014; Etzold et al., 2022; van der Maaten et al., 2018). This concept is counterintuitive to the idea that trees grow in a linear fashion; instead, the radial growth of an individual tree is stepwise (Knüsel et al., 2021; Zweifel et al., 2016). Herein lies a distinction between annual increment, which may be expressed as an absolute change in basal area or biomass, and radial changes on a more frequent timescale. On an hourly to daily timescale, radial changes may in fact be reversible, as elastic stem tissues shrink and expand with water loss and uptake (Deslauriers et al., 2003). During periods of ample moisture and favorable temperature, the stem radius may undergo irreversible increase from turgor-driven cell expansion followed by eventual maturation of xylem cells and lignification of cell walls (Cabon & Anderegg, 2022; Peters et al., 2021; Rathgeber et al., 2016). Interannually, optimal or sub-optimal average conditions drive overall seasonal increment, in addition to driving growth in subsequent seasons depending on availability of stored carbohydrates (Kannenberg, Novick, Alexander, et al., 2019; Körner, 2003).

While there is a rich body of research on seasonal and year-to-year relationships between climate and annual growth (Andrews et al., 2020; Martín-Benito et al., 2008; Teets et al., 2018; Van Den
Brakel & Visser, 1996), fewer studies have honed in on relationships between daily growth and climate conditions due to limitations related to cost and effort (Drew & Downes, 2009). Therefore, there is currently less information available on what causes a tree to actually exhibit radial increment on a given day, although the recent availability of inexpensive precision instruments for measuring high-frequency stem radius changes has led to increasing investigation into daily patterns of tree growth, particularly in response to the threat of extreme events under climate change (Deslauriers et al., 2003; Duchesne & Houle, 2011; Etzold et al., 2022).

2.2.2 Annual and Daily Drivers of Tree Radial Increment

In temperate and boreal mesic forests, there is growing evidence that the conditions suitable for daily radial increment depend on photoperiod, air temperature, soil moisture, and vapor pressure deficit (Duchesne & Houle, 2011; Etzold et al., 2022; Zweifel et al., 2021). Importantly, some evidence suggests that environmental conditions may have different effects on daily radial increment than on annual radial increment. For example, on a daily timescale, warmer temperatures tend to stimulate cambial division and cell expansion, but particularly at southern range margins overall warmer seasons may cause relatively lower annual growth compared to cooler growing seasons (Peters et al., 2021). There is also growing evidence that even in mesic forests high VPD drives reductions in leaf level physiology (Day, 2000; Ruehr et al., 2016) and daily radial increment (Etzold et al., 2022; Zweifel et al., 2021); however, annual growth (radial increment and biomass increment) rarely responds directly to this stressor (Teets et al., 2018). Stressors such as VPD, temperature, and soil water content may also interact to produce compounded effects on growth at both the annual and daily scale (Andrews et al., 2020; Etzold et al., 2022; Huang et al., 2020). Diurnal cellular expansion relies on a gradient of high to low
water potential from the functional xylem to the developing cell, which maintains the turgor of the cell (Peters et al., 2021; Rathgeber et al., 2016). In cases of high VPD, extremely negative atmospheric water potentials can quickly drive reduced xylem water potential (Ruehr et al., 2016; Schönbeck et al., 2022; Zweifel et al., 2021). These reductions in xylem water potential can be mitigated by the release of stored water (Cermak et al., 2007; Mcculloh et al., 2014) and uptake of soil water. However, species differ in their ability to release stored water and on days when soil water potential is low, the tree may be unable to replenish stem water to the degree needed to continue transpiration and maintain cell turgor for radial expansion (Peters et al., 2021). As the likelihood of extreme temperatures, drought, and VPD increases (Fernandez et al., 2020), it is now critical to resolve these discrepancies by determining the environmental drivers of daily tree radial increment (Kunert et al., 2022; Schönbeck et al., 2022; Teskey et al., 2015).

This study aims to determine how environmental variables drive probability of daily radial increment in mature Acer rubrum (red maple), Picea rubens (red spruce), and Tsuga canadensis (eastern hemlock) trees at Howland Research Forest, Maine, USA. These species represent two different wood types (diffuse porous and nonporous) and a range of sensitivities to climate extremes, with A. rubrum being classified as very tolerant and adaptable and P. rubens and particularly T. canadensis predicted to be generally more sensitive to climate extremes (Aubin et al., 2018; Boisvert-Marsh et al., 2020; Teets et al., 2018). Specifically, we tested (i) for synchrony of daily radial increment within and among species, (ii) the environmental drivers of daily radial increment, and (iii) how those drivers of daily radial increment compared to environmental drivers of annual radial increment.
2.3 Materials and Methods

2.3.1 Site Characteristics

We conducted this study at the Howland Research Forest (AmeriFlux Network US-Ho1; Fig. 2.1A), a site that is near the southern extent of the eastern North America ecotonal temperate-boreal forest in Maine, USA (Fig. 2.1B) (Hollinger et al., 1999, 2021). For this study, we used a 3-hectare (150 m × 200 m) fully stem-mapped forest plot, known as the NASA plot, which was established in Howland Research Forest in 1989 (Levine et al., 1994; Weishampel et al., 1994) and has since been re-mapped for verification (Teets et al., 2018). As part of a series of studies testing how tree growth responds to climate, increment cores were collected from 329 trees on this plot in 2016 (Fig. 2.1A). This sample represented 10% of total trees on the plot, and was selected using a random sample stratified by tree diameter class (Teets et al., 2018).
Figure 2.1 (A) Stem map of all cored trees on the 150 × 200 m ‘NASA Plot’ at Howland Research Forest. Circles represent locations of our focal species (gold = *Acer rubrum*, pink = *Picea rubens*, teal = *Tsuga canadensis*). Open circles are scaled by 2016 DBH (Teets et al., 2018); filled circles are scaled by 2022 DBH measured at the beginning of our study and represent trees in our 2022 study sample. × icons represent non-focal species on the plot and are not sized to scale. (B) Location of Howland Research Forest indicated by a red dot on a map of the state of Maine.

2.3.2 Tree Selection Criteria, Dendrometer Deployment and Protocol

In our study, we focused on *P. rubens*, *T. canadensis*, and *A. rubrum* to capture two of the most common conifer species in this region and the most abundant deciduous tree at this site. We randomly selected 10 individuals per species >8-m from the plot edge and with no visible damage, from the sample of previously cored trees. In our initial sample, all trees were in dominant or codominant canopy positions (hereafter referred to as “canopy”). However, to test for the effect of canopy position on radial increment responses to climate, three *P. rubens* and three *T. canadensis* were selected from sub-canopy classes (hereafter referred to as “sub-
canopy”), resulting in a sample of 7 canopy and 3 sub-canopy trees per species. All *A. rubrum* on the site were in the “canopy” category (Table B.2).

In mid-April 2022 we fitted each sample tree with an automatic point dendrometer (TOMST, Czech Republic) at breast height on the north side of the stem and programmed dendrometers to record horizontal pin displacement to the nearest 1 µm (± 0.27 µm) every 15 minutes.

Dendrometer data for this study were retrieved periodically throughout the season through October 30, 2022. However, we observed that the pressure of connecting to the dendrometer to download data caused erroneous fluctuations in the readings typically over the next two days. Therefore, raw data were inspected manually to remove the data (replaced with NAs) that were impacted by these instances. In total, this represented a loss of approximately 6% of the final data.

To determine daily radial increment, we first averaged 15-minute increments to hourly values and then calculated daily maximum and minimum values for displacement within each day. Days when tree radial increment occurred was calculated using the Zero-growth (ZG) method (Knüsel et al., 2021; Zweifel et al., 2005, 2016) whereby a tree is assumed to grow when the maximum radius of that tree in that day exceeds any previous maximum for that tree (Fig. B.1). We used the 5th and 95th percentiles of each tree’s maximum radial increment to delineate the growing season within which we would analyze how environmental variables impact the probability of daily radial increment (Etzold et al., 2022).

### 2.3.3 Climate and Environmental Variables

In order to test how environmental conditions impact the probability of radial increment $P_D$, we compiled temperature, relative humidity, and soil moisture data from the Howland Research Forest within 250 m of our research plot. Temperature was measured with platinum resistance
thermometers at 30-minute intervals 2 and 30 m above the ground on two flux towers and
relative humidity was determined by using eddy covariance methods (as described in Hollinger et al., 2021; Richardson et al., 2019). These parameters were used to calculate VPD following Ward and Trimble (2003) before being summarized to daily mean temperature ($T_{\text{avg}}$) and daily maximum vapor pressure deficit (VPD$_{\text{max}}$). Soil volumetric water content (VWC, proportion of 1) was measured at 30-minute intervals at 5, 10, and 20 cm depth in a soil profile pit approximately 250 m from the plot (Hydra Probe, Stevens Water Monitoring Systems, Inc.). We found that overall patterns in soil VWC was similar at all depths and therefore used only the 10-cm depth and averaged it to daily mean soil VWC. Daylength was calculated as a function of latitude and day of year using the R package ‘insol.’

2.3.4 Physiological Measurements

To compare how daily radial increment responses to environmental variables relate to physiology, we also measured photosynthesis, turgor loss point, and stem wood capacitance. In most cases, these samples came from nearby trees to facilitate sample collection and minimize destructive sampling on the research plot. To measure maximum photosynthetic rate ($A_{\text{max}}$) and turgor loss point (TLP), we randomly sampled from 19 canopy trees both in and outside the research plot on two days in early August 2022. Using a Bigshot slingshot, we harvested 5 to 7-cm diameter shoots from the sun-exposed canopies of at least four trees per species and removed 1 to 5 smaller shoots from each large shoot. Each smaller shoot used for measurements was cut from the larger shoot underwater and the cut end was kept hydrated in floral water tubes. The small shoots were then placed in a humidified opaque container for up to one hour before they were measured for $A_{\text{max}}$ ($\mu$mol CO$_2$ m$^{-2}$s$^{-1}$) (on A. rubrum, 2-3 leaves per shoot were used for $A_{\text{max}}$ measurements). $A_{\text{max}}$ was measured using an LI-6400/XT set at a fixed flow rate of 500
μmol/s and a light source set to 1500 μmol m$^{-2}$s$^{-1}$ with either a standard chamber fitted with a 2 × 3 cm cuvette or an opaque conifer chamber (LI-COR Biosciences, Lincoln, NE). For conifers, needles were removed from samples and projected leaf area was determined using a scanner and Fiji ImageJ software (Schindelin et al., 2012), then used to adjust values of photosynthesis and stomatal conductance to a leaf area basis. To estimate turgor loss point, 9 to 10 shoots per species were then transported back to the laboratory where we measured osmotic potential at full turgor and used that to predict the turgor loss point following Bartlett, Scoffoni, Ardy, et al. (2012) and Bartlett and Sack (2012).

In late October 2022, we collected increment cores (at breast height using a 5-mm diameter borer) from 10 trees per species within 250-m of the research plot to estimate stem wood capacitance – the ability of the stem wood to release water during drought (Mcculloh et al., 2014; Meinzer et al., 2003). The outermost 1-cm of xylem from each core was removed with a razor blade and transported to the lab in miniature centrifuge tubes, weighed, and vacuum saturated with de-ionized water for 24 hours. Next, we constructed water release curves (Mcculloh et al., 2014; Meinzer et al., 2003) with a sequence of paired measurements of sample mass and water potential as each sample progressively dried on the lab bench. Water potential was estimated using thermocouple psychrometers (Brown & Bartos, 1982). Once all samples exceeded -7.7 MPa (10-13 measurement rounds per sample) they were oven dried at 70 °C for 24 hours to collect dry mass. Water release curves were constructed for each sample by plotting water released (kg m$^{-3}$) as a function of water potential and capacitance was estimated for each sample as the slope of a linear regression fit to all data points above -0.5 MPa.
2.3.5 Statistical Analysis

Prior to analysis of our data, one canopy \textit{P. rubens} was removed from the dataset as its dendrometer data showed a season-long decline in growth that we determined to be a result of sampling error. This removal resulted in a final sample of 10 \textit{A. rubrum}, 9 \textit{P. rubens}, and 10 \textit{T. canadensis}. To describe the trees, we tested for differences in size, growth rate, and timing of radial increment initiation; we used ANOVA to test if start-of-season (SOS) diameter at breast height (DBH), basal area increment (BAI; from dendrometers; cm$^2$yr$^{-1}$), and initiation and cessation date of growth (DOY at 5$^{th}$ and 95$^{th}$ percentile of radial increment) differed among species and between canopy categories. Since there were no sub-canopy maples, we first conducted an ANOVA with species as the only predictor and conducted a second ANOVA on just the conifers that first tested a species by canopy category interaction. If that interaction was not significant, it was removed, and we assessed main effects. When significant effects were observed they were followed by Tukey’s Honest Significant Difference.

In order to determine the environmental variables associated with \(P_D\) we used generalized linear mixed effects models with daily radial increment as a binomial response variable (link = ‘logit’). For each species, we built a full model with fixed effects for all predictors (canopy category, daylength, VPD$_{\text{max}}$, VWC, and T$_{\text{avg}}$) as well as for daylength interacting with each predictor and VWC interacting with VPD$_{\text{max}}$. These interactions were selected to reduce the complexity of our full models and were based on previous research (Etzold et al., 2022) and suspected mechanisms. All fixed effects were scaled (mean = 0, standard deviation = 1) prior to model fitting and we verified that variance inflation factors (VIF) were below 4.0 in the final (see below) models for \textit{P. rubens} and \textit{T. canadensis}. Each final model included random effects for day of year (‘DOY’) and individual tree (‘series’) to account for autocorrelation and was optimized using Bound
Optimization BY Quadratic Association with 200,000 iterations. Canopy category was not included in the models for *A. rubrum*. For each species, the full model was progressively reduced by removing each non-significant interaction term (threshold of $p = 0.05$) until left with a species-level model including all individual predictors and only significant interactions (Table B.4). After model reduction, VIFs were checked, and one interaction was removed from the *A. rubrum* model that was above 4.0. Residuals were checked in final models by plotting against predictors and we calculated the marginal and conditional $R^2$ following Nakagawa & Schielzeth (2013) using the function ‘r.squaredGLMM’ in R package ‘MuMIn’.

In order to compare physiological variables associated with each species, we conducted ANOVA with species as predictor on calculated TLP and $A_{\text{max}}$ values. We identified the capacitance of each species as the slope of the linear model of volume of water released versus water potential.

All analyses were performed in R (ver. 4.2.3, R Core Team, 2023) using RStudio and the packages ‘lme4’, ‘insol’, ‘doBy’, ‘car’, and ‘MuMIn’ (Bates et al. 2023; Højsgaard and Halekoh, 2023; Corripio, 2019; Fox et al., 2023; Bartoń, 2023; RStudio Team 2023).

**2.4 Results**

During our study period in 2022, average daily maximum vapor pressure deficit ($\text{VPD}_{\text{max}}$) at the Howland Research Forest peaked in July and was rarely above 2.5 kPa (Fig. B.2). Average daily mean temperature ($T_{\text{avg}}$) also peaked in July, dropping slightly in August. Average soil moisture was highest in June (Table B.1).

We found no difference among the SOS DBH and BAI of canopy trees (Table 2.1; Fig. 2.2) but did find that sub-canopy *P. rubens* and *T. canadensis* had up to 51% lower DBH and 85-90% lower BAI than canopy trees (Table 2.1; Fig. 2.2B, C).
Table 2.1 Summary of tree characteristics by species and canopy category (C = canopy, S = sub-canopy): 2022 basal area increment (BAIavg) ± standard error (SE); April 2022 diameter at breast height (DBH) ± SE. Different lowercase letters indicate significant differences among canopy trees in each species ($p < 0.05$). (*) indicates significant differences between canopy categories in *Picea rubens* and *Tsuga canadensis*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Canopy category</th>
<th>$n$</th>
<th>2022 BAIavg (cm$^3$)</th>
<th>SE</th>
<th>April 2022 DBH (cm)</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acer rubrum</em></td>
<td>C</td>
<td>10</td>
<td>3.30*</td>
<td>0.73</td>
<td>23.03*</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>3</td>
<td>0.46*</td>
<td>0.11</td>
<td>13.8*</td>
<td>0.49</td>
</tr>
<tr>
<td><em>Picea rubens</em></td>
<td>C</td>
<td>6</td>
<td>4.68*</td>
<td>0.69</td>
<td>28.17*</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>3</td>
<td>0.46*</td>
<td>0.11</td>
<td>13.8*</td>
<td>0.49</td>
</tr>
<tr>
<td><em>Tsuga canadensis</em></td>
<td>C</td>
<td>7</td>
<td>2.34*</td>
<td>0.50</td>
<td>30.39*</td>
<td>2.27</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>3</td>
<td>0.34*</td>
<td>0.18</td>
<td>14.9*</td>
<td>1.07</td>
</tr>
</tbody>
</table>
Figure 2.2 Daily maximum stem radial increment (µm) of each sample tree across the full study period from 14 May (DOY 134) to 30 October (DOY 303) for (A) *Acer rubrum* (n = 10), (B) *Picea rubens* (n = 9), and (C) *Tsuga canadensis*. (B) and (C) are split into Canopy and Sub-canopy categories. Note different y-axis scales among panels.

In total, there were 42, 38, and 32 days on which over 50% of *A. rubrum*, *P. rubens*, and *T. canadensis* trees exhibited positive radial increment, respectively (Fig. 2.3). On 10, 10, and 7 of those days (*A. rubrum*, *P. rubens*, and *T. canadensis*, respectively), that species was the only one
with more than 50% of individuals with positive radial increment. Yet, roughly half (19) of those days were synchronous across all species suggesting some common environmental drivers of radial increment (Fig. 2.3).
Figure 2.3 Proportion of total trees per species of (A) *Acer rubrum*, (B) *Picea rubens*, and (C) *Tsuga canadensis* that exhibited stem radial increment on each day of the monitoring period, based on a binomial daily increment/no increment value which was used to build a generalized linear mixed and random effects model. Individual cutoff days for the start and end of the growth period of each tree were based on the day of year at which the tree reached the 5th and 95th percentile of its total radial increment. Gray bars represent proportion of trees with positive radial increment on a day outside of the individual’s growth period (period of growth that represented 5-95% of its total annual increment). Lighter portions of bars represent the proportion of conifer species trees in the ‘sub-canopy’ category (n = 3 per species) that showed radial increment, while darker portions represent the proportion of conifer species trees in the ‘canopy’ category (n$_{P. rubens}$ = 6; n$_{T. canadensis}$ = 7) that showed radial increment. As there were no sub-canopy maples in our study, panel (A) includes only dark ‘canopy’ category bars.
A. rubrum initiated secondary growth an average of 11 days later than canopy trees of either conifer species in our study (p = 0.03, Fig. 2.4A) but we found no difference among the species in average end of season (p = 0.6) and no effect of canopy class on either start or end of season dates (Fig.2.4A, 2.4B).

Figure 2.4 Day of year (DOY) of radial increment (A) initiation (Growth Period Start) and (B) cessation (Growth Period End) for each species and canopy category (Acer rubrum canopy [C]; Picea rubens canopy and sub-canopy [S]; Tsuga canadensis canopy and sub-canopy). A. rubrum radial increment started significantly later than in the other two species regardless of canopy class (p < 0.01). There was not a significant difference in the DOY each species ended radial increment (p > 0.2). Individual cutoff days for the start and end of the growth period of each tree were based on the day of year at which the tree reached the 5th and 95th percentile of its total increment.
When assessing the daily environmental drivers of $P_D$, for *A. rubrum* we found a significant main effect of daylength with $P_D$ increasing on longer days ($p < 0.001$, Fig. 2.5A, 2.6A), and a significant main effect of $T_{avg}$ with $P_D$ decreasing at higher temperatures ($p < 0.001$, Fig. 2.5A, 2.6B). We found a significant interaction effect of VWC and daylength in *A. rubrum* by which *A. rubrum* $P_D$ decreased with VWC on longer days, but increased with VWC on shorter days ($p = 0.002$) (Table B.4; *A. rubrum* full model marginal $R^2 = 0.31$; conditional $R^2 = 0.62$).
Figure 2.5 Effect sizes for each predictor (canopy category; maximum vapor pressure deficit, VPD$_{\text{max}}$; soil volumetric water content, VWC; daily average temperature, T$_{\text{avg}}$; daylength) of daily radial increment from our final generalized linear mixed effects models. Final models were developed separately for (A) Acer rubrum, (B) Picea rubens, and (C) Tsuga canadensis. All individual predictors were left in the final model, while only significant ($p < 0.05$) interaction effects for each species were retained. A label of n.s. indicates that an interaction effect was not included in the final model for the species as it was not significant. Labels of NA for Canopy Category in panel (A) indicate that the predictor was not tested in A. rubrum, as all trees of A. rubrum were in canopy positions. Asterisks indicate significance in the final model as follows: * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$). Canopy category coefficients represent how suppressed trees differed from canopy trees in P. rubens and T. canadensis. Coefficients scaled (mean = 0, SD = 1) prior to model fitting to facilitate comparisons. Conditional ($R^2_c$) and marginal ($R^2_m$) $R^2$ are listed beside the species name on each panel.
For *P. rubens*, high VPD\textsubscript{max} drove steep declines in $P_D$ ($p < 0.001$, Fig. 2.5B, 2.6A; *P. rubens* full model marginal $R^2 = 0.17$; conditional $R^2 = 0.63$). We also found that daylength interacted with soil moisture and canopy category. On long days, $P_D$ decreased at high VWC, whereas on short days, $P_D$ remained more stable across VWC levels (interaction effect $p < 0.001$, Fig. 2.6C). $P_D$ also declined drastically for sub-canopy trees at short daylengths (interaction effect $p < 0.001$, Fig. 2.6D).

For *T. canadensis*, sub-canopy trees were consistently less likely to grow than canopy trees ($p = 0.03$, Fig. 2.5C, 2.6D; *T. canadensis* full model marginal $R^2 = 0.16$; conditional $R^2 = 0.63$). *T. canadensis* also showed moderate increases in $P_D$ with increases in VWC ($p = 0.05$). During long days, high VPD\textsubscript{max} drove steeper declines in $P_D$ (interaction effect $p < 0.001$, Fig. 2.6A). However, on short days (later in the growing season) $P_D$ declined with decreasing VWC (interaction effect $p = 0.02$, Fig. 2.6C). *T. canadensis* was the only species with an interaction between VPD\textsubscript{max} and VWC. At low VPD\textsubscript{max}, $P_D$ remained relatively constant regardless of VWC; however, at high VPD\textsubscript{max}, $P_D$ declined steeply with decreasing VWC (interaction effect $p = 0.01$, Fig. 2.6E). Full final models of $P_D$ are listed in Table B.4.
Figure 2.6. Partial plots demonstrating significant interaction effects on daily probability of radial increment ($P_d$) for each species: (A) daily maximum vapor pressure deficit ($VPD_{max}$) interacted significantly with daylength for $T. canadensis$ ($p < 0.001$); (B) effect of daily average temperature ($T_{avg}$) did not interact significantly with daylength for any species; (C) soil moisture (as proportion of soil volumetric water content, VWC) interacted significantly with daylength for $A. rubrum$ ($p = 0.002$) $P. rubens$ ($p < 0.001$) and $T. canadensis$ ($p = 0.02$); (D) daylength interacted significantly with canopy category for $P. rubens$ ($p < 0.001$); (E) soil moisture significantly interacted with $VPD_{max}$ for $T. canadensis$ ($p = 0.01$). “Low” / “Short” and “High” / “Long” values of interaction predictors were defined as the 1st and 3rd quartile values for each species, respectively (actual values reported in Table B.3).

Capacitance of $A. rubrum$, $P. rubens$, and $T. canadensis$ (the slope of the linear model of the relationship between volume of water released and xylem water potential) was $582 \pm 123$ (mean ± SE) kg m$^{-3}$ MPa$^{-1}$, $379 \pm 221$ kg m$^{-3}$ MPa$^{-1}$, and $723 \pm 184$ kg m$^{-3}$ MPa$^{-1}$, respectively (Fig. B.3).

TLPs of the three species were not significantly different from one another ($p = 0.46$). TLP of $A. rubrum$ was $-1.91 \pm 0.07$ MPa (mean ± SE), TLP of $P. rubens$ was $-2.0 \pm 0.05$ MPa, and TLP of $T. canadensis$ was $-1.99 \pm 0.04$ MPa.
Finally, we found slight differences in midday $A_{\text{max}}$ between *A. rubrum* and *T. canadensis*, with *A. rubrum* having 56% higher rates of $A_{\text{max}}$ than *T. canadensis* during our sampling period ($p = 0.04$).

### 2.5 Discussion

In this study, we used point dendrometers to track sub-hourly changes in tree stem diameter and used these changes to determine days on which radial increment occurred in three characteristic species of the transitional temperate-boreal forest in Central Maine. We used these data to model daily radial increment as function of canopy position, daylength, VPD$_{\text{max}}$, VWC, and $T_{\text{avg}}$. Although we observed synchrony in radial increment among species on roughly 50% of growth days, the environmental drivers of radial increment differed significantly among species and often included interactions. Despite this mesic forest setting, however, daily maximum vapor pressure deficit (VPD$_{\text{max}}$) emerged as a consistent predictor of daily radial increment for conifer trees as either a main effect or in interaction with other environmental drivers.

#### 2.5.1 Timing and Synchrony Of Radial Increment

While all species completed their radial increment around the same time on average, *A. rubrum* began its growing season approximately 11 days later than the conifer species. This is consistent with other findings on secondary growth milestones; the lignification of cells making up new stem conduits in diffuse porous species may not occur until well after leaves are fully developed, in contrast to nonporous species (Takahashi et al., 2013). Furthermore, radial growth of diffuse porous species like red maple have been shown to peak up to a month later than nonporous species (D’Orangeville et al., 2022). Similar to our findings, prior comparisons of *A. rubrum* to
nonporous associates have found little difference among their EOS timing (D’Orangeville et al., 2022; Larysch et al., 2021; van Kampen et al., 2022).

During the growing season, there were 19 days on which at least 50% of all trees in all species exhibited radial increment, none of which were days on which VPD\textsubscript{max} exceeded 3.0 kPa. However, \textit{A. rubrum} had at least 50% of trees exhibit radial increment on two of the three days when VPD\textsubscript{max} exceeded 3.0 kPa, and \textit{T. canadensis} had 50% of trees exhibit radial increment on one of those three days (a day with moderate soil moisture). Combined with our other results suggesting VPD is an emerging driver of tree growth in the northeastern US (discussed more below), future investigation should consider testing for significant differences among species in growth on specific days of climate extremes. In the current study, the radial increment of \textit{A. rubrum} on days of very high VPD\textsubscript{max} coincides with our radial increment models, in which \textit{P}\textsubscript{D} of \textit{A. rubrum} did not fall below 0.50 up to a VPD\textsubscript{max} of 3.5 kPa. In fact, diffuse porous species such as \textit{A. rubrum} have been shown to exhibit diameter growth during periods of tree water deficit, when cells would hypothetically experience very low turgor pressure (D’Orangeville et al., 2022; Zweifel et al., 2016). Days of peak growth in \textit{A. rubrum} also tend to correspond with longer daylengths near the summer solstic, which is consistent with our observations on DOY 176 and 177 (June 25-26, 4-5 days after the summer solstice) and may have been a driving force behind radial increment occurring on these day despite high atmospheric demand (D’Orangeville et al., 2022). Given \textit{A. rubrum}’s classification as a generally tolerant and adaptable species (Aubin et al., 2018; Boisvert-Marsh et al., 2020), these observations may warrant future investigation into the role of VPD in growth.
2.5.2 Environmental Drivers of Daily Radial Increment

In our study, $P_D$ of *A. rubrum* showed a strong positive relationship to daylength. Daylength has been shown to be a highly significant predictor of growth in other temperate diffuse porous species such as European beech (*Fagus sylvatica*) (Etzold et al., 2022; Vitasse & Basler, 2013). However, the effect of daylength is not always observed in *Acer* species, the phenology of which may be insensitive to spring photoperiod (Basler & Körner, 2014; Way & Montgomery, 2015). On the other hand, in another study *A. rubrum* under artificial extended photoperiod showed higher rates of photosynthetic activity past the summer solstice than control trees, suggesting an effect of daylength independent of other variables that seasonally co-vary with photoperiod (Bauerle et al., 2012).

For *P. rubens*, the strong decline in $P_D$ with VPD$_{max}$ regardless of canopy category and other environmental conditions suggests a widespread sensitivity to this increasing environmental stressor. *P. rubens* is a sub-boreal conifer known to be constrained by physiological sensitivity to moisture limitation and temperature, thriving most often in cool, mesic environments (Dumais & Prévost, 2007; Vaughn et al., 2021). Indeed, VPD above 2.0 kPa has been found to be a threshold for significant photosynthetic decline (Day, 2000) and declines in biomass increment (Sanginés de Cárcer et al., 2018). In the current study, a VPD$_{max}$ of 2.0 kPa is approximately where modeled $P_D$ of *P. rubens* falls below our threshold of 0.5, suggesting a similar detrimental effect on radial increment. We did find some evidence that at long daylengths, spruce $P_D$ declined with increasing soil moisture. This may be due to a correlated effect not included in our models that is driving reduced increment rates at those times and, since radial increment of all species was generally not sensitive to soil moisture, it may suggest that these trees are not responding to soil water availability at this site. We also observed some evidence that sub-canopy
trees only grew on longer days, which could reflect generally lower radial increment overall or suggest a separation in how these size classes respond to daylength. Dendrochronological and ecological studies at our site have shown effects of canopy position on overall growth on an annual scale, finding as we did that there was a significant difference in seasonal increment between canopy and sub-canopy classes in *P. rubens* (Fien et al., 2019; Teets et al., 2018).

For *T. canadensis*, we found that canopy category was a dominant predictor of radial increment with sub-canopy trees less likely to grow on a given day. This aligns with hemlock’s high shade tolerance and ability to persist in shaded understories with low growth (Burns, 1990; Canham, 1989). However, we also still found some sensitivity to VPD and soil moisture. Interestingly, it appears that *T. canadensis* is sensitive to only the compounded effects of high VPD and low soil moisture but relatively resistant to VPD independently, and only moderately sensitive to VWC independently. The sensitivity to these compounded climate extremes may be driven by an inability to restore high water potentials to fuel turgor driven cell expansion at night during dry periods (Zweifel et al., 2021). However, this also suggests that mechanisms of drought-induced stomatal closure and other physiological adaptations may maintain growth during moderately dry soil moisture conditions and that, despite the potential for high VPD to induce water stress, this species may be able to quickly rehydrate stems and restore capacitance via soil water at night when VPD stress is reduced. The effect of VPD$_{\text{max}}$ on $P_D$ in *T. canadensis* was particularly negative on longer days (i.e., in early to mid-summer). If these longer days coincide with increasing VPD, and occasional soil moisture deficits, these may be conditions that are particularly likely to lead to declines in growth for this species (Dong et al., 2019; Schönbeck et al., 2022; Zweifel et al., 2016). Indeed, *T. canadensis* is often found in valleys and streamsides that are particularly unlikely to experience these conditions (Brantley et al., 2013; Elliott et al.,
Our results both support this ecological understanding but also emphasize the role of climate change stressors potentially further limiting this species to very specific climate refugia (Prasad & Potter, 2017).

### 2.5.3 Drivers Of Daily Vs. Annual Growth

While the scope of the current study does not include interannual growth, our observations of drivers of daily radial increment in these three species at Howland Forest join a larger body of work studying how annual growth responds to climate on this same research plot. Other research with these species at Howland Forest found no significant annual or interannual effects of climate parameters on growth of *A. rubrum*, suggesting that models based on daily patterns may overestimate the sensitivity of the total growth of *A. rubrum* (Teets et al., 2018) to environmental variables such as temperature and precipitation. Despite clearly finding that daily climate conditions drive the probability of daily increment, our results do not align with the predictors of annual growth from this site (Teets et al., 2018). For example, annual growth of *P. rubens* and *T. canadensis* is best in years following wet and cool summers (Kosiba et al., 2018; Teets et al., 2018; Wason et al., 2019). Yet our results suggest that high VPD, not temperature, is a key factor in reducing *P. rubens* and *T. canadensis* daily probability of stem increment.

Given the monthly and yearly lagged growth effects of climate demonstrated in a significant body of research (Cuny et al., 2015; Richardson et al., 2013; Teets et al., 2018; Wason et al., 2019), it may be reasonable to assume that much of the radial increment we observed on a daily scale is attributable to turgor-driven expansion of cells rather than addition of recently assimilated carbon. However, there is also the possibility of carbon limitation driving lowered probability of radial increment, particularly in sub-canopy *P. rubens* whose photosynthesis may
be limited to longer days in early summer. Additionally, we saw no differences among species-level TLP despite differing responses to VPD and VWC among species, suggesting that water limitations to cell expansion are not the only drivers of growth at this site. On an ecosystem scale, annual woody biomass increment (used as a proxy for tree ring width) at Howland is more closely associated with eddy flux-detected carbon uptake during the previous growing season than the current season (Teets et al., 2022). Future directions may include using eddy flux data to examine the relationship between intraseasonal carbon flux and probability of radial increment on a daily scale, or using analysis of non-structural carbohydrates to parse out source and sink limitations to growth. The discrepancies between predictors of daily and annual growth at this site suggest that longer-term studies of the daily drivers of growth, and emphasis on the mechanisms by which climate influences growth, are warranted.

2.5.4 Conclusions

With the progression of climate change, it is becoming more critical to examine the climatic drivers of tree growth at various scales of space and time in order to predict how future forests will respond to these complex variables (Etzold et al., 2022; Wason et al., 2017). In the current study of three tree species with different life histories, resistance strategies, and geographic ranges, we found that vapor pressure deficit reduced daily radial increment in red spruce and eastern hemlock, while daylength increased daily radial increment in red maple. The way in which a tree responds to daily climate patterns gives us insight into not only the sensitivity of that tree and potential mechanisms by which climate impacts growth, but also how its population may respond to future climate change. Future studies should continue to explore the mechanisms by which climate drives physiology and growth to improve our understanding of climate change effects in forests.
EPILOGUE: CONNECTING THE IMPORTANCE OF CLIMATE INTERACTIONS TO PREDICTIONS FOR FUTURE FORESTS

Climate change is threatening forests, and the Acadian Forest region of Maine is experiencing fast and dramatic changes in average temperatures, precipitation regimes, and incidence of extreme climate events (Fernandez et al., 2020). Future forests of the northeastern United States have the potential to look very different in terms of species composition and associations due to potential shifts in suitable habitat (Hobbs et al., 2018; Janowiak et al., 2018). However, short-term but dramatic fluctuations in climate may also have detrimental effects on tree recruitment and growth that are not as apparent in models of long-term change (Bauweraerts et al., 2013; Jackson et al., 2009; Wright et al., 2018). As such, it is important to assess the effects of singular extreme climate events (i.e., drought and heat waves) on the growth and physiology of trees in order to predict how forests of the near future will respond to climate change extremes, in addition to examining the general responses of forest trees to a range of climate variables.

In these two chapters, we used two different approaches to examine the effects of climate conditions on tree growth and physiology. Chapter 1 used the flexibility and convenience of a greenhouse experiment to manipulate climate conditions experienced by seedlings in order to determine responses to singular extreme events. Because climate extremes increasingly co-occur in the form of hot drought, we designed a full factorial experiment examining the effects of individual stressors (drought and heat) as well as interactions of those stressors (Adams et al., 2009; Mitchell et al., 2014; Teskey et al., 2015). While we performed more intensive measurements on our seedlings in Chapter 1 than was possible in Chapter 2, our comparison of seedling growth was based on end-of-season values, likely after cell expansion and lignification had completed for all species (D’Orangeville et al., 2022; Duchesne & Houle, 2011; Rathgeber et
al., 2016). Conversely, Chapter 2 used a field study of mature trees to observe stem radius changes on a daily basis and model the probability of radial increment based on a given day’s climate conditions. Because of the differences in our study design and scale between these two chapters, it is not possible to directly compare one to the other. However, it bears noting the importance of manipulative experiments as well as observational studies in determining tree responses to climate, the limitations of each, and what can be learned using these diverse approaches.

In Chapter 1, we found that growth of red oak was not sensitive to heat or drought, although photosynthesis of red oak showed greater sensitivity to increasing temperatures at low VWC. This interaction was also apparent in red spruce, but not in paper birch, which was consistently sensitive to both temperature and VWC as individual drivers. Analysis in Chapter 1 benefited from the inclusion of species from all wood types (ring porous, diffuse porous, and nonporous) and varied geographic ranges. Chapter 2 was somewhat more limited in this manner, having only nonporous and diffuse porous wood types represented. Because research suggests that phenology and intraseasonal growth rates are quite different among all three wood types, future studies should take these differences into account by including a wider assortment of species in assessments of drivers of daily increment (D’Orangeville et al., 2022; Etzold et al., 2022). Future research with wider geographic range across the northeastern United States and Canadian maritime provinces may also capture a wider range of in situ climate conditions as well as a more diverse set of species.

In each chapter, we identified a species that was relatively insensitive to climate conditions in terms of its growth. In Chapter 1, our analysis of sensitivity was based on responses to imposed climate events: two droughts and a heat wave, in addition to the potential stress of species
associations within our planting treatments. In Chapter 2, we based our conclusions on species climate sensitivity on responses to observed climate patterns within a single season at a single site, which is closer to a case study approach than in Chapter 1. Although comparisons between studies with vastly different observational methods should be made with reservations, we did see that both species shown to be relatively “insensitive” to climate variables were ones predicted to thrive under climate change conditions, or to be resistant to stress of extreme climate events (Janowiak et al., 2018; Reich et al., 2018). In our Chapter 1 study, red oak was not sensitive to drought, heat, or their combination in its growth responses, although its sensitivity to compounded heat and water limitation in photosynthetic response should be taken into account during recovery year measurements, due to the possibility of reduced carbohydrate storage. In our Chapter 2 study, red maple showed greatest probability of growth on longer days, and a negative relationship with temperature, but was the only species to not show interaction effects with VPD_{max} or VWC. Red oak and red maple are also the species in each study with the broadest and most southerly geographic ranges (Sander, 1990; Weston & Bauerle, 2007), suggesting that our findings may support future predictions of the success of these species under climate change scenarios.

Red spruce was a common species between Chapter 1 and 2. In Chapter 1, we found that red spruce growth and vigor were sensitive to long drought but not heat, while red spruce photosynthesis was increasingly sensitive to heat under conditions of low soil moisture. In Chapter 2, red spruce showed a negative response to increasing soil moisture on early summer days. Interestingly, we observed some foliage dieback in well-watered spruce in Chapter 1, which we attributed to overwatering; however, this dieback was observed in the late summer and early fall. Future studies combining manipulative experiments with observational studies may
benefit from basing their tests of interaction effects in mature trees on sensitivities observed in seedlings. Future directions of this study may include testing for temperature and VWC interaction effects on daily growth to determine whether the interactions we observed in effects on photosynthesis scale to turgor-driven radial increment in mature trees. The single non-daylength interaction we tested in Chapter 2 was significant only for eastern hemlock, which was more likely to show radial increment on days of high \( VPD_{\text{max}} \) when VWC was also high, suggesting significant sensitivity to increasing atmospheric demand with climate change (Sanginés de Cárcer et al., 2018; Schönbeck et al., 2022).

It is not possible to predict the forests of the future with total accuracy based on models of what has been observed in the past (Jackson et al., 2009). However, it is apparent that the use of various observational methods and timescales can provide robust information about the ways in which trees respond to current and future conditions, both average and extreme, at various life stages. Short-term physiological and increment responses observed in this thesis shed light on the significance of compounded climate conditions as drivers of complex changes in trees that may not be apparent on a larger or longer-term scale. At the same time, understanding long-term responses to climate events is critical for predicting future species and their interactions in forests under climate change. As climate conditions shift rapidly and unpredictably in the northeastern United States, it will become more important than ever to take diverse approaches to research on forest responses to extremes.
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Takahashi, S., Okada, N., & Nobuchi, T. (2013). Relationship between the timing of vessel formation and leaf phenology in ten ring-porous and diffuse-porous deciduous tree


Figure A.1 (A) Heat chamber (left) and open-sided ambient control chamber (right) installed over two halves of an experimental block. All chambers were installed on day 8 of the drought period (DOY 189) to begin the heat wave period, and the heat wave period was ended by removing the side panels of the heat chambers (DOY 206). (B) A radiation shield-covered iButton datalogger deployed inside a chamber.
Figure A.2 Linear regression of number of red spruce needles vs. projected area, from a sample of 14 red spruce shoots sampled in July 2022. Dashed blue lines represent 95% confidence interval of the regression. Dashed green lines represent prediction interval for values of projected area at 95% CI.
Figure A.3 Average daily maximum vapor pressure deficit (VPD) in heat wave and ambient control chambers throughout the study period (DOY 182-234). Error bars represent ±1 standard error of the mean. Pink, orange, and red bars at the bottom of the plot indicate the duration of the heat wave, short drought, and long drought, respectively.
Figure A.4 Relative height growth (represented as a percentage of diameter at the beginning of the growing season) of each species: (A) red spruce (*Picea rubens*), (B) red oak (*Quercus rubra*), (C) paper birch (*Betula papyrifera*). Irrigated trees were kept above 10% VWC throughout the experiment, short and long drought trees received 25% of their container volume in water every four days for 15 days, and long drought trees continued to be droughted for another 15 days after the short drought, receiving 25% of their container volume of water once during this period. Single planted trees were planted alone in a 1-liter container; double planted trees were planted with an individual of red spruce in a 2-L container. Horizontal bars along the bottom of each panel denote the period of time during which we implemented the heatwave and drought treatments. Error bars represent ± 1 standard error of the mean.
**Figure A.5** End-of-season (EOS, DOY 273) relative diameter growth of red spruce (*Picea rubens*) by species that red spruce was planted with (planting treatment: double planted with red spruce, planted with red oak (*Quercus rubra*), planted with paper birch (*Betula papyrifera*), or planted singly). Single planted trees were planted alone in a 1-liter container; double planted trees were planted in a 2-L container. Different lowercase letters at the top of the figure indicate statistically significant differences among planting treatments.
Figure A.6 End-of-season (EOS, DOY 266) percent brown foliage assessment of (A) red spruce (*Picea rubens*), (B) red oak (*Quercus rubra*), and (C) paper birch (*Betula papyrifera*). Irrigated trees were kept above 10% VWC throughout the experiment, short and long drought trees received 25% of their container volume in water every four days for 15 days, and long drought trees continued to be droughted for another 15 days after the short drought, receiving 25% of their container volume of water once during this period. Percent brown foliage data were logit transformed prior to running statistics. Different lowercase letters indicate statistically significant differences between treatments.
Figure A.7 Calculated average minimum epidermal conductance ($g_{\text{min}}$) of red spruce (*Picea rubens*; $n = 9$), red oak (*Quercus rubra*; $n = 10$), and paper birch (*Betula papyrifera*; $n = 7$). Different lowercase letters indicate statistically significant differences among species.
Table A.1 Average starting heights and diameters by species (mean ± SE), for *Picea rubens*, *Quercus rubra*, and *Betula papyrifera*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Avg. starting height (cm)</th>
<th>Avg. starting diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. rubens</em> (n = 120)</td>
<td>28.3 ± 0.50</td>
<td>3.91 ± 0.09</td>
</tr>
<tr>
<td><em>Q. rubra</em> (n = 48)</td>
<td>21.7 ± 0.77</td>
<td>4.27 ± 0.19</td>
</tr>
<tr>
<td><em>B. papyrifera</em> (n = 48)</td>
<td>34.6 ± 1.62</td>
<td>3.28 ± 0.14</td>
</tr>
</tbody>
</table>

Table A.2 Best-fit models of stomatal conductance ($g_s$) as a function of leaf water potential ($\Psi_{leaf}$), used to determine $\Psi_{leaf}$ at 90% stomatal closure ($g_{s90}$) for study species red spruce (*Picea rubens*), red oak (*Quercus rubra*), and paper birch (*Betula papyrifera*).

<table>
<thead>
<tr>
<th>Species</th>
<th>Best fit model</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. rubens</em></td>
<td>$g_{s(P. rubens)} = \frac{0.15}{1 + e^{-743 \times \Psi_{leaf} - 804}} + 0.01$</td>
</tr>
<tr>
<td><em>Q. rubra</em></td>
<td>$g_{s(Q. rubra)} = \frac{0.11}{1 + e^{-23.6 \times \Psi_{leaf} - 54.9}} + 0.002$</td>
</tr>
<tr>
<td><em>B. papyrifera</em></td>
<td>$g_{s(B. papyrifera)} = \frac{0.18}{1 + e^{-53.8 \times \Psi_{leaf} - 63.7}}$</td>
</tr>
</tbody>
</table>
Figure B.1 Visual example of the zero-growth (ZG) concept of tree growth, under which the tree does not grow during periods of stem shrinkage, and exhibits radial increment when the new stem radius exceeds the previous maximum. Distance between the overall maximum (red dashed line) and daily maximum (black solid line) represents the magnitude of the tree water deficit, usually expressed in liters.
Figure B.2 Daily average values of (A) maximum vapor pressure deficit ($\text{VPD}_{\text{max}}$), (B) temperature ($T_{\text{avg}}$), (C) daylength, and (D) soil moisture as volumetric water content (VWC, %) at 10 cm belowground, for Howland Research Forest, May 18 to October 30, 2022.
Figure B.3 Water release curves of each species: (A) Acer rubrum (n = 10), (B) Picea rubens (n = 10), (C) Tsuga canadensis (n = 10) based on water release dry-down procedure (McCulloh et al., 2014; Meinzer et al., 2003). Capacitance (C, kg m\(^{-3}\) MPa\(^{-1}\)) was calculated with a linear model of the straight part of the curve, at \(\Psi\) values greater than -0.55 MPa.
Table B.1 Average environmental conditions by month at Howland Forest, Maine: maximum vapor pressure deficit (VPD\textsubscript{max}), daylength, soil moisture (% volumetric water content, VPD), and average temperature (T\textsubscript{avg}). Values are mean $\pm$ one standard error (SE).

<table>
<thead>
<tr>
<th>Month</th>
<th>VPD\textsubscript{max} (kPa)</th>
<th>Daylength (hours)</th>
<th>VWC (%)</th>
<th>T\textsubscript{avg} (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>1.55 ± 0.14</td>
<td>15.58 ± 0.01</td>
<td>25.0 ± 0.3</td>
<td>17.1 ± 0.1</td>
</tr>
<tr>
<td>July</td>
<td>2.23 ± 0.09</td>
<td>15.27 ± 0.04</td>
<td>20.0 ± 0.4</td>
<td>21.8 ± 0.09</td>
</tr>
<tr>
<td>August</td>
<td>1.56 ± 0.13</td>
<td>14.09 ± 0.08</td>
<td>18.6 ± 0.7</td>
<td>20.7 ± 0.09</td>
</tr>
</tbody>
</table>
Table B.2 Characteristics of all trees in the study at Howland Research Forest, ME: individual dendrometer ID, species, canopy class (2 = dominant; 3 = co-dominant; 5 = overtopped; 6 = suppressed), canopy category (C = canopy, classes 2-3; S = sub-canopy, classes 5-6), 2022 basal area increment (BAI), and 2022 start-of-season diameter at breast height (DBH).

<table>
<thead>
<tr>
<th>Dendrometer ID</th>
<th>Species</th>
<th>Canopy class</th>
<th>Canopy category</th>
<th>2022 BAI (cm²)</th>
<th>April 2022 DBH (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>92219201</td>
<td>Acer rubrum</td>
<td>3</td>
<td>C</td>
<td>2.104989</td>
<td>22.0</td>
</tr>
<tr>
<td>92222372</td>
<td>Acer rubrum</td>
<td>2</td>
<td>C</td>
<td>8.830571</td>
<td>31.0</td>
</tr>
<tr>
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<td>Acer rubrum</td>
<td>2</td>
<td>C</td>
<td>3.596875</td>
<td>35.7</td>
</tr>
<tr>
<td>92222382</td>
<td>Acer rubrum</td>
<td>2</td>
<td>C</td>
<td>2.524897</td>
<td>17.7</td>
</tr>
<tr>
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<td>Acer rubrum</td>
<td>3</td>
<td>C</td>
<td>4.322443</td>
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</tr>
<tr>
<td>92222387</td>
<td>Acer rubrum</td>
<td>3</td>
<td>C</td>
<td>4.456236</td>
<td>24.0</td>
</tr>
<tr>
<td>92222390</td>
<td>Acer rubrum</td>
<td>3</td>
<td>C</td>
<td>2.013647</td>
<td>22.0</td>
</tr>
<tr>
<td>92222391</td>
<td>Acer rubrum</td>
<td>3</td>
<td>C</td>
<td>2.003396</td>
<td>11.8</td>
</tr>
<tr>
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<td>Acer rubrum</td>
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<td>C</td>
<td>2.779169</td>
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<tr>
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<td>C</td>
<td>0.361086</td>
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<tr>
<td>92219202</td>
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<td>C</td>
<td>4.726775</td>
<td>31.4</td>
</tr>
<tr>
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<td>C</td>
<td>7.554607</td>
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</tr>
<tr>
<td>92222375</td>
<td>Picea rubens</td>
<td>6</td>
<td>S</td>
<td>0.287902</td>
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</tr>
<tr>
<td>92222378</td>
<td>Picea rubens</td>
<td>5</td>
<td>S</td>
<td>0.65354</td>
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</tr>
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<tr>
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<tr>
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<td>S</td>
<td>0.695845</td>
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<tr>
<td>92222377</td>
<td>Tsuga canadensis</td>
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<td>S</td>
<td>0.252717</td>
<td>15.0</td>
</tr>
<tr>
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<td>2</td>
<td>C</td>
<td>2.5168</td>
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<td>Tsuga canadensis</td>
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<td>S</td>
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<td>C</td>
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<td>C</td>
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<td>C</td>
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<tr>
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<td>Tsuga canadensis</td>
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<td>C</td>
<td>3.254792</td>
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Table B.3  Environmental predictor statistics (mean, 1\textsuperscript{st} quartile value, 3\textsuperscript{rd} quartile value) used in prediction models for visualization of modeled relationships for each species (\textit{Acer rubrum}, \textit{Picea rubens}, \textit{Tsuga canadensis}): maximum vapor pressure deficit (VPD\textsubscript{max}), daylength, soil moisture (% volumetric water content, VWC), and average temperature (T\textsubscript{avg}). 1\textsuperscript{st} and 3\textsuperscript{rd} quartile values of each predictor were used for “low” and “high” or “short” and “long” values, while means of each predictor were used as constants when that variable was not used in the prediction dataset. Values were scaled for comparisons during analysis, then un-scaled and actual values used for visualization.

<table>
<thead>
<tr>
<th>Species</th>
<th>Predictor</th>
<th>Statistic</th>
<th>Scaled value</th>
<th>Actual value</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{A. rubrum}</td>
<td>VPD\textsubscript{max} (kPa)</td>
<td>Mean</td>
<td>0.01</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.66</td>
<td>1.31</td>
</tr>
<tr>
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<td>3\textsuperscript{rd} Qu. Value</td>
<td>0.84</td>
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<tr>
<td></td>
<td>Daylength (hours)</td>
<td>Mean</td>
<td>-0.05</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.39</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3\textsuperscript{rd} Qu. Value</td>
<td>0.69</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>VWC (%)</td>
<td>Mean</td>
<td>-0.08</td>
<td>21.1</td>
</tr>
<tr>
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<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.61</td>
<td>19.0</td>
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<tr>
<td></td>
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<td>3\textsuperscript{rd} Qu. Value</td>
<td>-0.74</td>
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<tr>
<td></td>
<td>T\textsubscript{avg} (°C)</td>
<td>Mean</td>
<td>0.04</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.49</td>
<td>17.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3\textsuperscript{rd} Qu. Value</td>
<td>0.68</td>
<td>22.1</td>
</tr>
<tr>
<td>\textit{P. rubens}</td>
<td>VPD\textsubscript{max} (kPa)</td>
<td>Mean</td>
<td>0.0002</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.66</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3\textsuperscript{rd} Qu. Value</td>
<td>0.81</td>
<td>2.40</td>
</tr>
<tr>
<td></td>
<td>Daylength (hours)</td>
<td>Mean</td>
<td>0.03</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.39</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3\textsuperscript{rd} Qu. Value</td>
<td>-0.65</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>VWC (%)</td>
<td>Mean</td>
<td>-0.04</td>
<td>21.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.68</td>
<td>18.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3\textsuperscript{rd} Qu. Value</td>
<td>-0.89</td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td>T\textsubscript{avg} (°C)</td>
<td>Mean</td>
<td>0.04</td>
<td>19.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.54</td>
<td>17.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3\textsuperscript{rd} Qu. Value</td>
<td>0.67</td>
<td>22.1</td>
</tr>
<tr>
<td>\textit{T. canadensis}</td>
<td>VPD\textsubscript{max} (kPa)</td>
<td>Mean</td>
<td>-0.01</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.67</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3\textsuperscript{rd} Qu. Value</td>
<td>-0.83</td>
<td>2.41</td>
</tr>
<tr>
<td></td>
<td>Daylength (hours)</td>
<td>Mean</td>
<td>0.02</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.13</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3\textsuperscript{rd} Qu. Value</td>
<td>0.68</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>VWC (%)</td>
<td>Mean</td>
<td>0.11</td>
<td>21.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
<td>-0.49</td>
<td>19.5</td>
</tr>
<tr>
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<td></td>
<td>3\textsuperscript{rd} Qu. Value</td>
<td>-0.95</td>
<td>25.3</td>
</tr>
<tr>
<td></td>
<td>T\textsubscript{avg} (°C)</td>
<td>Mean</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1\textsuperscript{st} Qu. Value</td>
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<td>16.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3\textsuperscript{rd} Qu. Value</td>
<td>0.67</td>
<td>22.1</td>
</tr>
</tbody>
</table>
Table B.4 Final generalized linear mixed effects models for each species (*Acer rubrum*, *Picea rubens*, *Tsuga canadensis*) with random effects for day of year (‘DOY’) and individual tree (‘series’), their predictors and significance values, and marginal (m) and conditional (c) $R^2$ values of each final model calculated using R package ‘MuMIn’ in RStudio.

<table>
<thead>
<tr>
<th>Species</th>
<th>Predictor/expression</th>
<th>$p$-value</th>
<th>Final model of $P_D$</th>
<th>$R^2_m$</th>
<th>$R^2_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. rubrum</em></td>
<td>$T_{avg}$</td>
<td>&lt; 0.001 ***</td>
<td>$P_D \sim T_{avg} + VPD_{max} + VWC + \text{daylength} + (VWC \times \text{daylength}) + (1 \mid \text{DOY}) + (1 \mid \text{series})$</td>
<td>0.31</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>VPD$_{max}$</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VWC</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>daylength</td>
<td>&lt; 0.001 ***</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>canopy category</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VWC $\times$ daylength</td>
<td>0.006 **</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. rubens</em></td>
<td>$T_{avg}$</td>
<td>0.51</td>
<td>$P_D \sim T_{avg} + VPD_{max} + VWC + \text{daylength} + \text{canopy cat.} + (VWC \times \text{daylength}) + (\text{canopy cat.} \times \text{daylength}) + (1 \mid \text{DOY}) + (1 \mid \text{series})$</td>
<td>0.17</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>VPD$_{max}$</td>
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<td>canopy category</td>
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<tr>
<td></td>
<td>VWC $\times$ daylength</td>
<td>0.02 *</td>
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<tr>
<td></td>
<td>canopy $\times$ daylength</td>
<td>&lt; 0.001 ***</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>T. canadensis</em></td>
<td>$T_{avg}$</td>
<td>0.53</td>
<td>$P_D \sim T_{avg} + VPD_{max} + VWC + \text{daylength} + \text{canopy cat.} + (VPD_{max} \times \text{daylength}) + (VWC \times \text{daylength}) + (VPD_{max} \times VWC) + (1 \mid \text{DOY}) + (1 \mid \text{series})$</td>
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<td>VPD$_{max}$ $\times$ daylength</td>
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<tr>
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<td>VWC $\times$ daylength</td>
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<td>VPD$_{max}$ $\times$ VWC</td>
<td>0.01 *</td>
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<td></td>
</tr>
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</table>
BIOGRAPHY OF THE AUTHOR

Alexandra (Alley) Barry was born in North Carolina and raised in California and Virginia. She has been hiking since she could walk, and before that used her parents as pack mules to explore the likes of Big Sur and Shenandoah National Park. Originally a pre-medicine biology major at Virginia Commonwealth University (VCU), Alley realized her passion for ecological research after working as a field assistant for vegetation surveys as a sophomore. She completed her senior thesis on reproductive dynamics of the spongy moth (*Lymantria dispar* L.) with Dr. Derek Johnson and spent three summers as a field technician and collaborator on the Forest Resilience Threshold Experiment (FoRTE) project at the University of Michigan Biological Station with Dr. Chris Gough.

Alley graduated from VCU in 2019 with a B.S. in Biology and Psychology and spent a year teaching middle school life science before moving to Orono, Maine to pursue an M.S. in Forest Resources with Dr. Jay Wason. Alexandra is a candidate for the Master of Science degree in Forest Resources from the University of Maine in August 2023.