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Tree Responses to Moderate and Extreme Drought in the Northeastern United States

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TREE RESPONSES TO MODERATE AND EXTREME DROUGHT IN THE NORTHEASTERN UNITED STATES

By

Ruth van Kampen

B.S. Biology, Bates College, 2019

A Thesis

Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science
(in Forest Resources)

The Graduate School
The University of Maine
August 2021

Advisory Committee:

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TREE RESPONSES TO MODERATE AND EXTREME DROUGHT IN THE NORTHEASTERN UNITED STATES

By Ruth A. van Kampen

Thesis Advisor: Dr. Jay Wason

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
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Climate change is expected to lead to novel drought conditions in the Northeastern United States. Therefore, experimental studies that mimic these conditions are crucial to understand the potential impact on forests. Further, recent large scale dendrochronological studies suggest that spring and summer droughts may immediately impact tree growth while fall droughts may cause delayed impacts on growth the following growing season. Therefore, in this study, we investigated the impacts of six-week-long spring, summer, and fall droughts on the physiology and intra-annual growth on 288 saplings of six tree species native to the Northeastern United States. These species (deciduous broadleaf angiosperms, hereafter "broadleaf": *Acer rubrum* L., *Betula papyrifera* Marsh., *Prunus serotina* Ehrh.; and coniferous evergreen gymnosperms, hereafter "conifer": *Juniperus virginiana* L., *Pinus strobus* L., and *Thuja occidentalis* L.) represent different anticipated drought tolerances and projected abundances with

climate change according to previous studies. Additionally, we used experimental dry-downs of seventy-one leafy shoots and seventeen xylem segments to assess how structural and physiological adaptations of each species relate to water use during an extreme drought.

We observed marked differences in how the growth patterns of these six species responded to seasonal droughts. Spring and summer droughts generally caused height growth rate reductions for all species. Negative impacts on height growth were stronger for trees that had higher water-use and therefore experienced drought sooner. Importantly, some species such as *A. rubrum*, *Pr. serotina*, and *T. occidentalis* were able to compensate for these height growth reductions during spring and summer droughts with more rapid post-drought height growth. We also found that spring and summer droughts for *Pr. serotina*, *Pi. strobus*, and *T. occidentalis* resulted in reductions in diameter growth rates but only post-drought. Interestingly, these three species were not able to compensate for this decrease in diameter growth, which remained low throughout the rest of the growing season. These high-resolution data on intra-annual growth rates of trees in response to seasonal droughts reveal details about the growth phenology that supports and extends our understanding of annual resolution tree ring studies at larger scales.

In the benchtop dry-down experiment that simulated an extreme drought, we found that leafy shoots of conifer species dried more slowly than leafy shoots of broadleaf species. In general, conifer species lost water at equal rates between leaves and stems. In contrast, deciduous species lost water very quickly and experienced larger reductions in leaf water content compared to stem water content. We saw evidence of drought-deciduousness in our greenhouse experiment where *B. papyrifera* was the fastest to dry-down, and in two instances, its cambia remained hydrated enough to re-flush an additional cohort of leaves post-drought. On the other hand, conifers were slow to dry-down in the greenhouse experiment, only experiencing moderate drought by the end of each drought period. The clear division in response between fast-drying broadleaved deciduous angiosperm species and slow-drying needle-leaved evergreen conifers may be partly driven by lower leaf area per shoot of conifer species, which we observed in the simulated extreme drought experiment.

In the mixed wood forests common in the northeastern United States, stands may respond to drought in different ways depending on the species present. For example, we observed very different responses to growth and to some extent, recovery, in our species, and in a stand with species showing different responses, competitive dynamics may be altered among these species as the climate continues to change. Acknowledging that tree responses to drought as individuals and as communities may not align will be important moving forward from studies like these, which define drought responses of individual species, to studies which observe drought responses of entire forests.

UNIVERSITY OF MAINE LAND ACKNOWLEDGEMENT

The University of Maine recognizes that it is located on Marsh Island in the homeland of the Penobscot Nation, where issues of water and territorial rights, and encroachment upon sacred sites, are ongoing. Penobscot homeland is connected to the other Wabanaki Tribal Nations — the Passamaquoddy, Maliseet, and Micmac — through kinship, alliances and diplomacy. The university also recognizes that the Penobscot Nation and the other Wabanaki Tribal Nations are distinct, sovereign, legal and political entities with their own powers of self-governance and self-determination.

ACKNOWLEDGEMENTS

I would like to acknowledge the community of researchers, colleagues, friends, and family who have helped me along in graduate school and my life beside it for the past two years. First, to my advisor Jay Wason, who has read thousands of words in drafts, replied to numerous Slack messages, and who is understanding beyond belief, thank you for your mentorship.

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LIST OF ABBREVIATIONS

MPa	Megapascal
NE US	Northeastern United States
P_{50}	Pressure at which a stem loses 50% of its conductivity
PLC	Percent loss of conductivity
RCBD	Randomized complete block design
RDG	Relative diameter growth
RHG	Relative height growth
RWC	Relative water content
RWC_{leaf}	Leaf relative water content
RWC_{shoot}	Shoot relative water content
RWC_{stem}	Stem relative water content
SMA	Standardized major axis
TCP	Thermocouple psychrometer
VPD	Vapor pressure deficit
VWC	Volumetric water content
Ψ_{leaf}	Leaf water potential
Ψ_{MD}	Midday water potential

Ψ_{PD}	Predawn water potential
Ψ_{shoot}	Shoot water potential
Ψ_{stem}	Stem water potential

CHAPTER 1: DROUGHT TIMING IMPACTS ON INTRA-ANNUAL GROWTH RATES OF SIX NORTHEASTERN UNITED STATES FOREST TREE SPECIES

DroughtTIME
Drought Timing Impacts in Maine

Study logo designed by Douglas A. van Kampen

1.1 ABSTRACT

Recent large-scale dendrochronological studies suggest that spring and summer droughts may impact tree growth in the current year while fall droughts may cause delayed impacts on growth the following growing season. Although this information is critical to better anticipate climate change impacts on trees, we lack a mechanistic understanding of how plant water relations drive these impacts on growth. Therefore, in this study, we investigated the impacts of spring, summer, and fall droughts on the physiology and intra-annual growth on 288 saplings of six tree species native to the Northeastern United States. These species (deciduous broadleaf angiosperms (hereafter “broadleaf”: *Acer rubrum, Betula papyrifera, Prunus serotina;* and coniferous evergreen gymnosperms (hereafter “conifer”: *Juniperus virginiana, Pinus strobus,* and *Thuja occidentalis*) have a variety of drought tolerances, predicted abundances, and wood types. Soil moisture levels reached similarly low levels during all droughts, and led to marked differences in how the growth patterns of these six species responded to seasonal droughts. Spring and summer droughts generally caused height growth reductions for all species; importantly, some species such as *A. rubrum*, *Pr. serotina*, and *T. occidentalis* were able to compensate for these growth reductions later in the growing season. We did see temporary diameter growth reductions in *Pr. serotina*, *Pi. strobus*, and *T. occidentalis* following spring and summer drought. Interestingly, these species were not able to compensate for this decrease and growth, and their diameter growth remained lower than the controls through the growing season. The results of this study define the seasonal growth patterns of the six study species and provide not only detailed diameter measurements, but also high-resolution height measurements during seasonal drought.

1.2 INTRODUCTION

In the Northeastern United States (hereafter NE US), average summer temperatures and the likelihood of summer drought are expected to increase (Vose et al. 2019), and short- and long-term drought events are expected to increase in frequency by two to three times (Wake et al. 2014) partly due to declines in summer rainfall, increased heat, and an increase in evapotranspiration (Hayhoe et al. 2007, Vose et al. 2019). Drought can lead to decreased gross primary productivity and carbon storage (Allen et al. 2010, McDowell and Allen 2015, D’Orangeville et al. 2018). Importantly, many tree species in this region may lack adaptations to withstand the expected novel future drought conditions (Liénard et al. 2016). As the climate continues to change, the likelihood of extreme drought is expected to increase. Therefore, there is a need for experimental research that simulates novel future climate conditions to better predict tree responses.

Studies of recent droughts suggest that the timing of drought relative to species phenology is key for predicting diameter growth responses in current and subsequent years (Noormets et al. 2008, Huang et al. 2018, Kannenberg, Maxwell, et al. 2019). Early in the growing season, trees primarily allocate resources to diameter growth. Therefore, diameter growth is often sensitive to spring droughts (D'Orangeville et al. 2018). In contrast, later in the growing season when less diameter growth tends to occur, diameter growth is less sensitive to droughts of the same length (D'Orangeville et al. 2018). Further, studies conducted on lagged (or "legacy") effects of droughts reveal that immediate effects of drought may be greatest for early summer droughts, while late-summer droughts have greater effects the following year (Foster et al. 2014, Kannenberg, Maxwell, et al. 2019, Au et al. 2020). These studies have been retrospective studies of tree rings, without focusing on the physiology behind the impacts of drought timing and other aspects of growth like height, biomass, and allocation to roots and shoots. Therefore, the physiology explaining these widespread patterns and how they differ by key functional types is not clear.

The impacts of drought timing are likely to vary between functional groups due to variation in tree phenology. Bud break and the initiation of vertical growth occurs in the spring, and is usually later for conifer species than deciduous species (Richardson et al. 2009, Osada 2017). Therefore, droughts in the spring may impact the growth of conifer and deciduous species differently. Further, in some conifer species, the maximum diameter growth rate occurs in early June (balsam fir; Duchesne et al. 2012), and seasonal variation in peak diameter growth rate is largely driven by water availability (Delpierre et al. 2016, Oberhuber 2017). Therefore, seasonal water use differences may drive differential responses of trees to droughts occurring at different points in the growing season and either stall or delay those effects depending on their functional group and species-specific phenology.

Much of the NE US is covered by mixed-wood forests of both conifer coniferous and broadleaved deciduous species. Therefore, understanding how conifers and deciduous tree species will respond to novel drought conditions is important to consider for the future management of these forests. Importantly, most studies of drought timing have focused only on the radial growth of large canopy trees. Therefore, there is limited knowledge on how drought timing impacts other aspects of growth like height and biomass allocation to roots and shoots. Additionally, small size classes of trees like saplings may be even more sensitive to the timing of drought since they usually have shallower roots and less carbon storage. To investigate the effects of drought timing on northeastern United States tree species, we conducted a greenhouse study to estimate at what time of year saplings of six species, *Acer rubrum, Betula papyrifera, Prunus serotina, Juniperus virginiana, Pinus strobus,* and *Thuja occidentalis* (Table 1) are most vulnerable to moderate droughts. Our objectives were to 1) discern how droughts occurring in the spring, summer, and fall may impact height and diameter growth, and 2) determine the differences in severity between droughts in the spring, summer, or fall.

1.3 MATERIALS & METHODS

1.3.1 Experimental Design

To determine the effects of drought timing on the hydraulics and growth of tree species native to the NE US, we studied six species with a range of drought tolerances to include species with low, moderate, and high drought resistance (Table 1). Additionally, some of these species are expected to gain, lose, or maintain suitable habitat in the northeast as climatic conditions shift and as habitats in Maine become more or less suitable for their survival (Prasad et al. 2007, Janowiak et al. 2018). This study used a balanced design of species with each of these traits.

Table 1.1. Study species, degree of drought tolerance (Peters et al. 2015), predicted change in New England (Janowiak et al. 2018), leaf longevity, tree type, and relative position within each species' range found in central Maine (Burns & Honkala, 1990; Matthews et al., 2018).

Species	Drought tolerance	Projected change in habitat suitability	Leaf longevity	Tree type	Maine's location within native range
Acer rubrum	Moderate	Stable	Deciduous	Angiosperm	Central
Betula papyrifera	Low	Decrease	Deciduous	Angiosperm	Southern
Prunus serotina	High	Increase	Deciduous	Angiosperm	Northern
Juniperus virginana	High	Increase	Conifer	Gymnosperm	Northern
Pinus strobus	Moderate	Stable	Conifer	Gymnosperm	Central
Thuja occidentalis	Low	Decrease	Conifer	Gymnosperm	Southern

A 6m × 11m high-tunnel greenhouse was constructed on the University of Maine campus in Orono, Maine in May 2019, in which 288 individually irrigated saplings were planted in individual 19-liter containers with seven 1-inch drainage holes. The saplings were arranged in a randomized complete block design with 12 experimental blocks of 24 saplings each (288 saplings in total; Fig. 1.1A). Thirty- to sixty-cm tall saplings (Cold Stream Farm, Free Soil, MI) were planted on May 29, 2019 in a nursery mix consisting of fine aged pine bark and sphagnum peat (Jolly Gardener, Poland Spring, ME) with fertilizer (5.9 g/L Osmocote 18-6-12). For the first year of growth in the greenhouse, the greenhouse cover was left open, and saplings experienced ambient rainfall and climatic conditions and were each irrigated 7.6 L three times per week.

To avoid freezing damage to roots of containerized plants, individual blocks were insulated with straw in between the containers and a thin layer on top of the soil surface, and 1"-thick foam insulation boards surrounding the block when nighttime temperatures were consistently below freezing (November-May). Soil temperature was monitored over winter using iButtons (iButtonLink LLC., DS1921G Thermochron, Whitewater, WI) buried in the center of six randomly chosen containers. iButtons were deployed on November 4, 2019 and set to log every 180 minutes beginning at 19:00 and suggested that soil temperatures reached below -5 °C only five times throughout the winter (Fig. A1.1). Soil temperatures down to -5 °C have been shown to not directly damage cold-hardened fine roots in a northern hardwood forest (Tierney et al. 2001), and we did not observe obvious mortality of any of our trees from freezing damage to roots.

Prior to initiating the drought treatments, 98% of the *A. rubrum*, 71% of *Pr. serotina*, and 2% of *T. occidentalis* experienced either frost damage, browse, or vole damage to above ground shoots. The remaining species were unaffected. Therefore, in all the following analyses, cross-species comparisons including these species are interpreted with this potential confounding effect in mind. However, due to the regrowth of most damaged trees, treatment comparisons within species are still possible. Trees that were dead at the start of the drought experiment (4.5% across all species) were removed and not included in the analysis, as well as their experimental block.

1.3.2 EXPERIMENTAL TREATMENTS

To understand tree responses to drought at different points in the growing season, three different 6-week droughts were imposed starting on June 2 (spring), July 14 (summer), and August 25, 2020 (fall). We chose six-week droughts since we aimed to impose extreme droughts on the saplings. Each experimental block (Fig. 1.1B) contained all six species and four individuals of each species, such that each block only had one individual replicate of each unique species and treatment combination. Species and treatment combinations were randomly arranged within each block. To occlude precipitation and impose the drought, a polyvinyl greenhouse covering was installed over the structure in the spring of 2020, and 1.2 m sidewalls were left open to limit heating and facilitate air circulation (Fig. 1.1C). Weeds growing in containers with saplings were removed weekly by cutting at the soil surface. Trees were irrigated 3.8 L nightly in 2020, and soil moisture (volumetric water content; VWC) was measured twice weekly in each container using a soil moisture meter with 20 cm probes (HydroSense II Handheld Soil Moisture Sensor, Campbell Scientific, Logan, UT) inserted at the soil surface.

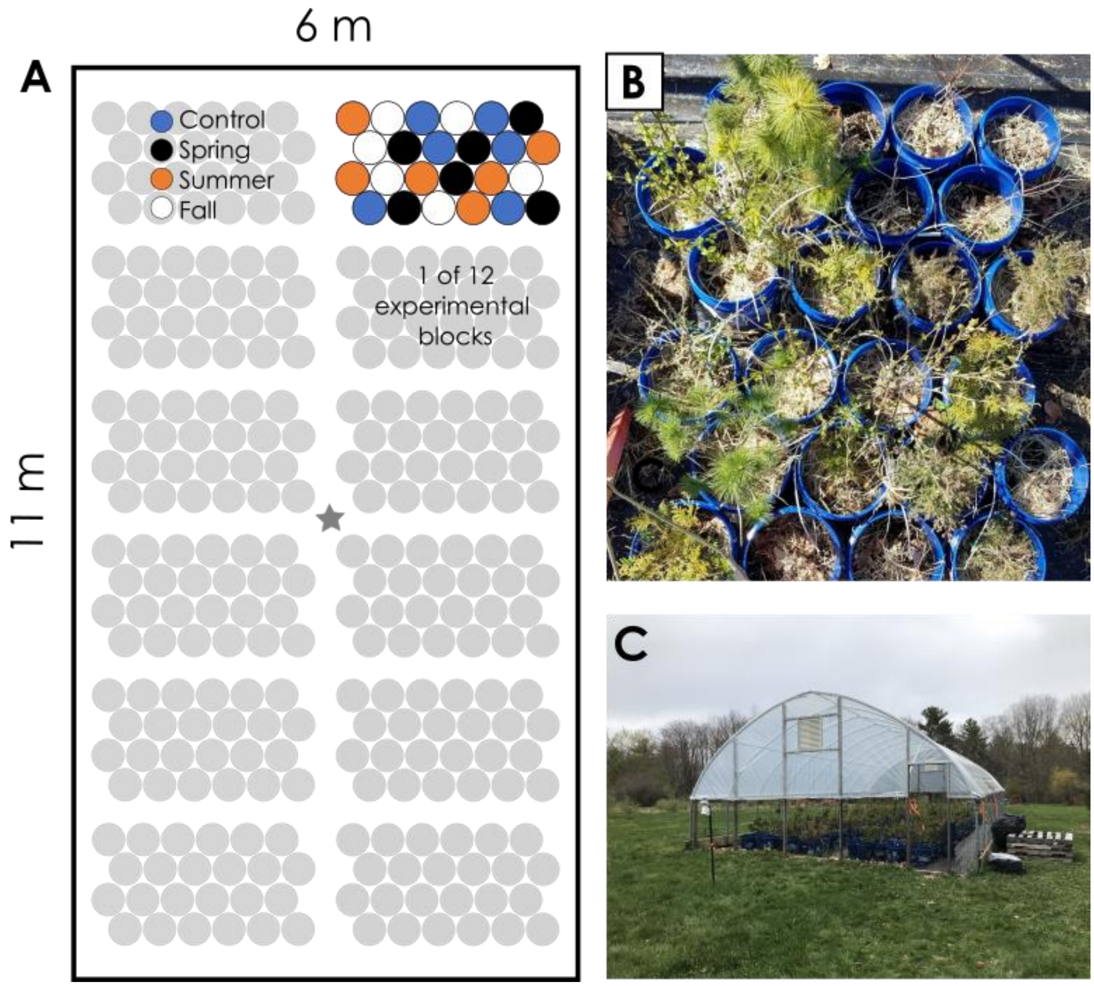

Figure 1.1. A) Experimental layout of 6 m × 11 m high tunnel greenhouse. 288 saplings were arranged in 12 blocks in a randomized complete block design (RCBD), with each species and treatment combination appearing once per block, for a total of 12 replicates per species and treatment combination. Stars denote the location of data loggers with temperature and humidity sensors. B) One experimental block of saplings in May 2020. C) Greenhouse in May 2020 after installing the greenhouse covering.

Air temperature and relative humidity were logged hourly inside and outside of the greenhouse using dataloggers (HOBO Onset #MX2302A, Bourne, MA; and iButtons). Temperatures inside and outside the greenhouse were monitored through the entirety of the experiment with iButtons, while temperature and humidity were logged inside and outside of the greenhouse in June using the HOBO dataloggers. In June, the average hourly temperature inside the greenhouse ranged from -1.18° to 4.72°C warmer than ambient (Fig. A1.2A; mean ± SE: 1.19°C ± 0.055°C). Also, the hourly vapor pressure deficit (VPD, units = kPa) inside the greenhouse ranged from -0.2 kPa to 1.3 kPa higher than ambient (Fig. A1.2B; mean ± SE: 0.2 kPa ± 0.01 kPa). We found that the maximum daily temperature in the greenhouse was higher in the spring and summer months compared to the fall (Fig. A1.3A, TukeyHSD, $p < 0.001$), while VPD was higher in the summer than in the spring (Fig. A1.3B, t-test, $p < 0.001$). The hotter and drier conditions in the greenhouse over the summer mimicked future climate conditions saplings in the NE US are expected to experience.

1.3.3 PHYSIOLOGICAL MEASUREMENTS

To estimate water stress in saplings, predawn leaf water potential (Ψ_{PD}) was measured on a subset of trees from each species and treatment during the first day of the drought and each week thereafter. Samples were collected at least one hour before the first light (3:30 AM - 5:30 AM) from two randomly selected blocks by cutting one leaf from the control and droughted tree from each species with a fresh razor blade, totaling 24 leaves in each sampling effort. On conifer species, a one-year-old fascicle was collected for *Pi. strobus,* and for *J. virginiana and T. occidentalis*, a two-centimeter sample was collected from the terminal end of a lateral branch. Samples were immediately stored in foil-lined bags and kept in a cooler until they were transported to the lab. Water potentials were then measured using a pressure chamber (PMS Instruments; Model 1000; Albany, Oregon). Predawn relative water content of the leaves (RWC_{leaf}) was estimated using the fresh mass of the leaf prior to the Ψ_{PD} measurement compared to the oven dry mass of the leaf, using the equation $RWC_{leaf} = (fresh – dry)/dry$. To avoid water loss of the leaf before the water potential measurement, leaves were kept in bags during the mass measurements, and bag mass was subtracted later. Samples were weighed using an analytical balance (Sartorius Practum 224-1S, 0.0001 g, Goettingen, Germany). Midday leaf water potentials (Ψ_{MD}) were also measured in the third and sixth weeks of each drought to determine the maximum water stress saplings experienced. Samples were collected as above between 12:30 and 2:30 PM and brought back to the lab as described above for Ψ_{MD} and RWC_{MD} measurements.

To determine how droughts may impact leaf photosynthetic ability, predawn quantum yield was measured on leaves in situ immediately before they were excised for Ψ_{PD} measurements using a Fluorpen (FP 110, Drásov, Czech Republic). Additionally, chlorophyll content was estimated during each drought using a chlorophyll meter once per week on the broadleaved species (OptiSciences CCM-200, Hudson, NH). Three measurements per sapling were taken and later averaged.

1.3.4 GROWTH ESTIMATES

The height and diameter of each sapling were measured weekly throughout the growing season. Height was measured vertically from the soil surface to the tallest living bud. Two perpendicular diameter measurements were conducted with manual calipers at 10 cm above the soil surface and averaged to account for non-circular stems.

The week of October 13, 2021, each sapling was harvested for biomass estimates. Roots and shoots were separated in the field and roots were rinsed twice in water to remove excess soil. All samples were kept in drying rooms for at least one week and then dried at 60°C in a drying oven for at least an additional 48 hours before weighing. Broadleaved trees were split into roots and shoots (many leaves had fallen before this date), and conifers were split into roots, shoots, and leaves. Individual *Pi. strobus* fascicles were counted as leaves and *T. occidentalis* and *J.*

virginiana leaves were defined as any part of the shoot that was still green. Therefore, total biomass was estimated using the sum of the shoot mass and root mass for each individual. Samples were weighed using an analytical balance (Sartorius Practum 3102-1S, 0.01 g).

1.3.5 STATISTICAL ANALYSIS

To test the difference in temperature and VPD inside the greenhouse between the spring and summer drought treatments, we used Welch's t-test. To determine the rate of soil moisture decline among species and treatment over the course of each drought, we initially used a linear mixed effects model with species, treatment, and day of drought as covariates using the R package 'nlme' (DebRoy et al. 2021). We then modeled the relationship between soil moisture and water potential using negative exponential models to Ψ_{PD} or Ψ_{MD} as a function of soil moisture for each species and estimated the soil moisture at the end of each drought using species-level ANOVAs. To capture differences in the initial decline in soil moisture, we also tested soil moisture in each species and treatment at approximately two weeks into each drought using an ANOVA with Tukey's post-hoc test. We then evaluated the effect of biomass on final soil moisture by plotting the residuals of the above-described ANOVA models to biomass measurements to determine if biomass was important in explaining the variation in final soil moisture for the study species.

To describe the effect of treatment on height and diameter growth, we used species-level Welch's t-tests to test the relative height or diameter for each treatment on each day of measurement as a percent of its starting height or diameter compared to that of the control. Individual measurements that were clearly errors (major increase in height followed by immediate decrease and vice versa) were removed prior to analysis (13.75% diameter measurements and 1.9% height measurements were removed). We compared the relative height or diameter growth of the treatment trees to the control on each day for each species. Finally, end-of-season biomass and root to shoot ratio differences among species and treatments were tested using an ANOVA. All data and figures were analyzed ($\alpha = 0.05$) and produced using R Version 4.0.3 (R Core Team 2021). Other packages used in data organization, visualization, and analysis were: ‘dplyr’ (Wickham et al. 2018), ‘lubridate’ (Spinu et al. 2021), ‘pivottabler’ (Bailiss 2021), ‘doBy’ (Halekoh 2021), ‘multcomp’ (Hothorn et al. 2021), and ‘emmeans’ (Lenth 2021).

1.4 RESULTS

We found that the initial differences in soil moisture were smaller during the spring drought than during the summer and fall droughts in all species except *Pi. strobus* and *J. virginiana* (on day 13/14 of the drought; Fig. 1.2, $p < 0.05$). Soil moisture values on the final day of each treatment (day 41 or 42) did not differ among drought treatments, but all treatments were significantly lower than the control (Fig. 1.2 & Fig. 1.3). Leaf Ψ_{PD} declined for all species when the soil moisture reached values below ~5% regardless of the season of drought (Fig. 1.4). We found a significant interaction between day of drought, treatment, and species on Ψ_{PD} ($p < 0.001$). Due to the complexity of interpreting three-way interactions that suggest important effects of all three predictors that depend on the values of the others, we focus our results on within-species comparisons of treatment effects on minimum soil moisture at the end of each drought.

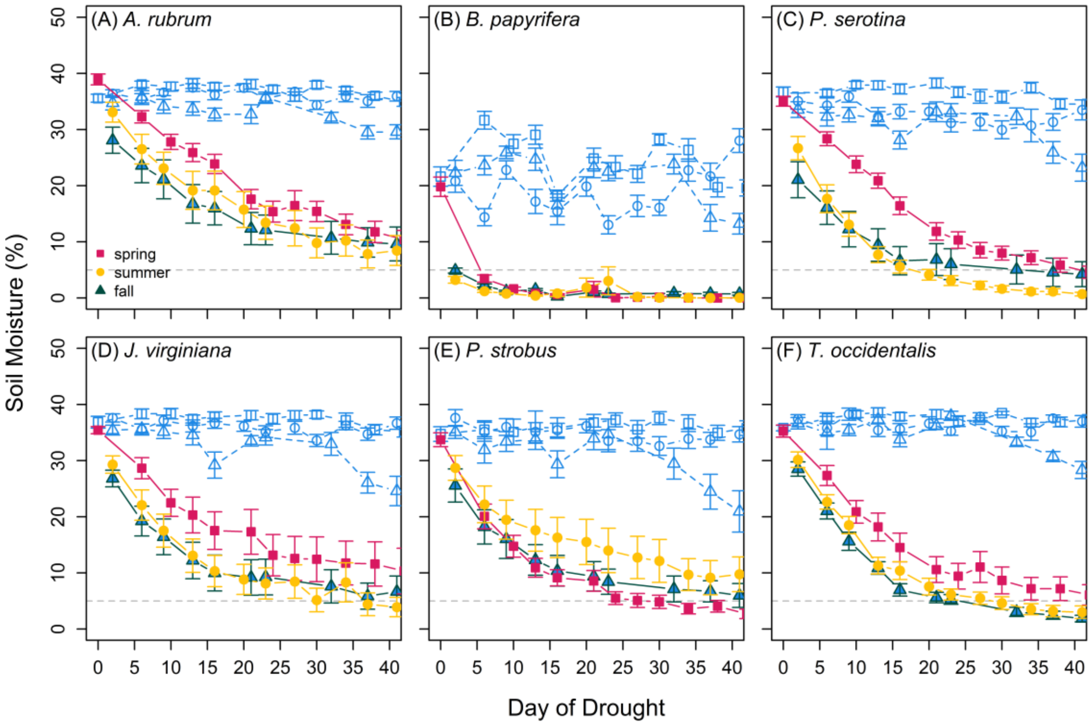

Figure 1.2. Patterns of declining soil moisture (mean ± SE) as a function of date of drought initiated during spring (red filled squares), summer (yellow filled circles), fall (green filled triangles) relative to their respective controls (blue empty matching symbols). Each panel represents the soil moisture measurements from containers containing one of the six study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)). Droughts are all standardized to the same axis to facilitate comparison in the rate of dry down during each time of year for each species. A light-gray dashed line at 5% soil moisture is on each panel to facilitate the comparison of rates of dry-down for each treatment.

Although we found that the treatments appeared to have different patterns of declining soil moisture (Fig. 1.2), within each species the treatments had similar final soil moisture estimates that were always lower than the control (Figure 1.3). *B. papyrifera* dried down the fastest (Figure 1.3B) and had low survival (8%) in the spring and summer droughts whereas the other five species had high survival (43%-100%) during the droughts (Table A1.1). Because we saw variable rates of declining soil moisture within a species that appeared to be driven by tree size, we also tested if total sapling biomass impacted the final soil moisture reading for each species by adding it as a covariate. We found that larger trees lead to lower final soil moisture for *A. rubrum*, *Pr. serotina*, and *J. virginiana* (Fig. A1.4, $p < 0.05$) but not the other three species.

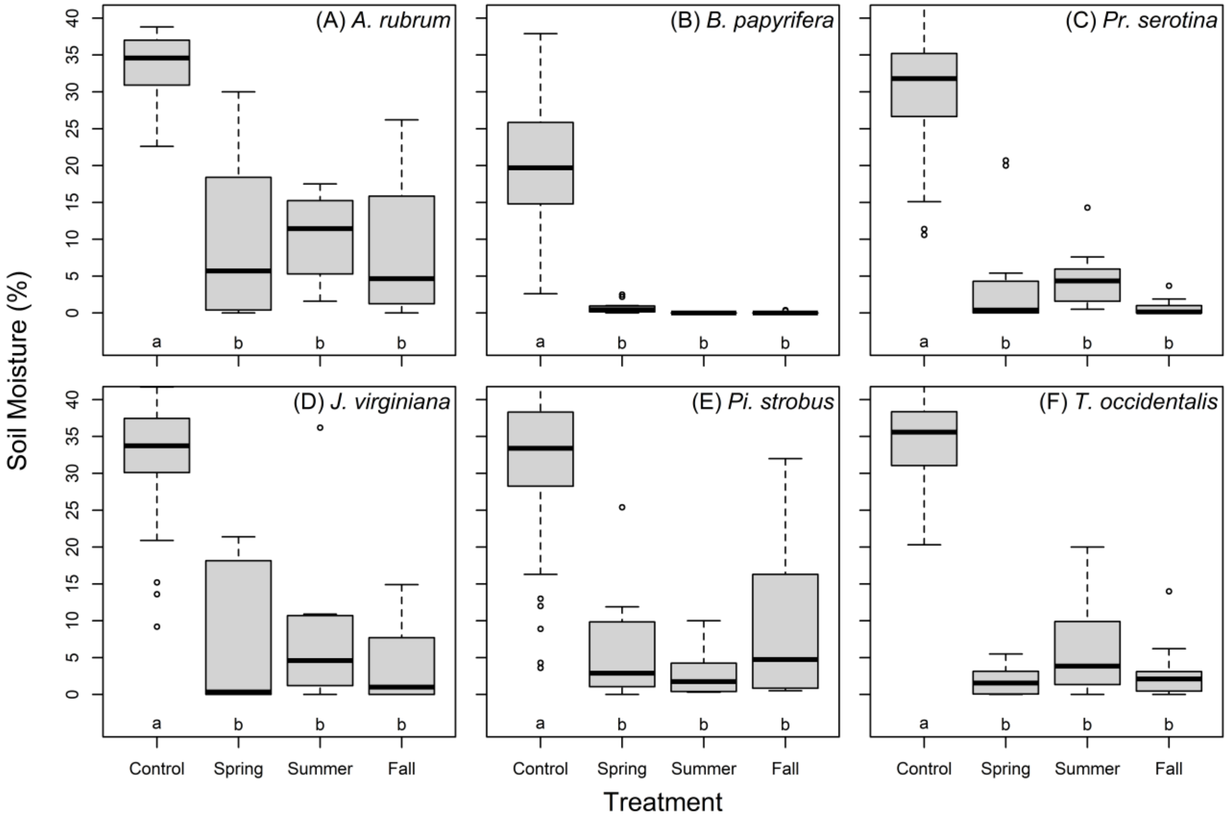

Figure 1.3. Soil moisture on the final day of each seasonal drought as a function of treatment for each species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)). Significant differences ($\alpha = 0.05$) are indicated by treatments that do not share a letter.

At high soil moisture (30-45%), average Ψ_{PD} was between 0 and -0.7 MPa and average Ψ_{MD} was between -0.8 and -1.6 MPa for all species (Fig. 1.4 and Table 1.2). *Pr. serotina* had the greatest range between Ψ_{PD} and Ψ_{MD}, while *T. occidentalis* had the smallest range overall. In addition to these patterns in soil moisture and Ψ_{PD}, we saw evidence of declining quantum yield and chlorophyll content in *B. papyrifera* saplings (Fig. A1.12 & Fig. A1.13). For example, over the spring and summer droughts, chlorophyll content declined and was lower than the control by the end of the droughts; interestingly, at week three of the fall drought, chlorophyll content was lower than the control, but as leaf senescence occurred, the chlorophyll content of the control saplings also declined.

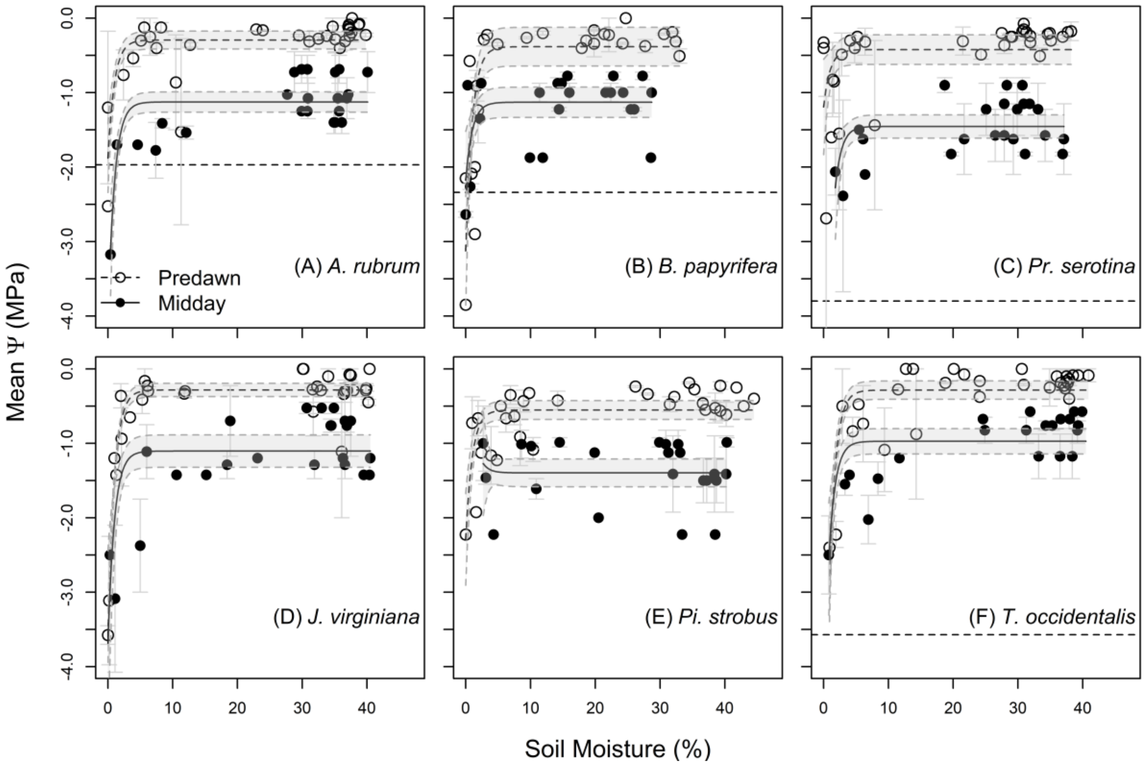

Figure 1.4. Mean ± SE Ψ_{PD} (predawn water potential; open circles) or Ψ_{MD} (midday water potential; filled circles) as a function of measured soil moisture within two days of measurement. Negative exponential models (solid black line) with 95% confidence intervals (light gray band bounded with dashed gray lines) were fit for each study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)) and for each measurement type (Ψ_{PD} or Ψ_{MD}). Horizontal dashed line represents the P_{50} value, the water potential at which a stem loses 50% of its hydraulic conductivity, indication embolization. The P_{50} values for *J. virginiana* (-6.6 MPa) and *Pi. strobus* (-5.25 MPa) go beyond the limits of the data represented and are thus not represented here.

Table 1.2. Predicted mean (SE) Ψ_{PD} (predawn water potential) and Ψ_{MD} (midday water potential) at high soil moistures (30-45%) for the six study species (deciduous broad-leaved trees: *Acer rubrum*, *Betula papyrifera*, and *Prunus serotina*; evergreen coniferous trees: *Juniperus virginiana*, *Pinus strobus*, and *Thuja occidentalis*). These values were predicted using negative exponential models fitted to the water potential and soil moisture data (see Fig. 4)

Species	Predicted Ψ_{PD} (MPa)	Predicted Ψ_{MD} (MPa)	Difference (MPa)
A. rubrum	-0.29 (0.06)	-1.1 (0.07)	-0.81
B. papyrifera	-0.38 (0.14)	-1.1 (0.11)	-0.72
Pr. serotina	-0.42 (0.10)	-1.5 (0.08)	-1.08
J. virginiana	-0.28 (0.05)	-1.1 (0.11)	-0.82
Pi. strobus	-0.55 (0.06)	-1.4 (0.10)	-0.85
T. occidentalis	-0.29 (0.07)	-0.97 (0.9)	-0.68

The declines in soil moisture and associated declines in water potential had important effects on height and diameter growth that differed by both species and season. Despite some control trees at high soil moisture showing lower growth, a lower minimum soil moisture during a drought generally resulted in lower height and diameter growth than in the controls for all species (Fig. A1.8 and Fig. A1.9). *A. rubrum* experienced extensive browse before initiating the droughts and we found that drought had minimal effects on height and diameter growth of these small individuals (Fig 1.5A and 1.6A, respectively). In contrast, *B. papyrifera* was unaffected by browse and the irrigated saplings had the highest height and diameter growth in the spring (Fig A1.10B and Fig A1.11B, respectively). We found that both height (Fig. 1.5B) and diameter (Fig. 1.6B) growth of *B. papyrifera* was lower in the spring and summer droughts. Low survival of *B. papyrifera* following all droughts limited our ability to assess lagged effects. Finally, *Pr. serotina*, which also experienced extensive browse damage before initiating the experiment, reached peak height growth in early spring (Fig A1.10C) and despite reaching low soil moisture levels its height was unaffected by drought in any season (Fig. 1.5C). At the end of the spring drought, and until approximately seven days after rewatering, diameter growth was lower (Fig. 1.6C).

Conifers did not experience browse or frost damage. Irrigated *J. virginiana* saplings had the highest height growth in the spring and summer (Fig A1.10D). We found a lagged drought effect on height growth of *J. virginiana* with decreased height growth in the last days of a summer drought and into the fall after rewatering (Fig. 1.5D). *Pi. strobus* saplings had the most height and diameter growth in the spring (Fig A1.10E and Fig A1.11E); in *Pi. strobus* saplings, we saw more immediate effects of the spring drought leading to lower height growth in the spring continuing through the growing season (Fig. 1.5E). We also saw a decrease in diameter growth at the end of the spring drought and near the end of the summer drought, with a recovery of diameter growth after rewatering (Fig. 1.6E). Finally, *T. occidentalis* reached peak height growth in the spring (Fig A1.10F); saplings experiencing a spring drought had lower height growth at the end of a spring drought and for about a week post-rewatering (Fig. 1.5F).

Despite the clear effects of drought timing on height and diameter growth for some species, we found little evidence that these moderate droughts impacted final biomass (except in *B. papyrifera* and *Pr. serotina*; Table A1.1), likely due to the large variation in initial height (Fig A1.5) and ability of some species to compensate with additional growth later in the year. However, we did see that total biomass in *B. papyrifera* was significantly impacted by a spring and a summer drought (Table A1.1). These effects are likely a result of mortality of trees during drought treatments and reflect the amount of time the trees had to grow before mortality. We also found total biomass in *Pr. serotina* was lower than the control following a summer drought, but not from a spring or fall drought (Table A1.1).

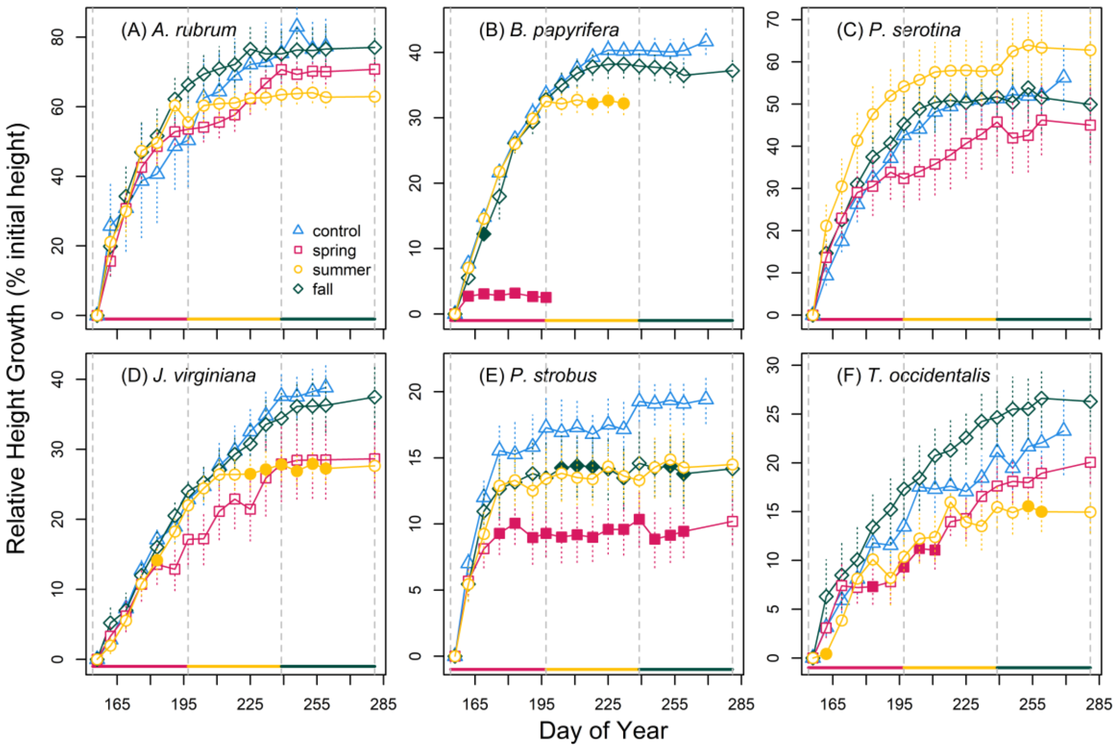

Figure 1.5. Relative height growth (Percent of initial height; mean ± SE) over the course of the growing season for each study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)). Solid lines at the bottom of each panel denote the span of each treatment (red = spring, yellow = summer, and green = fall). Treatments are color coded (blue triangle = control, red square = spring, yellow circle = summer, green rhombus = fall). If the mean relative height was significantly different from the control at α = 0.05, the symbol is filled.

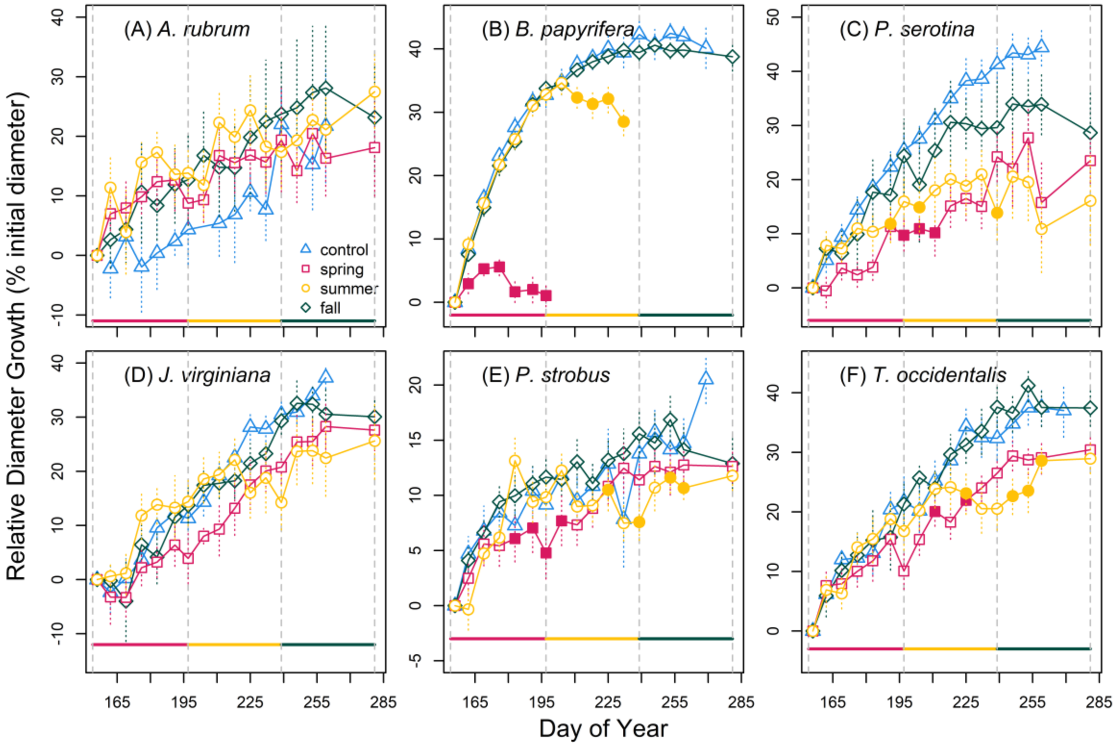

Figure 1.6. Relative diameter growth (Percent of initial diameter; mean ± SE) over the course of the growing season for each study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)). Solid lines at the bottom of each panel denote the span of each treatment (red = spring, yellow = summer, and green = fall). Treatments are color coded (blue triangle = control, red square = spring, yellow circle = summer, green rhombus = fall). If the mean relative diameter was significantly different from the control at α = 0.05, the symbol is filled.

1.5 DISCUSSION

In this study we tested how the impacts of seasonal droughts impacted the growth and survival of six tree species native to the NE US. We found clear patterns in the phenology of growth with height and diameter growth peaking in spring. When drought occurred during these seasons, growth was reduced and in some cases could recover after rewatering. Our results provide important detailed observations that extend seasonal drought research on temperate tree species using dendrochronological data that focuses on long-term series of diameter growth by contributing height growth response to seasonal drought, which is critical in competition dynamics in forests (D'Orangeville et al. 2018, Kannenberg, Maxwell, et al. 2019).

1.5.1 SOIL MOISTURE AND Ψ_{PD}

We found a complex interaction between species, treatment, and day of drought driving the decline in Ψ_{PD}. Although we were unable to measure Ψ_{PD} on all individuals at each sample interval, we did have high-frequency measurements of patterns in soil moisture decline that we were able to use to model the link between soil moisture and water potential. This link suggested that some saplings did, in fact, experience extremely low Ψ_{PD} and severe drought stress. Saplings of *A. rubrum* and *B. papyrifera* experiences water potentials at low soil moisture values (less than 5%) which reached or surpassed published P_{50} values for these species (see Fig. 1.4 and Table A1.2), suggesting that at low soil moisture values, these two species experienced embolism. However, many of the trees in this study experienced only moderate drought stress and did not surpass published P_{50} values. Interestingly, despite only experiencing moderate drought stress, we did still see patterns of declining growth that are likely related to stomatal closure (Allen et al. 2010, McDowell and Allen 2015) to prevent declines in water potential. We found strong effects of sapling size on the soil moisture, and thus Ψ_{PD}, that each sapling experienced, especially for the species that experienced herbivory and frost damage. Variability in starting height and final biomass (Fig. A1.4 & A1.5) likely also led to the variability in soil moisture and Ψ_{PD} during each drought treatment.

1.5.2 SEASONAL GROWTH PATTERNS

We hypothesized that spring and summer droughts would impact height and diameter growth more than a fall drought. Indeed, we observed important differences in relative height growth rate (RHG) across seasons in the controls of our study species. All species had the highest RHG during the spring and it decreased over the growing season. We observed decreases in RHG in *B. papyrifera, Pi. strobus,* and *T. occidentalis.* In *B. papyrifera*, we were unable to assess height growth after rewatering since all but one of these saplings died. *T. occidentalis* was able to recover after rewatering, but *Pi. strobus* RHG remained lower than the control for the entirety of the growing season. While not significantly different from the control, we did see that the RHG *Pr. serotina* and *J. virginiana* was reduced for the remainder of the growing season following a spring drought, which could be due to less sugar accumulation during a critical phenological phase.

We did not discern differences in relative diameter growth among seasons (RDG) in the controls of our study species except in *B. papyrifera* and *Pi. strobus,* which both had peak RDG in the spring. During a spring drought, both of these species had declines in RDG, and while *Pi. strobus* was able to recover in the summer, *B. papyrifera* did not due to mortality. These species also had decreased RDG during a summer drought. In *T. occidentalis*, spring and summer droughts resulted in slightly lagged effects on RDG in the summer and fall, respectively. Similarly, *Pr. serotina* had a roughly three-week period of reduced RDG following rewatering after the spring drought. *Pr. serotina* and *A. rubrum* are characterized by continuous, indeterminate growth (Canham et al. 1999), and in *Pr. serotina* we observed cessation of height growth in the spring and summer droughts, with an increase in height growth after rewatering, consistent with the literature. One possible reason we did not observe very strong differences in RHG and RDG in *A. rubrum* and *Pr. serotina* is the herbivory and frost damage these saplings experienced, which strongly reduced their initial size relative to the soil water available (Fig. S5). It is difficult to determine whether the growth responses we did see in these two species are simply recovery post-herbivory or the nature of their indeterminate, continuous growth strategies.

We observed immediate and same-season lagged effects on height and diameter growth. Lagged same-season effects have been observed for some species (Kannenberg, Novick, et al. 2019) and next-season legacy, or “carry-over” effects have been shown to be greater after a late-season drought (Babst et al. 2012, Kannenberg, Maxwell, et al. 2019), since carbon is usually redistributed to the roots and stem during this period (Sperling et al. 2017). We saw limited immediate effects of a fall drought in our study, but this fall drought could have had consequences for these saplings during the next growing season. Had we continued this experiment for at least one additional growing season, we likely would have been able to measure the same suite of physiological and growth estimates to identify any lagged effects of seasonal droughts in the following year. Detailed response patterns for each species and treatment can be found in Tables A1.3 and A1.4.

The high-frequency height measurements we conducted on these six species provide new information in addition to what dendrochronological studies have elucidated on the effects of drought timing on tree growth. We observed temporary reductions in RHG during drought for most species, with these height growth reductions being prolonged or compensated for later in the growing season. This compensation could be why we did not see large-scale reductions in biomass across species and treatments. Additionally, we can now begin to understand the physiological mechanisms behind the growth reductions seen in the tree ring record. For example, according to Michelot et al. (2012), *Pinus sylvestris* earlywood growth is sensitive to water deficits in the spring, and latewood is the most sensitive component to water deficits and warm temperatures in the fall. This could explain why we saw growth in *Pi. strobus* drastically decrease during a spring drought (height) and decrease during a fall drought (diameter). In addition, height is very important in forests where there is intense competition for light. Therefore, including height in future research may improve our understanding of how climate change may impact competitive dynamics beyond inference gained from radial growth alone.

Interestingly, one *B. papyrifera* each from the spring and summer droughts flushed a new cohort of leaves following rewatering, after having shed at least 95% of their leaves during the drought. This is a potential adaptation to extreme drought conditions (Hochberg et al. 2017), as part of the cambium tissue and xylem had to have been kept hydrated for leaves to re-flush. Although we could not assess this in the other species, in another study on *Juglans regia* saplings, petioles lost 87% of their hydraulic conductivity and leaves were shed after only a 5-day drought; while stems only lost 14% of their hydraulic conductivity (Tyree et al. 1993, Hochberg et al. 2017). Drought-deciduousness has been observed in other broadleaved angiosperm species during drought stress (Marchin et al. 2010), despite there being other contrasting evidence that hydraulic segmentation does not occur in broadleaved angiosperm species (Johnson et al. 2016, Jin et al. 2019, Li et al. 2020). Clearly, more work is needed using both in situ and ex vivo methods to determine interspecies differences in the presence of hydraulic segmentation.

Given the range of drought tolerances, predicted future abundance, wood type, and location in its native range within our study species, we expect each of the study species to react differently to drought when they are considered as part of a forest. *A. rubrum* saplings experienced extreme browse prior to the beginning of this study; however, it grows well in cool, wet springs after a wet fall and cool winter (Hart et al. 2012). Thus, we expected to observe large growth reductions in height and diameter growth due to a summer drought and following a dry fall. However, the extreme browse *A. rubrum* saplings experienced may have overwhelmed growth responses to drought and there was weak evidence for growth reductions due to drought in this species. *B. papyrifera* is a fast-growing, short-lived pioneer species (Burns and Honkala 1990) and 10% of its daily water flux has been attributed to nighttime transpiration (Daley and Phillips 2006), thus using large amounts of water. Unsurprisingly, this species is water-limited during the growing season, and diameter growth is positively correlated with summer water availability (Huang et al. 2010). Also, *B. papyrifera* saplings in this study were larger and had greater visual leaf area than other saplings in this study. Spring and summer droughts both ceased height and diameter growth in *B. papyrifera*, with only one sapling from each of these two treatments surviving.

Diameter growth in *J. virginiana* is sensitive to low summer precipitation and to the previous year's summer and fall precipitation (Aus Der Au et al. 2018). We did see generally lower diameter growth in saplings of this species following a summer drought. If the trees were grown for a second-year post-drought, we expect that diameter would also be low in this one-year experiment. *Pi. strobus* diameter growth has also been shown to be sensitive to low summer precipitation (Chhin et al. 2018), which we did observe in the diameter measurements for this species during the summer drought. This species is usually found on the well-drained sandy soils of glacial moraines (Lancaster and Leak 1978, Burns and Honkala 1990) and may have been over-watered in this study, since some irrigated individuals experienced needle-browning and low quantum yield values. We found that *T. occidentalis* saplings were quite resilient to a moderate drought, with their growth being only temporarily affected by drought and their ability to compensate with additional growth after rewatering. *T. occidentalis* trees growing in cedar swamps respond positively to summer precipitation and negatively to excessive precipitation in the fall and winter (Housset et al. 2015). However, this species can be found both in swamps with frequently wet soil and on exposed cliffs with dry soil (Burns and Honkala 1990), which may be why we observed some degree of drought resilience.

1.5.3 STUDY DESIGN

This study used fixed-length droughts of six weeks to impose moderate droughts during the spring, summer, and fall. Alternative methods of simulated drought include watering a small amount each day to avoid the rapid dry-down of high water-use species (Mitchell et al. 2013, Hartmann et al. 2013) and drying-down to target water potentials (Hammond et al. 2019). These designs are useful in order to target specific measures of water stress; however, they are logistically demanding. Another method that has begun to be used more often is the mortality threshold study design, which uses rewatering at target increments of drought length or predawn water potential to determine mortality thresholds (Brodribb and Cochard 2009, Hammond et al. 2019). Our study design allowed us to measure individual saplings and quantify what each individual was experiencing. The RCBD we used in this study also demonstrated that this approach may be challenging, especially if there is mortality that is unrelated to the experimental treatments, which necessitates excluding these blocks from the analysis. Our approach did emphasize the importance of tracking physiological markers that indicate water stress, since tree size was clearly a factor in determining when and how a sapling will experience drought.

While we used Ψ_{PD} to determine water stress, other proxies of water stress could be used to monitor drought progression in a less-invasive manner, such as stem thermocouple psychrometers, which have been used to monitor Ψ_{stem} continuously in both herbaceous and woody plant species (Coffey et al. 1997, Dixon and Tyree 2006). Also, optical vulnerability curves have recently been used to estimate important predictors of water stress such as P_{50}, the water potential at which a stem loses 50% of its hydraulic conductivity (Choat et al. 2016, Brodribb et al. 2017, Gauthey et al. 2020). Both of these methods can estimate the loss of conductivity in stems, which is an important indicator of water stress. Additional minimally invasive techniques, such as tracking RWC (Jackson et al. 1995) and water storage (Zhang et al. 2013), can help forest managers determine if their trees are stressed.

1.5.4 CONCLUSIONS

This study provides high-frequency height and diameter measurements for saplings of six NE US tree species throughout one growing season in irrigated and drought conditions and shows that intra-annual height and diameter growth is impacted by seasonal droughts. Further, some of these species showed the capacity to compensate for decreased growth during a drought after rewatering that these species were able to recover after a moderate drought. These data also provide insights into the patterns of regeneration of these species, especially as the changing climate shifts the suitable habitats of these species north. Additionally, these data help explain patterns that the dendrochronological record cannot and can lead to better understandings of how climate change may impact growth, carbon uptake, and carbon storage at larger scales.

CHAPTER 2: WHOLE-SHOOT AND STEM SEGMENT DRY-DOWNS REVEAL STRUCTURAL AND PHYSIOLOGICAL DIFFERENCES IN DROUGHT TOLERANCE STRATEGIES OF NORTHEASTERN FOREST TREES

2.1 ABSTRACT

Climate change is expected to lead to novel climate conditions in the northeastern United States. Therefore, experimental studies that mimic these conditions are crucial to understand the potential impact on forest trees. The goal of this research was to determine how tree hydraulic traits relate to drought resistance and resilience of six northeastern forest tree species. We used experimental dry-downs of leafy shoots and xylem segments to assess how structural and physiological adaptations of each species relate to water use during drought. A total of seventy-one leafy shoots from six species (*Acer rubrum, Prunus serotina, Betula papyrifera, Pinus strobus, Juniperus virginiana, and Thuja occidentalis*) were subjected to an experimental bench-top dry down to simulate extreme drought. Conifer species dried to -2.5 MPa 51× slower than broadleaved species and in general, lost water at equal rates between leaves and stems; in contrast, deciduous species experienced larger reductions in leaf water content compared to stem water content. The clear division between fast-drying broadleaved deciduous angiosperm species and slow-drying needle-leaved evergreen conifer species was further supported by morphological and physiological traits of leaves and stems. Broadleaves had about 2× the standardized leaf area per shoot than conifers. Understanding that conifer and deciduous tree species in NE US forests have differing hydraulic strategies that confer varying degrees of drought resistance and resilience will be key to managing these species as their suitable habitats shift with climate change.

2.2 INTRODUCTION

Mesic forests in the Northeastern United States (NE US) are not historically drought-prone (Peters et al. 2015). Therefore, many of the tree species in this region may not be well adapted to future drought conditions that are more likely with climate change (Liénard et al. 2016). Despite this risk, it is still unclear how tree species native to the NE US may respond to future novel climate conditions and drought (Vose et al. 2019). Therefore, we need a better understanding of the structural and physiological adaptations of trees that help them to survive extreme drought.

To prevent excessive water loss during extreme drought and to avoid low water potentials that drive embolism formation and spread, many trees close their stomata during moderate droughts (Davies and Kozlowski 1977, Meinzer et al. 2009, Liénard et al. 2016, Johnson et al. 2016, Hochberg et al. 2017). Closing stomata comes with a clear cost of reduced photosynthesis in favor of conserving water. Importantly, after stomatal closure, leaves still lose water through leaky stomata and the cuticle (minimum epidermal conductance; Duursma et al. 2019). However, in addition to leaf characteristics that conserve water during drought, total stem xylem water storage and release (stem capacitance) may be key for some species to withstand extreme drought conditions. Although high capacitance is commonly associated with low wood density (Meinzer et al. 2003, McCulloh et al. 2014, Jupa et al. 2016, Fu et al. 2019, Siddiq et al. 2019) and large amounts of flexible xylem parenchyma cells (Borchert and Pockman 2005) that are not common in trees in the NE US, the water stored in other xylem cells (such as tracheids, fibers, and vessels) can be released and may be critical during extreme drought (McCulloh et al. 2014, Fu et al. 2019). However, we know very little about how patterns of water storage and release in stems may be coordinated with rates of water loss from leaves in species of the NE US that rarely experience extreme drought.

Shoot dry-downs can provide a mechanistic understanding of how leaves, stems, and entire shoots coordinate hydraulic strategies during extreme drought. Recent studies have highlighted the utility of using branch-level traits such as leaf area, shoot conductance, and relative water content (RWC) at a critical water potential to estimate whole-plant survival following stomatal closure during extreme drought (Gleason et al. 2014, Blackman et al. 2016). In general, evergreen conifer species dry more slowly than broadleaved species due to wider hydraulic safety margins and lower minimum stomatal conductance values (Blackman, Creek, et al. 2019). In addition to differences between functional groups, leaf water is released readily as water potential drops in species from both arid and wet climates, while stem water is released more readily per drop in water potential in species from only wet climates (Gleason et al. 2014). Despite these studies on leaf and stem hydraulics during drought, relatively little is known about the coordination of leaf and stem hydraulic traits during extreme droughts in temperate needle-leaved evergreen conifer species and broadleaved deciduous angiosperm species, especially those found in the NE US (Tyree and Yang 1990, Carrasco et al. 2015).

In this study, we combine detailed measurements of whole-shoot dry-downs with estimates of xylem water storage and release to determine how leaf and stem hydraulic traits are coordinated to resist drought. We included three needle-leaved evergreen conifer (hereafter “conifer”) species (*Juniperus virginiana, Pinus strobus,* and *Thuja occidentalis*) and three broadleaved deciduous angiosperm (hereafter “deciduous”) species (*Acer rubrum, Betula papyrifera,* and *Prunus serotina*) native to the NE US that have a range of drought tolerance and predicted future abundance (See Table 1.1 in Chapter 1). We hypothesized that 1) conifer species would have a slower rate of desiccation during extreme drought than deciduous species, and 2) morphological and anatomical differences would be the driving force behind differences in xylem capacitance and leafy-shoot dry-down dynamics.

2.3 MATERIALS & METHODS

2.3.1 STUDY DESIGN

To test how branch and leaf hydraulics are coordinated for six species native to the NE US we conducted a series of dry-down experiments on stems and leafy shoots. Plant material for these dry-down experiments was collected from saplings grown in a 6m × 11m high-tunnel greenhouse in Orono, Maine (Chapter 1). Trees were planted in May 2019, in individual 19-liter containers with seven one-inch drainage holes. Thirty- to sixty-cm-tall saplings (Cold Stream Farm, Free Soil, MI) were planted in a nursery mix consisting of fine aged pine bark and sphagnum peat (Jolly Gardener, Poland Spring, ME) and fertilizer (5.92 g/L Osmocote 18-6-12). Trees were irrigated 15.1 L three times a week in 2019 and 7.6 L per night in 2020 during the growing season. For more details about the high-tunnel greenhouse, reference Chapter 1.

2.3.2 SHOOT DRY-DOWNS

Prior to leaf senescence in October 2020, we initiated the shoot dry down experiment. Between ten and thirteen leafy shoots (Fig 2.1A) 30-46 cm long containing all of the current year of growth and approximately 5 cm of the prior year growth were collected from 8:00 h to 9:00 h and immediately bagged and transported to the lab. Before beginning the experiment, we sampled approximately one cm of stem tissue from the base of the shoot and removed the bark to estimate fresh RWC_{stem}; these samples were then vacuum infiltrated with distilled water overnight (vacuum pressure 70 kPa) to estimate saturated RWC_{stem}. The cut ends of each shoot were then sealed with petroleum jelly and shoots were kept in open paper bags throughout the remainder of the experiment. Every two to six hours, the shoots were weighed to estimate shoot water loss over time (Sartorius Practum 3102-1S, 0.01 g, Goettingen, Germany). At the first, last, and 3-6 systematically chosen measurements in between, shoots from each species were destructively sampled to estimate shoot water potential (Ψ_{shoot}), stem relative water content (RWC_{stem}), and leaf relative water content (RWC_{leaf}; Fig 1B and 1C).

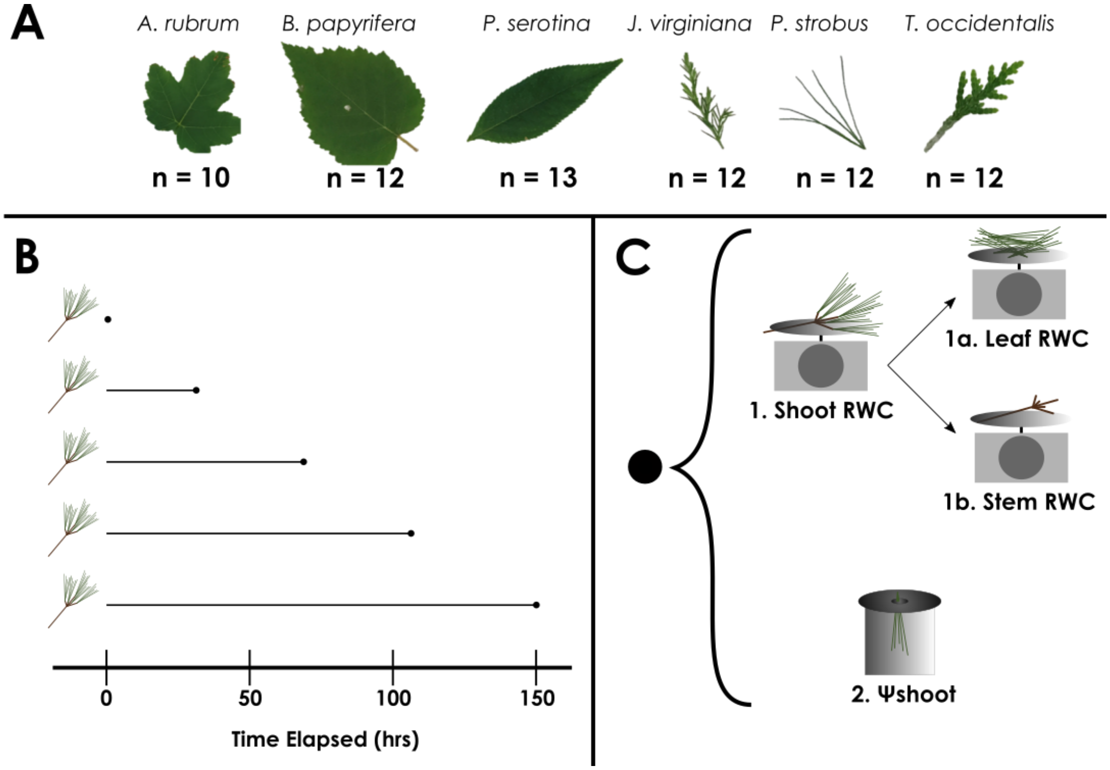

Figure 2.1. Schematic diagram of the shoot dry-down experiment, which was conducted in order to measure patterns of shoot, leaf, and stem RWC relative to Ψ_{shoot} during a simulated drought. A) Sample sizes for each study species, left to right are: *A. rubrum* (n = 10), *B. papyrifera* (n = 12), *Pr. serotina* (n = 13), *J. virginiana* (n = 12), *Pi. strobus* (n = 12), *T. occidentalis* (n = 12). B) At the end of each shoot's dry down, the shoot was equilibrated for 30 minutes in a plastic bag, shoot mass was collected (1), and the shoot's leaves and stem were separated, and their masses collected (1a and 1b, respectively). RWC was calculated as ($Mass_{fresh}$ – $Mass_{dry}$)/ $Mass_{dry}$. Two leaves were then collected to estimated Ψ_{shoot}. C) Ten to thirteen shoots per species started the dry-down at the same time and were systematically sampled through the duration of the experiment, represented by the black circle at the end of each line. One to two shoots were sampled immediately (0 hrs), while other shoots went through the experiment for variable lengths of time and sampled accordingly. The remaining shoots at the end of the dry-down were all sampled. The experiment was continued until water loss substantially slowed (2.1-3.3 days for deciduous species and 6.25 days for conifer species).

Ψ_{shoot} was measured by equilibrating the whole shoot in a sealed plastic bag in a cooler for ~30 minutes. Two leaves were then severed using a razor blade and Ψ_{shoot} was measured using a pressure chamber and later averaged (PMS Instruments; Model 1000; Albany, Oregon).

RWC_{shoot}, RWC_{leaf}, and RWC_{stem} were calculated using the fresh mass of the shoot, leaves, or stem, and the dry mass after drying in the drying oven at 60°C for 48 hours using the equation RWC = (fresh – dry)/dry. Relative humidity and temperature were monitored during the experiment (Onset HOBO UX100-003, Bourne, MA) and these data were used to calculate vapor pressure deficit (VPD). Average temperature during the experiment was 27.9 ± 1.0 °C (mean ± SD) and average VPD was 0.12 ± 0.03 kPa over the course of the dry down. Desiccation time was standardized to VPD-hrs (kPA x hours; Blackman, Li, et al. 2019). To quantify leaf area per dry mass of each shoot, dry leaf mass per area (LMA; g^2 m^{-2}) estimates were calculated using 3-4 leaves per species. The estimated leaf area per shoot was calculated by dividing the total dry mass (g) of leaves from the shoot dry-down experiment by the estimated LMA (g m^{-2}) for each species.

2.3.4 WATER RELEASE CURVES

To quantify how xylem structural characteristics relate to patterns in stem water storage and release, water release curves (Meinzer et al. 2003) were conducted on current year stem samples (Fig. S1A). Xylem segments were collected from irrigated saplings in early October and were trimmed to 1 cm and stripped of bark. Samples were stored in a freezer at -20°C for two weeks before beginning the water release curves. The fresh volume of each segment was calculated using two diameter estimates (one from each end) and length measurements using digital calipers (Mitutoyo CD-6” ASX, Kanagawa, Japan). Fresh mass was collected (Sartorius Practum 224-1S, 0.0001 g, Goettingen, Germany) to estimate the starting RWC_{stem} of the xylem segments, and then xylem segments were vacuum infiltrated with distilled water overnight (vacuum pressure 70 kPa). Water potential was estimated using thermocouple psychrometers (TCPs; JRD Merrill Specialty Equipment, Logan, UT, USA). The TCP chambers were bagged and kept in a circulating water bath that was kept at room temperature for 6-8 hours until readings stabilized. The cycle of drying, weighing, and measuring water potential was repeated five to thirteen times for each stem segment until water potentials reached below -7.0 MPa. TCPs were calibrated against NaCl solutions of known water potential (Brown and Bartos 1982). Water release curves were constructed using mass of water released per volume of tissue relative to the decline in water potential (Meinzer et al. 2003, McCulloh et al. 2014). Wood density of the stem segments was estimated using the volume estimates explained above and the oven-dry mass (g) collected at the end of the experiment.

2.3.5 STATISTICAL ANALYSIS

To relate the decline in water potential to the length of the shoot dry-down we used a generalized linear model using the functional group (conifer vs. broadleaf) as a grouping variable. We used the R package ‘nlme’ (DebRoy et al. 2021) to compare the pattern of long-term dry down between conifer and broadleaved species. We used all data points in the generalized linear model for deciduous species and excluded the first data point in conifer species to avoid the rapid non-linear decline in those samples. To test patterns in water loss from leaf and stem tissue during the shoot dry downs, we plotted RWC_{stem} as a function of RWC_{leaf}. A slope of one would indicate that the rate of decrease in RWC_{stem} and RWC_{leaf} was equal during the dry down. Therefore, for each species, we used linear regression to test if the slopes were different from one. In order to explain the differences in dry down rate between conifers and deciduous species, we tested which functional group had a higher ratio of leaf area per stem dry mass (cm g^{-1}) with a Welch’s t-test. Finally, to determine how species differed in fresh RWC_{stem} we used an ANOVA with Tukey’s HSD post-hoc.

To calculate xylem capacitance (water released per decline in water potential) in the water release curve experiment, Michaelis-Menten models (see Fig. A2.1B) were fit to each sample in the water release curve experiment. Using the inflection point of that model, a linear model was fit to a subset of the data from each sample from 0 to the inflection point. The slope of this line was used as the capacitance value, following Meinzer (2003). Finally, a standardized major axis (SMA) regression was used to determine the relationship between xylem capacitance and wood density, using the R package ‘smatr’ (Warton et al. 2012). All data and figures were analyzed ($\alpha = 0.05$) and produced using RStudio Version 4.0.3 (R Core Team 2021). Other packages used in data analysis, visualization, and organization are ‘tidyr’ (Wickham and RStudio 2021), ‘dplyr’ (Wickham et al. 2018), and ‘emmeans’ (Lenth 2021).

2.4 RESULTS

2.4.1 WATER LOSS FROM LEAFY SHOOTS

We found that the rate of decline in water potential for conifer shoots was 51× slower than in deciduous shoots (Fig. 2.2; strong interaction between functional group and VPD-hrs, $p = 0.006$). Despite a short period with a rapid decline to moderate water potentials, conifer shoots then continued to slowly lose water throughout the entire six-day experiment, whereas deciduous shoots reached very lower water potential quickly and shoot relative water content stabilized after 2-3 days (Fig S2.2). When accounting for the effect of VPD on dry time, conifers took approximately 22.3 VPD-hrs to dry to $\Psi_{stem} = -2.5$ MPa, whereas broadleaves reached that same water potential approximately 51× faster (0.44 VPD-hrs; Fig. 2.2). The slower decline in water potential and RWC of the whole shoot (RWC_{shoot}) for conifers was likely partially driven by an almost 2× lower shoot leaf area per stem dry mass, with conifers averaging 0.0025 m^2 g^{-1} and broadleaves averaging 0.0047 m^2 g^{-1} (Welch’s t-test, $t = 3.584$, $df = 38.483$, $p = 0.0009$). Further, only *A. rubrum* and *B. papyrifera* water potentials declined past published P_{50} values (see Table A1.2) over the course of the experiment, suggesting that only these species experienced significant embolism.

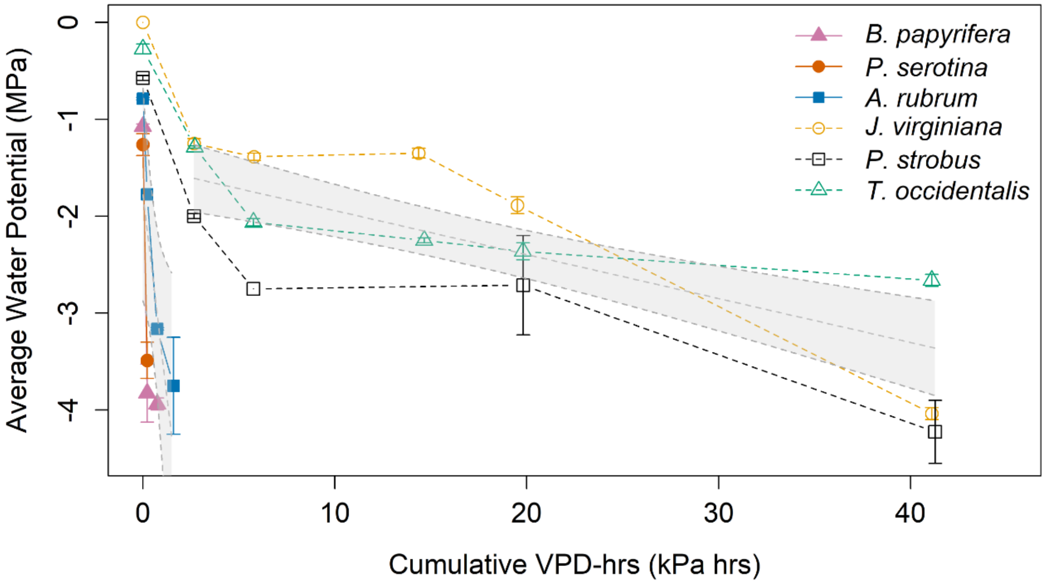

Figure 2.2. Mean ± SE Ψ_{stem} (MPa) over the course of the leafy shoot dry-down experiment in VPD-hrs (kPa x hours) in the six study species (deciduous species have solid symbols and solid lines; conifer species have open symbols and dashed lines). The first point was excluded for conifer species. Gray-shaded 95% confidence intervals surround each functional group, bounded with dark gray dashed lines, with the mean model included as a light gray dashed line. We tested for an interaction of functional group with VPD-hrs using a generalized linear model.

Next, we tested how leaf and stem water storage pools were depleted during the shoot dry-down. We found that at the start of the dry-down, despite similar RWC_{leaf}, conifer species appeared to have more stem water storage represented by higher RWC_{stem} (RWC_{stem} = 1.4-1.7) than deciduous species (RWC_{stem} = 0.8-1.2; Fig. 2.3). Interestingly, patterns of water loss between leaves and stems were not consistent across functional groups. *Pi. strobus* and *J. virginiana* (conifers) lost water at similar rates between leaf and stem tissues (slope not significantly different from 1), whereas *T. occidentalis* and the three broadleaved species experienced more rapid drops in RWC_{leaf} than RWC_{stem} (Fig. 2.3).

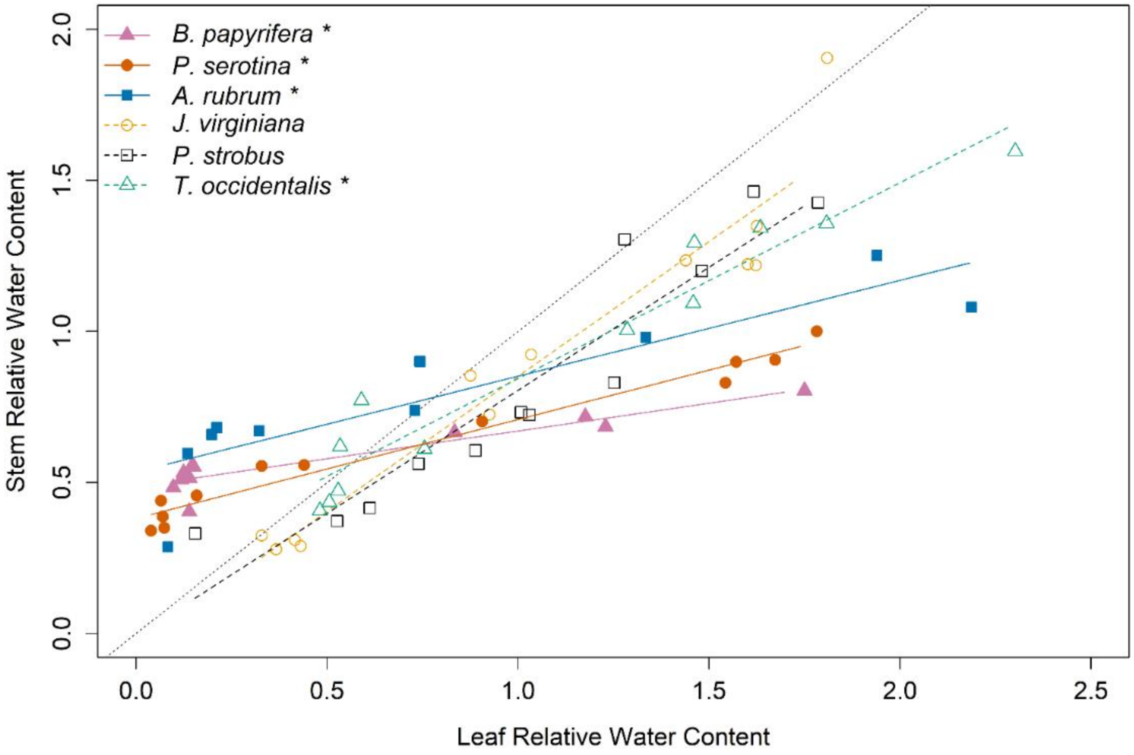

Figure 2.3. Stem relative water content as a function of leaf relative water content from the shoot dry-down experiment. Linear regressions were calculated, and these are represented using dashed lines for conifers and solid lines for broadleaves. The slopes of the regression lines were tested against the light gray 1:1 line using a linear regression t-test; if slopes were different from 1, this was denoted using an asterisk next to the species name in the upper left-hand corner.

2.4.2 WATER LOSS FROM XYLEM SEGMENTS

When testing water storage and release in excised xylem segments with xylem water release curves, we found that species with lower wood density had higher xylem capacitance (Fig. 2.4; $R^2 = 0.72$, $p = 0.029$). We also found that water release curves revealed a wide range of xylem capacitance between 0 MPa and the inflection point of their water release curve (Figure 2.5 and Figure B2.1). In contrast, although the shoot dry-downs started at slightly lower initial water potentials, they suggested much lower xylem capacitance in part due to shallow regression slopes but also because a large amount of stored water was still remaining in the shoot at low water potential (Fig. 2.5). For example, in the range of water potentials covered by both methods, we found that that shoots typically had ~2× more stored water available compared to the xylem segments in the water release curves.

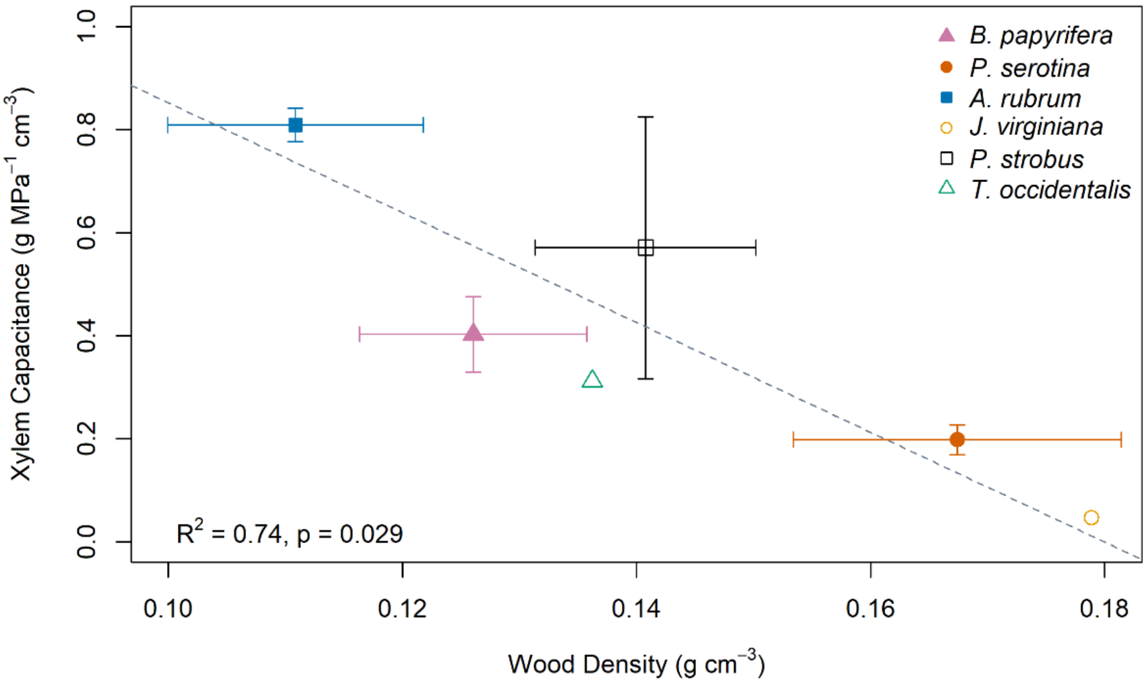

Figure 2.4. Mean ± SE xylem capacitance (g MPa^{-1} cm^{-3}) as a function of mean ± SE wood density (g cm^{-3}) for *B. papyrifera* (n = 4), *Pr. serotina* (n = 4), *A. rubrum* (n = 2), *J. virginiana* (n = 1), *Pi. strobus* (n = 5), *and T. occidentalis* (n = 1). Some species have less replication due to outliers. A standardized major axis regression (gray dashed line) was used to account for variation in the x and y axes ($R^2 = 0.74$, p = 0.029).

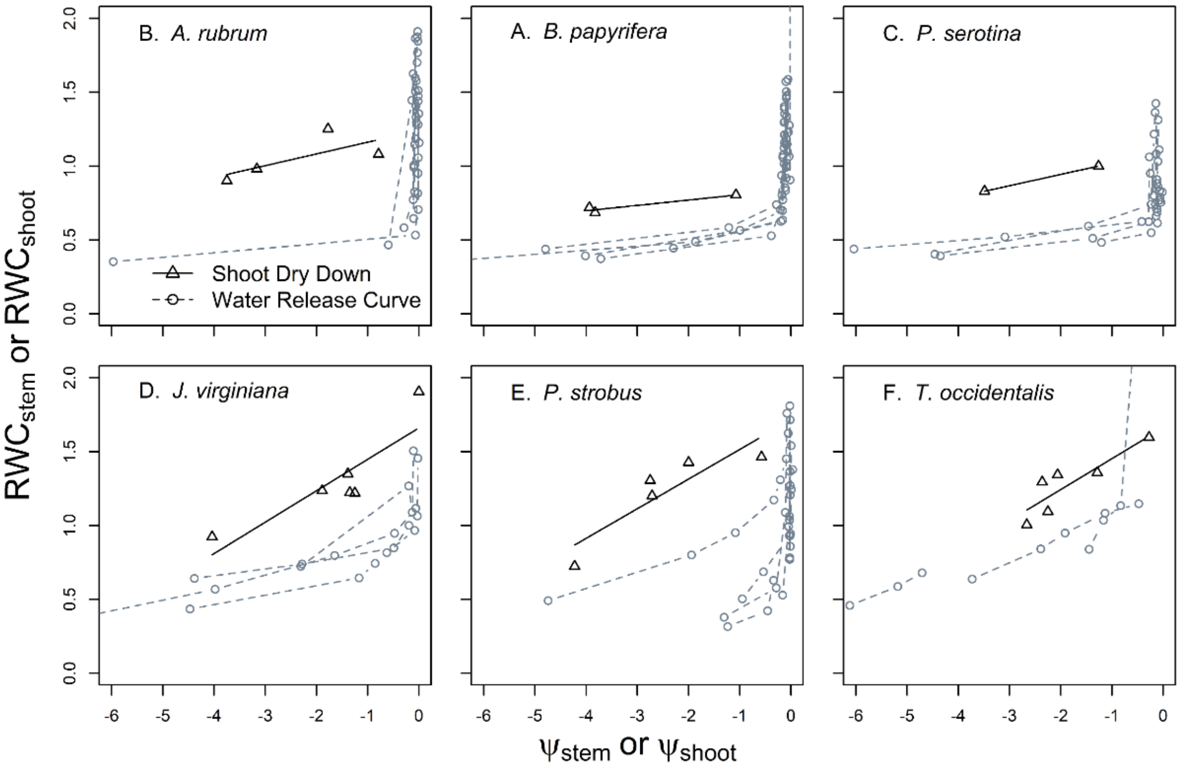

Figure 2.5. A) $\mathrm{RWC}_{\mathrm{stem}}$ or $\mathrm{RWC}_{\mathrm{xylem}}$ as a function of Ψ_{stem} or Ψ_{shoot} for shoot dry-downs conducted with 30-46 cm long leafy shoots (black triangles, solid line) and water release curves conducted on 1 cm debarked stem segments (gray circles, dashed line), respectively, for each study species (A-F, *A. rubrum*, *B. papyrifera*, *Pr. serotina*, *J. virigiana*, *Pi. strobus*, and *T. occidentalis*).

2.5 DISCUSSION

2.5.1 SHOOT WATER LOSS DURING AN EXTREME DROUGHT

This study combined whole-shoot dry downs with estimates of xylem water storage and release to determine how leaf and stem are coordinated to resist drought. In the shoot dry-down experiment, we found that conifers took 51× longer than deciduous species to reach low water potentials. Other studies testing differences in dry down patterns find similar differences between conifers and deciduous species (Blackman, Li, et al. 2019); however, our study now clarifies that this may be a widespread trait in species in the NE US and provides physiological traits and patterns for these study species.

We also found that in general, deciduous species experienced rapid drops in RWC_{leaf} relative to declines in RWC_{stem}, compared to conifer species (except for *T. occidentalis*), which had similar rates of dry down between leaves and stems. One explanation for the difference in dry down patterns between deciduous and conifer species is the segmentation hypothesis, which suggests that some plants make distal organs, such as leaves, more likely to experience drought-induced embolism formation to isolate those organs from the perennial main stem during drought and limit further desiccation. Temperate conifer species tend to have greater leaf to branch segmentation than temperate deciduous species (Johnson et al. 2016, Jin et al. 2019), which have been shown to have limited segmentation (Wason et al. 2018). However, the natural gradient in midday water potential still often results in embolism formation in the petiole in most species, which would still isolate the leaves from the stem (Wason et al. 2018). The rapid desiccation of leaves in the broadleaved shoots in this simulated extreme drought, therefore, may be a form of segmentation or drought deciduousness that could conserve stem water and keep cambia and xylem hydrated for post-drought recovery (Marchin et al. 2010). In contrast, the conifer species in this study maintain needles for multiple years are therefore of higher value to maintain during drought. This may explain what we observed in this study including the lower overall rates of water loss during drought, slower declines in leaf water potential, and the release of stem water to maintain needle hydration. Conifers have also been shown to have lower minimum stomatal conductance than broadleaved deciduous species, which may play an additional role in the slow dry down of the conifer species in this study (Duursma et al. 2019).

Further, differences in leaf capacitance, leaf area and minimum stomatal conductance likely drove the differences in dry-down between deciduous and coniferous species. Tropical angiosperms have been shown to have lower leaf capacitance than conifers, and that conifers took much longer to reach low water potentials than angiosperms (Brodribb et al. 2005). We found that broadleaved species had approximately 2× more standardized leaf area per stem than the conifers. Further, species in the order Pinales (the conifers in this species) tend to have lower minimum stomatal conductance values than species in the orders Rosales and Fagales (Pr. serotina and B. papyrifera, respectively Duursma et al. 2019), and these two traits combined could have led to conifers drying down more slowly than the broadleaves. Within the broadleaved species, *B. papyrifera* dried down the fastest. This species has a nighttime stomatal conductance more than 3× higher than that of *A. rubrum* and *Quercus rubra* (Daley and Phillips 2006), which can also help explain why shoots of this species dried down the fastest among broadleaves in this study.

Our results also support previous findings that xylem anatomy plays a role in stem water storage and release during drought (Borchert and Pockman 2005, Carrasco et al. 2015). Water can be stored in xylem cells such as parenchyma, tracheids, fibers, and vessels. Importantly, conifers have a relatively simple xylem anatomy consisting primarily of tracheids. As embolisms form in tracheids during drought, that water can be released to the transpiration stream by the numerous pit connections between neighboring tracheids. For example, in old-growth *Pseudotsuga menziesii* trees, transpiration can be maintained for up to a week using freely available water in the tree (Čermák et al. 2007). Tracheids in conifers are generally more connected than the more complex xylem anatomy of the broadleaved species in this study that have vessels for water transport interspersed among a matrix of supporting fibers. If those fibers are filled at maturity and connected to vessels with pits they may release stored water during drought (Knipfer et al. 2017). However, pit connections between fibers and vessels are not always common (Carlquist 1984, Cai et al. 2014) and often non-vessel xylem cells are observed to be fully hydrated even after embolism formation and spread in vessels (Morris et al. 2018). This anatomy may be related to patterns we observed during the shoot dry-down experiment, where stored water pools may not have been completely accessible to the transpiration stream either due to limited pit connections allowing water transport or by being blocked by existing or new embolisms.

2.5.2 WATER STORAGE AND RELEASE FROM XYLEM SEGMENTS

In the xylem water release curve experiment on excised xylem segments, we found that xylem capacitance was negatively correlated with wood density, which is consistent with other studies (Meinzer et al. 2003, McCulloh et al. 2014, Jupa et al. 2016, Fu et al. 2019). The deciduous species had capacitance values between 200 and 850 kg m^{-3} Mpa^{-1}, while the conifer study species had values between 50 and 800 kg m^{-3}. These values are similar and fall within the range of other estimates of xylem capacitance; other studies had ranges of 83 to 416 kg m^{-3} in tropical forest trees (Meinzer et al. 2003), ~100 to 900 kg m^{-3} in four conifer species (McCulloh et al. 2014), and ~75 to 450 kg m^{-3} in six angiosperms (Fu et al. 2019). In stem-succulent species, which have flexible parenchyma cells surrounding xylem cells, most water storage is intracellular and water is released from tissues such as fibers, parenchyma, and intracellular spaces (Borchert and Pockman 2005, Meinzer et al. 2008), In species with lower parenchyma fractions such as our study species, most water storage is extracellular (Borchert and Pockman 2005). Capillary water and stored water released during cavitation events have been shown to be important in water-stressed saplings and tissues (Knipfer et al. 2019, Yazaki et al. 2020, respectively), and in cavitation events occurring below -2.5 MPa (Tyree and Yang 1990). This trait may be why we saw some survival in two *B. papyrifera* saplings (Chapter 1), as they had enough hydrated cambia to re-flush a second cohort of leaves. Interestingly, we did not see average differences in xylem capacitance between broadleaved and conifer species. This is similar to the findings of Tyree and Yang (1990) when studying *Tsuga*, *Thuja*, and *Acer* stems and may be due to the relatively low parenchyma fraction in the xylem of NE US tree species relative to other regions, regardless of the functional group.

In contrast to leafy shoots that primarily lose water through the transpiration stream, excised xylem segments used for water release curves can also lose water from cut ends and exposed surfaces after bark removal. These exposed surfaces may induce an experimental artifact that explains why shoot dry-downs in this study typically had ~2× more water storage than excised xylem segments at similarly low water potentials that are likely approaching critical levels. In addition, leaf capacitance is generally higher than xylem capacitance, at least in broadleaved species (Fu et al. 2019). Thus, excised xylem segments may overestimate the amount of water that can be released during drought by ~2× in our species. Taken together, these results suggest that, although excised xylem segments tell us about the structure of the xylem and how it relates to its ability to store and release water during drought, shoot dry-downs may more accurately represent the total available stored water that can be accessed by the transpiration stream during drought and the important role of leaves in regulating the rate of dry-down. Finally, these patterns need to be considered in the context of long-term survival during drought. Achieving lower RWC_{stem} by drawing down stored water pools may be detrimental if it results in excessive drying and mortality of sapwood, cambium, and other sensitive tissues. Therefore, maintaining high RWC_{stem} and limiting capacitive release may be advantageous in some cases (Meinzer et al. 2003, McCulloh et al. 2014).

Water release curves on excised segments may also start at unrealistically high levels of saturation relative to the fresh RWC from stems in situ. Indeed, when comparing fresh RWC to saturated RWC of current-year stem samples in this study we found that saturated RWC was up to 50% higher than fresh RWC despite consistent irrigation of all trees for the past two years. Therefore, more water may have been present in the saturated samples at the start of the water release curve analysis than they would normally experience in situ and would therefore lead to unrealistically high estimates of capacitance as that water is quickly lost during the initial decline in water potential. Further, we also saw differences between fresh RWC and saturated RWC between the two functional groups. The fresh RWC of conifers was only 1-17% lower than the saturated RWC, whereas fresh RWC for deciduous species was 15-50% lower than saturated RWC ($p < 0.001$). This suggests that the impact of over-saturation may be larger in angiosperm species with more complex anatomy and more cells that may not be hydrated at maturity (Umebayashi et al. 2010).

2.5.3 CONCLUSIONS

In this study, we combined shoot dry-downs with water release curves to assess how leaf and stem are coordinated during extreme drought. We identified at least two distinct strategies employed by tree species in the temperate forests of the NE US to withstand drought. Firstly, conifer species appear to take a more conservative strategy of maintaining hydration of all perennial tissues, including leaves. This may come partly at the expense of losing more stored water released from xylem; however, in most cases, this may not be enough to drastically reduce water transport and lead to mortality. In contrast, broadleaved species lost water from leaves very rapidly and had about twice the standardized leaf area per branch of conifers and appeared to maintain relatively high levels of water in stems. This may represent a segmentation type strategy that sacrifices ephemeral leaves in favor of maintaining hydration of xylem and other tissues in the main stem for regrowth post drought. Taken together, these two contrasting strategies suggest that in mixed wood forests such as the forest types commonly found in the NE US, deciduous and conifer species may have vastly different responses to future drought conditions which may push trees beyond physiological thresholds such as their P_{50} values. Further, while this study focused on stem and shoot-level responses to extreme drought, future research must seek to focus on the effects of extreme droughts on larger forest trees. While a similar study to this one could be done on whole branches of large trees, the dynamics of dry-down associated with roots and associates would not be captured, necessitating the need to work with large trees. Additionally, plant organs, such as the stomata and xylem, may behave differently in vivo and ex vivo. Therefore, future research should continue to connect small scale physiological data on subsets of plants to whole organism responses to better inform how whole trees and forests will respond to future climate change.

EPILOGUE: WHAT CAN STEM, SHOOT, AND SAPLING DRY-DOWNS TELL US ABOUT HOW FORESTS WILL RESPOND TO FUTURE DROUGHTS?

Using leaf, stem, shoot, and whole plant dry-downs to predict how forests will respond to droughts is challenging (Kannenberg, Novick, et al. 2019). In this study we measured shoot- and stem-segment-level droughts (Chapter 2) and found a disconnect between the shoot dry-down and water release curve experiments. It appears that the more realistic shoot dry-downs, which primarily only allow water loss by transpiration through the leaves, result in less total water release during drought than in the water release curves on individual xylem segments. Cut stems can also dry quickly, depending on how they are dried; for example, excised and de-barked stems such as the ones used in water release curve experiments (Meinzer et al. 2003, Jupa et al. 2016) can dry extremely quickly due to the severed vessels and tracheids that are not usually exposed to the atmosphere. This method does provide estimates of important physiological traits such as xylem capacitance and water storage that are related to easily measured structural traits, such as wood density (Meinzer et al. 2003, McCulloh et al. 2014), as well as xylem structural traits, such as number and size of xylem conduits. However, our results suggest that water release curves overestimate the amount of water that is released during drought by up to 2×. Therefore, the xylem connections to the transpiring water stream via leaves are critical and can serve as buffers of water loss from the xylem and need to be considered during experimental dry-downs. Perhaps beginning water release curves with xylem segments at their starting relative water content could provide a clearer picture into xylem dry-down dynamics.

Entire shoot dry-downs can begin to account for the leaf-petiole-stem connection, and shoot dry-downs have proven to have some utility in predicting the time to mortality after stomatal closure during drought (Gleason et al. 2014, Blackman et al. 2016). The shoots used in this study only contained one full year of growth, which may not account for access to additional water in older growth rings that can be access by cross-ring connections in some species (Wason et al. 2019). Future studies using shoot-dry downs could use larger branches containing several years of growth, which may begin to account for possible connections between growth rings. Even in one experiment which disrupted 50% of tree sapwood via chainsaw cutting, mature trees were able to maintain high water potentials (Dietrich et al. 2018). Additionally, drought deciduousness, such as what we saw in *B. papyrifera* saplings, can keep xylem hydrated enough to keep cambia alive for post-drought recovery. While it is useful for intra-season growth, a second flush of leaves can come at a large carbon cost, resulting in drought legacy effects in future growing seasons.

While the above-mentioned dry-down methods can be useful for identifying and comparing physiological strategies among different species, they fail to account for tree size. We observed extensive variation in the rate of whole-sapling dry-down in the Chapter 1 experiment that was due in part to sapling biomass. Whole-sapling dry-down can be useful since each organ of the tree is involved in buffering or succumbing to drought, and the complex dynamics of root-soil interactions can be accounted for. Additionally, studies using whole saplings and modeled after recent studies on survival thresholds would be useful in the context of seasonal droughts (Hartmann et al. 2013, Blackman et al. 2016, Hammond et al. 2019, Blackman, Creek, et al. 2019). While this method is useful and relatively logistically simple, it can’t incorporate the complex intra-tree interactions in a stand of trees—such as interconnected root systems (Schoonmaker et al. 2008), varying life history strategies (Burns and Honkala 1990), and water tables (Kannenberg, Maxwell, et al. 2019).

Indeed, more work is needed to elucidate the relationships between each of these methods and how they can inform what we know about how entire forests and ecosystems respond to drought. While using each of these methods, we need to keep in mind their strengths and their limitations, as well as the assumptions made when linking physiological strategies to whole-ecosystem processes. Entire forests serve as massive carbon storage pools (Pan et al. 2011), and droughts threaten their ability to store carbon (McDowell and Allen 2015). While carbon starvation has rarely been seen as a cause of tree mortality at the individual level (Hartmann et al. 2013, Adams et al. 2017), carbon sinks in forests are vital for the health of the ecosystem and the planet. In order to better understand how carbon pools and hydraulic functioning interact, research must be done that seeks to establish the relationships between the patterns we observe during small-scale dry downs, such as xylem segments and shoots, and large-scale dry downs, such as whole-sapling and whole-forest drought, while also including carbon dynamics. There is no doubt that individual traits can be useful in forest modelling tools (He et al. 2008, Aubin et al. 2016, Kolus et al. 2019); however, it has been established that measuring stand-level response to fluctuations in climate can be more informative to forest management recommendations than using only the responses of individuals (Teets et al. 2018). Forest systems are extremely complex, and the challenges facing scientists to understand how climate change will affect them are myriad. Approaches that seek to explain multi-scale linkages of drought effects on forest ecosystems are an important avenue to consider in future research endeavors in order to address this challenge.

REFERENCES

Adams HD, Zeppel MJB, Anderegg WRL, Hartmann H, Landhäusser SM, Tissue DT, Huxman TE, Hudson PJ, Franz TE, Allen CD, Anderegg LDL, Barron-Gafford GA, Beerling DJ, Breshears DD, Brodribb TJ, Bugmann H, Cobb RC, Collins AD, Dickman LT, Duan H, Ewers BE, Galiano L, Galvez DA, Garcia-Forner N, Gaylord ML, Germino MJ, Gessler A, Hacke UG, Hakamada R, Hector A, Jenkins MW, Kane JM, Kolb TE, Law DJ, Lewis JD, Limousin J-M, Love DM, Macalady AK, Martínez-Vilalta J, Mencuccini M, Mitchell PJ, Muss JD, O'Brien MJ, O'Grady AP, Pangle RE, Pinkard EA, Piper FI, Plaut JA, Pockman WT, Quirk J, Reinhardt K, Ripullone F, Ryan MG, Sala A, Sevanto S, Sperry JS, Vargas R, Vennetier M, Way DA, Xu C, Yepez EA, McDowell NG (2017) A multi-species synthesis of physiological mechanisms in drought-induced tree mortality. Nat Ecol Evol 1:1285–1291.

Allen C, Macalady A, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears D, Hogg EH, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim J-H, Allard G, Running S, Semerci A, Cobb N (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For Ecol Manag 259:660–684.

Asbjornsen H, McIntire CD, Vadeboncoeur MA, Jennings KA, Coble AP, Berry ZC (2021) Sensitivity and threshold dynamics of Pinus strobus and Quercus spp. in response to experimental and naturally occurring severe droughts. Tree Physiol. https://doi.org/10.1093/treephys/tpab056 (3 June 2021, date last accessed).

Au TF, Maxwell JT, Novick KA, Robeson SM, Warner SM, Lockwood BR, Phillips RP, Harley GL, Telewski FW, Therrell MD, Pederson N (2020) Demographic shifts in eastern US forests increase the impact of late-season drought on forest growth. Ecography 43:1475–1486.

Aubin I, Munson AD, Cardou F, Burton PJ, Isabel N, Pedlar JH, Paquette A, Taylor AR, Delagrange S, Kebli H, Messier C, Shipley B, Valladares F, Kattge J, Boisvert-Marsh L, McKenney D (2016) Traits to stay, traits to move: a review of functional traits to assess sensitivity and adaptive capacity of temperate and boreal trees to climate change. Environ Rev 24:164–186.

Aus Der Au R, Awada T, Hiller J, Battipaglia G, Saurer M, Cherubini P (2018) Tree Rings of Pinus ponderosa and Juniperus virginiana Show Different Responses to Stand Density and Water Availability in the Nebraska Grassland. Pap Nat Resour. https://digitalcommons.unl.edu/natrespapers/927

Babst F, Carrer M, Poulter B, Urbinati C, Neuwirth B, Frank D (2012) 500 years of regional forest growth variability and links to climatic extreme events in Europe. Environ Res Lett 7:045705.

Bailiss C (2021) pivottabler: Create Pivot Tables. https://CRAN.R-project.org/package=pivottabler (30 April 2021, date last accessed).

Blackman CJ, Creek D, Maier C, Aspinwall MJ, Drake JE, Pfautsch S, O'Grady A, Delzon S, Medlyn BE, Tissue DT, Choat B (2019) Drought response strategies and hydraulic traits contribute to mechanistic understanding of plant dry-down to hydraulic failure. Tree Physiol 39:910–924.

Blackman CJ, Li X, Choat B, Rymer PD, Kauwe MGD, Duursma RA, Tissue DT, Medlyn BE (2019) Desiccation time during drought is highly predictable across species of Eucalyptus from contrasting climates. New Phytol 224:632–643.

Blackman CJ, Pfautsch S, Choat B, Delzon S, Gleason SM, Duursma RA (2016) Toward an index of desiccation time to tree mortality under drought. Plant Cell Environ 39:2342–2345.

Borchert R, Pockman WT (2005) Water storage capacitance and xylem tension in isolated branches of temperate and tropical trees. Tree Physiol 25:457–466.

Brodribb TJ, Carriqui M, Delzon S, Lucani C (2017) Optical Measurement of Stem Xylem Vulnerability. Plant Physiol 174:2054–2061.

Brodribb TJ, Cochard H (2009) Hydraulic failure defines the recovery and point of death in water-stressed conifers. Plant Physiol 149:575–584.

Brodribb TJ, Holbrook NM, Zwieniecki MA, Palma B (2005) Leaf hydraulic capacity in ferns, conifers and angiosperms: impacts on photosynthetic maxima. New Phytol 165:839–846.

Brown RW, Bartos DL (1982) A calibration model for screen-caged Peltier thermocouple psychrometers. U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station, Ogden, UT. https://www.fs.usda.gov/treesearch/pubs/31297 (9 March 2021, date last accessed).

Burns RM, Honkala BH (1990) Silvics Manual Volume 1-Conifers and Volume 2-Hardwoods. United States Department of Agriculture Forest Service, Washington, DC, USA. https://www.srs.fs.usda.gov/pubs/misc/ag_654/table_of_contents.htm (6 March 2021, date last accessed).

Cai J, Li S, Zhang H, Zhang S, Tyree MT (2014) Recalcitrant vulnerability curves: methods of analysis and the concept of fibre bridges for enhanced cavitation resistance. Plant Cell Environ 37:35–44.

Canham CD, Kobe RK, Latty EF, Chazdon RL (1999) Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. Oecologia 121:1–11.

Carlquist S (1984) Vessel Grouping in Dicotyledon Wood. Aliso 10:505–525.

Carrasco LO, Bucci SJ, Di Francescantonio D, Lezcano OA, Campanello PI, Scholz FG, Rodríguez S, Madanes N, Cristiano PM, Hao G-Y, Holbrook NM, Goldstein G (2015)

Water storage dynamics in the main stem of subtropical tree species differing in wood density, growth rate and life history traits. Tree Physiol 35:354–365.

Čermák J, Kučra J, Bauerle WL, Phillips N, Hinckley TM (2007) Tree water storage and its diurnal dynamics related to sap flow and changes in stem volume in old-growth Douglas-fir trees. Tree Physiol 27:181–198.

Chhin S, Zalesny RS, Parker WC, Brissette J (2018) Dendroclimatic analysis of white pine (Pinus strobus L.) using long-term provenance test sites across eastern North America. For Ecosyst 5:18.

Choat B, Badel E, Burlett R, Delzon S, Cochard H, Jansen S (2016) Noninvasive Measurement of Vulnerability to Drought-Induced Embolism by X-Ray Microtomography1. Plant Physiol 170:273–282.

Coffey WLP, Gordon RJ, Dixon MA (1997) Patterns of stem water potential in field grown potatoes using stem psychrometers. Potato Res 40:35–46.

Daley MJ, Phillips NG (2006) Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. Tree Physiol 26:411–419.

Davies WJ, Kozlowski TT (1977) Variations among woody plants in stomatal conductance and photosynthesis during and after drought. Plant Soil 46:435–444.

DebRoy S, R Core Team, Sarkar D, Pinheiro J, Bates D (2021) Linear and Nonlinear Mixed Effects Models.

Delpierre N, Berveiller D, Granda E, Dufrêne E (2016) Wood phenology, not carbon input, controls the interannual variability of wood growth in a temperate oak forest. New Phytol 210:459–470.

Dietrich L, Hoch G, Kahmen A, Körner C (2018) Losing half the conductive area hardly impacts the water status of mature trees. Sci Rep 8:15006.

Dixon M, Tyree M (2006) A new stem hygrometer, corrected for temperature gradients and calibrated against the pressure bomb. Plant Cell Environ 7:693–697.

D’Orangeville L, Maxwell J, Kneeshaw D, Pederson N, Duchesne L, Logan T, Houle D, Arseneault D, Beier CM, Bishop DA, Druckenbrod D, Fraver S, Girard F, Halman J, Hansen C, Hart JL, Hartmann H, Kaye M, Leblanc D, Manzoni S, Ouimet R, Rayback S, Rollinson CR, Phillips RP (2018) Drought timing and local climate determine the sensitivity of eastern temperate forests to drought. Glob Change Biol 24:2339–2351.

Duchesne L, Houle D, D’Orangeville L (2012) Influence of climate on seasonal patterns of stem increment of balsam fir in a boreal forest of Québec, Canada. Agric For Meteorol 162–163:108–114.

Duursma RA, Blackman CJ, Lopéz R, Martin-StPaul NK, Cochard H, Medlyn BE (2019) On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. New Phytol 221:693–705.

Foster TE, Schmalzer PA, Fox GA (2014) Timing matters: the seasonal effect of drought on tree growth. J Torrey Bot Soc 141:225–241.

Fu X, Meinzer FC, Woodruff DR, Liu Y-Y, Smith DD, McCulloh KA, Howard AR (2019) Coordination and trade-offs between leaf and stem hydraulic traits and stomatal regulation along a spectrum of isohydry to anisohydry. Plant Cell Environ 42:2245–2258.

Gauthey A, Peters JMR, Carins-Murphy MR, Rodriguez-Dominguez CM, Li X, Delzon S, King A, López R, Medlyn BE, Tissue DT, Brodribb TJ, Choat B (2020) Visual and hydraulic techniques produce similar estimates of cavitation resistance in woody species. New Phytol 228:884–897.

Gleason SM, Blackman CJ, Cook AM, Laws CA, Westoby M (2014) Whole-plant capacitance, embolism resistance and slow transpiration rates all contribute to longer desiccation times in woody angiosperms from arid and wet habitats. Tree Physiol 34:275–284.

Halekoh SH and U (2021) doBy: Groupwise Statistics, LSmeans, Linear Contrasts, Utilities. https://CRAN.R-project.org/package=doBy (30 April 2021, date last accessed).

Hammond WM, Yu KailiangL, Wilson LA, Will RE, Anderegg WRL, Adams HD (2019) Dead or dying? Quantifying the point of no return from hydraulic failure in drought-induced tree mortality. New Phytol 0. https://doi.org/10.1111/nph.15922 (7 June 2019, date last accessed).

Hart JL, Buchanan ML, Clark SL, Torreano SJ (2012) Canopy accession strategies and climate-growth relationships in Acer rubrum. For Ecol Manag 282:124–132.

Hartmann H, Ziegler W, Kolle O, Trumbore S (2013) Thirst beats hunger – declining hydration during drought prevents carbon starvation in Norway spruce saplings. New Phytol 200:340–349.

Hayhoe K, Wake CP, Huntington TG, Luo L, Schwartz MD, Sheffield J, Wood E, Anderson B, Bradbury J, DeGaetano A, Troy TJ, Wolfe D (2007) Past and future changes in climate and hydrological indicators in the US Northeast. Clim Dyn 28:381–407.

He HS, Keane RE, Iverson LR (2008) Forest landscape models, a tool for understanding the effect of the large-scale and long-term landscape processes. For Ecol Manag 254 371-374 254. https://www.fs.usda.gov/treesearch/pubs/13414 (5 October 2019, date last accessed).

Hochberg U, Windt CW, Ponomarenko A, Zhang Y-J, Gersony J, Rockwell FE, Holbrook NM (2017) Stomatal Closure, Basal Leaf Embolism, and Shedding Protect the Hydraulic Integrity of Grape Stems. Plant Physiol 174:764–775.

Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S (2021) multcomp: Simultaneous Inference in General Parametric Models. https://CRAN.R-project.org/package=multcomp (30 April 2021, date last accessed).

Housset JM, Girardin MP, Baconnet M, Carcaillet C, Bergeron Y (2015) Unexpected warming-induced growth decline in Thuja occidentalis at its northern limits in North America. J Biogeogr 42:1233–1245.

Huang J, Tardif JC, Bergeron Y, Denneler B, Berninger F, Girardin MP (2010) Radial growth response of four dominant boreal tree species to climate along a latitudinal gradient in the eastern Canadian boreal forest. Glob Change Biol 16:711–731.

Huang M, Wang X, Keenan TF, Piao S (2018) Drought timing influences the legacy of tree growth recovery. Glob Change Biol 24:3546–3559.

Jackson GE, Irvine J, Grace J (1995) Xylem cavitation in Scots pine and Sitka spruce saplings during water stress. Tree Physiol 15:783–790.

Janowiak MK, D'Amato AW, Swanston CW, Iverson L, Thompson FR, Dijak WD, Matthews S, Peters MP, Prasad A, Fraser JS, Brandt LA, Butler-Leopold P, Handler SD, Shannon PD, Burbank D, Campbell J, Cogbill C, Duveneck MJ, Emery MR, Fisichelli N, Foster J, Hushaw J, Kenefic L, Mahaffey A, Morelli TL, Reo NJ, Schaberg PG, Simmons KR, Weiskittel A, Wilmot S, Hollinger D, Lane E, Rustad L, Templer PH (2018) New England and northern New York forest ecosystem vulnerability assessment and synthesis: a report from the New England Climate Change Response Framework project. https://www.nrs.fs.fed.us/pubs/55635 (21 October 2019, date last accessed).

Jin Y, Wang C, Zhou Z (2019) Conifers but not angiosperms exhibit vulnerability segmentation between leaves and branches in a temperate forest. Tree Physiol 39:454–462.

Johnson DM, Wortemann R, McCulloh KA, Jordan-Meille L, Ward E, Warren JM, Palmroth S, Domec J-C (2016) A test of the hydraulic vulnerability segmentation hypothesis in angiosperm and conifer tree species. Tree Physiol 36:983–993.

Jupa R, Plavcová L, Gloser V, Jansen S (2016) Linking xylem water storage with anatomical parameters in five temperate tree species. Tree Physiol 36:756–769.

Kannenberg SA, Maxwell JT, Pederson N, D'Orangeville L, Ficklin DL, Phillips RP (2019) Drought legacies are dependent on water table depth, wood anatomy and drought timing across the eastern US. Ecol Lett 22:119–127.

Kannenberg SA, Novick KA, Alexander MR, Maxwell JT, Moore DJP, Phillips RP, Anderegg WRL (2019) Linking drought legacy effects across scales: From leaves to tree rings to ecosystems. Glob Change Biol 25:2978–2992.

Knipfer T, Cuneo IF, Earles JM, Reyes C, Brodersen CR, McElrone AJ (2017) Storage Compartments for Capillary Water Rarely Refill in an Intact Woody Plant. Plant Physiol 175:1649–1660.

Knipfer T, Reyes C, Earles JM, Berry ZC, Johnson DM, Brodersen CR, McElrone AJ (2019) Spatiotemporal Coupling of Vessel Cavitation and Discharge of Stored Xylem Water in a Tree Sapling. Plant Physiol 179:1658–1668.

Kolus HR, Huntzinger DN, Schwalm CR, Fisher JB, McKay N, Fang Y, Michalak AM, Schaefer K, Wei Y, Poulter B, Mao J, Parazoo NC, Shi X (2019) Land carbon models underestimate the severity and duration of drought's impact on plant productivity. Sci Rep 9:1–10.

Lancaster KF, Leak WB (1978) A silvicultural guide for white pine in the northeast. https://www.nrs.fs.fed.us/pubs/6190 (25 April 2021, date last accessed).

Lenth RV (2021) emmeans: Estimated Marginal Means, aka Least-Squares Means. https://CRAN.R-project.org/package=emmeans

Li X, Delzon S, Torres-Ruiz JM, Badel E, Burlett R, Cochard H, Jansen S, King A, Lamarque L, Lenoir N, Martin-Stpaul N, Choat B (2020) Lack of vulnerability segmentation in four angiosperm tree species: evidence from direct X-ray microtomography observation. Ann For Sci 77

Liénard J, Harrison J, Strigul N (2016) US forest response to projected climate-related stress: a tolerance perspective. Glob Change Biol 22:2875–2886.

Maherali H, Moura CE, Caldeira MC, Willson CJ, Jackson RB (2006) Functional coordination between leaf gas exchange and vulnerability to xylem cavitation in temperate forest trees. Plant Cell Environ 29:571–583.

Marchin R, Zeng H, Hoffmann W (2010) Drought-deciduous behavior reduces nutrient losses from temperate deciduous trees under severe drought. Oecologia 163:845–54.

McCulloh K, Johnson DM, Meinzer FC, Woodruff DR (2014) The dynamic pipeline: hydraulic capacitance and xylem hydraulic safety in four tall conifer species. Plant Cell Environ 37:1171–1183.

McDowell NG, Allen CD (2015) Darcy's law predicts widespread forest mortality under climate warming. Nat Clim Change 5:669–672.

Meinzer FC, James SA, Goldstein G, Woodruff D (2003) Whole-tree water transport scales with sapwood capacitance in tropical forest canopy trees. Plant Cell Environ 26:1147–1155.

Meinzer FC, Johnson DM, Lachenbruch B, McCulloh KA, Woodruff DR (2009) Xylem hydraulic safety margins in woody plants: coordination of stomatal control of xylem tension with hydraulic capacitance. Funct Ecol 23:922–930.

Meinzer FC, Woodruff DR, Domec J-C, Goldstein G, Campanello PI, Gatti MG, Villalobos-Vega R (2008) Coordination of leaf and stem water transport properties in tropical forest trees. Oecologia 156:31–41.

Michelot A, Bréda N, Damesin C, Dufrêne E (2012) Differing growth responses to climatic variations and soil water deficits of Fagus sylvatica, Quercus petraea and Pinus sylvestris in a temperate forest. For Ecol Manag 265:161–171.

Mitchell PJ, O'Grady AP, Tissue DT, White DA, Ottenschlaeger ML, Pinkard EA (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. New Phytol 197:862–872.

Morris H, Plavcová L, Gorai M, Klepsch MM, Kotowska M, Schenk HJ, Jansen S (2018) Vessel-associated cells in angiosperm xylem: Highly specialized living cells at the symplast–apoplast boundary. Am J Bot 105:151–160.

Noormets A, McNulty SG, DeForest JL, Sun G, Li Q, Chen J (2008) Drought during canopy development has lasting effect on annual carbon balance in a deciduous temperate forest. New Phytol 179:818–828.

Oberhuber W (2017) Soil water availability and evaporative demand affect seasonal growth dynamics and use of stored water in co-occurring saplings and mature conifers under drought. Trees 31:467–478.

Osada N (2017) Relationships between the timing of budburst, plant traits, and distribution of 24 coexisting woody species in a warm-temperate forest in Japan. Am J Bot 104:550–558.

Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG, Ciais P, Jackson RB, Pacala SW, McGuire AD, Piao S, Rautiainen A, Sitch S, Hayes D (2011) A Large and Persistent Carbon Sink in the World's Forests. Science 333:988–993.

Peters MP, Iverson LR, Matthews SN (2015) Long-term droughtiness and drought tolerance of eastern US forests over five decades. For Ecol Manag 345:56–64.

Prasad AM, Iverson LR, Matthews S, Peters M (2007) A Climate Change Atlas for 134 Forest Tree Species of the Eastern United States [database]. Northern Research Station, USDA Forest Service, Delaware, Ohio. https://www.nrs.fs.fed.us/atlas/tree

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Richardson AD, Hollinger DY, Dail DB, Lee JT, Munger JW, O'keefe J (2009) Influence of spring phenology on seasonal and annual carbon balance in two contrasting New England forests. Tree Physiol 29:321–331.

Schoonmaker A, Teste F, Simard S, Guy R (2008) Tree proximity, soil pathways and common mycorrhizal networks: Their influence on the utilization of redistributed water by understory seedlings. Oecologia 154:455–66.

Siddiq Z, Zhang Y-J, Zhu S-D, Cao K-F (2019) Canopy water status and photosynthesis of tropical trees are associated with trunk sapwood hydraulic properties. Plant Physiol Biochem 139:724–730.

Sperling O, Silva LCR, Tixier A, Théroux-Rancourt G, Zwieniecki MA (2017) Temperature gradients assist carbohydrate allocation within trees. Sci Rep 7:3265.

Sperry JS, Hacke UG (2004) Analysis of circular bordered pit function I. Angiosperm vessels with homogenous pit membranes. Am J Bot 91:369–385.

Sperry JS, Nichols KL, Sullivan JEM, Eastlack SE (1994) Xylem Embolism in Ring-Porous, Diffuse-Porous, and Coniferous Trees of Northern Utah and Interior Alaska. Ecology 75:1736–1752.

Spinu V, Grolemund G, Wickham H, Lyttle I, Costigan I, Law J, Mitarotonda D, Larmarange J, Boiser J, Lee CH, Inc G (2021) lubridate: Make Dealing with Dates a Little Easier. https://CRAN.R-project.org/package=lubridate (30 April 2021, date last accessed).

Teets A, Fraver S, Weiskittel AR, Hollinger DY (2018) Quantifying climate–growth relationships at the stand level in a mature mixed-species conifer forest. Glob Change Biol 24:3587–3602.

Tierney GL, Fahey TJ, Hardy JP, Fitzhugh RD, Driscoll CT (2001) Soil freezing alters fine root dynamics in a northern hardwood forest. :16.

Tyree M, Cochard H, Cruiziat P, Sinclair B, Ameglio T (1993) Drought-induced leaf shedding in walnut: evidence for vulnerability segmentation. Plant Cell Environ 16:879–882.

Tyree MT, Dixon MA (1986) Water stress induced cavitation and embolism in some woody plants. Physiol Plant 66:397–405.

Tyree M, Yang S (1990) Water-storage capacity of Thuja, Tsuga and Acer stems measured by dehydration isotherms. Planta 182:420–426.

Umebayashi T, Utsumi Y, Koga S, Inoue S, Matsumura J, Oda K, Fujikawa S, Arakawa K, Otsuki K (2010) Xylem water-conducting patterns of 34 broadleaved evergreen trees in southern Japan. Trees 24:571–583.

Vose JM, Peterson DL, Luce CH, Patel-Weynand T (2019) Effects of drought on forests and rangelands in the United States: translating science into management responses. U.S. Department of Agriculture, Forest Service, Washington, DC. https://www.fs.usda.gov/treesearch/pubs/59158 (22 February 2020, date last accessed).

Wake CP, Keeley C, Burakowski EA, Wilkinson P, Hayhoe K (2014) Climate Change in Northern New Hampshire: Past, Present and Future. Sustain Inst Univ N H:79.

Warton DI, Duursma RA, Falster DS, Taskinen S (2012) smatr 3– an R package for estimation and inference about allometric lines. Methods Ecol Evol 3:257–259.

Wason JW, Anstreicher KS, Stephansky N, Huggett BA, Brodersen CR (2018) Hydraulic safety margins and air-seeding thresholds in roots, trunks, branches and petioles of four northern hardwood trees. New Phytol 219:77–88.

Wason JW, Brodersen CR, Huggett BA (2019) The functional implications of tracheary connections across growth rings in four northern hardwood trees. Ann Bot 124:297–306.

Wickham H, Francois R, Henry L, Muller K (2018) dplyr: A grammar of data manipulation. R package version 0.7.7. https://CRAN.R-project.org/package=dplyr

Wickham H, RStudio (2021) tidyr: Tidy Messy Data. https://CRAN.R-project.org/package=tidyr (30 April 2021, date last accessed).

Willson C, Manos P, Jackson R (2008) Hydraulic traits are influenced by phylogenetic history in the drought-resistant, invasive genus Juniperus (Cupressaceae). Am J Bot 95:299–314.

Yazaki K, Levia DF, Takenouchi A, Watanabe M, Kabeya D, Miki NH, Taneda H, Ogasa MY, Oguro M, Saiki S-T, Tobita H, Fukuda K (2020) Imperforate tracheary elements and vessels alleviate xylem tension under severe dehydration: insights from water release curves for excised twigs of three tree species. Am J Bot 107:1122–1135.

Zhang Y-J, Meinzer FC, Qi J-H, Goldstein G, Cao K-F (2013) Midday stomatal conductance is more related to stem rather than leaf water status in subtropical deciduous and evergreen broadleaf trees. Plant Cell Environ 36:149–158.

APPENDICES

APPENDIX 1.

Table A1.1. Mean ± SE total biomass (root plus shoot), and root:shoot biomass data for each species and treatment combination (n = 8-12 each row). Letters denote significance within a species. Survival ratios are also included in the last column, which do not include saplings that began the season dead. We saw biomass differences among treatments in *B. papyrifera* and *Pr. serotina*.

Species	Treatment	Total biomass (g)	Root:Shoot	Survival
A. rubrum	Control	27.78 ± 9.40	2.39 ± 0.66	3/7
	Spring	43.91 ± 14.06	1.24 ± 0.15	9/9
	Summer	32.22 ± 7.58	1.63 ± 0.13	8/9
	Fall	51.97 ± 15.58	0.98 ± 0.19	12/12
B. papyrifera	Control	514.17 ± 32.11 (A)	0.75 ± 0.05	12/12
	Spring	89.04 ± 11.25 (B)	0.30 ± 0.30	1/12
	Summer	262.44 ± 26.88 (C)	0.43 ± 0.43	1/12
	Fall	490.54 ± 50.49 (A)	1.15 ± 1.15	12/12
Pr. serotina	Control	182.94 ± 42.43 (A)	3.54 ± 1.58	10/10
	Spring	108.54 ±17.03 (A)	2.25 ± 0.13	12/12
	Summer	64.08 ±12.22 (B)	1.58 ± 0.30	12/12
	Fall	76.13 ± 23.22 (A)	1.38 ± 0.23	10/10
J. virginiana	Control	70.18 ± 16.00	1.28 ± 0.17	12/12
	Spring	45.23 ± 11.24	1.39 ± 0.08	12/12
	Summer	42.83 ± 13.01	1.25 ± 0.13	12/12
	Fall	91.86 ± 17.81	1.36 ± 0.15	12/12
Pi. strobus	Control	124.54 ± 33.86	1.65 ± 0.28	11/12
	Spring	152.13 ± 21.30	1.36 ± 0.19	12/12
	Summer	118.59 ± 22.47	1.44 ± 0.48	11/11
	Fall	203.97 ± 33.75	1.15 ± 0.12	11/12
T. occidentalis	Control	70.58 ± 13.80	1.05 ± 0.10	12/12
	Spring	61.93 ± 16.40	1.42 ± 0.28	12/12
	Summer	98.21 ± 26.82	1.84 ± 0.61	12/12
	Fall	93.52 ± 11.60	1.10 ± 0.09	12/12

Table A1.2. Published P_{50} values for the six study species with associated reference. Published values for *Pr. serotina* could not be found, so a related species, *Pr. virginiana* was used in its place.

Species	P_{50} (MPa)
A. rubrum	-1.97 (Maherali et al. 2006)
B. papyrifera	-2.34 (Sperry et al. 1994)
Pr. virginiana	-3.8 (Sperry and Hacke 2004)
J. virginiana	-6.6 (Willson et al. 2008)
Pi. strobus	-5.25 (Asbjornsen et al. 2021)
T. occidentalis	-3.57 (Tyree and Dixon 1986)

Table A1.3. Height growth responses to drought in spring, summer, or fall. Possible responses denoted are “stop” meaning that growth stopped during this period, “increase” meaning that growth was increasing during this period, “decrease” meaning that growth decreased during this period, and “NA” meaning that no discernable difference in growth compared to the control was occurring.

Species	Season	Treatment		
		Spring	Summer	Fall
A. rubrum	Spring	Stop	NA	NA
	Summer	Increase	Stop	NA
	Fall	NA	NA	NA
B. papyrifera	Spring	Stop	NA	NA
	Summer	NA	Stop	NA
	Fall	NA	NA	Decrease
Pr. serotina	Spring	Stop	NA	NA
	Summer	Increase	Stop	NA
	Fall	NA	Increase	NA
J. virginiana	Spring	NA	NA	NA
	Summer	NA	Stop	NA
	Fall	NA	NA	NA
Pi. strobus	Spring	Stop	NA	NA
	Summer	NA	Stop	NA
	Fall	NA	NA	NA
T. occidentalis	Spring	Stop	NA	NA
	Summer	Increase	Stop	NA
	Fall	NA	NA	NA

Table A1.4. Diameter growth responses to drought in spring, summer, or fall. Possible responses denoted are “stop” meaning that growth stopped during this period, “increase” meaning that growth was increasing during this period, “decrease” meaning that growth decreased during this period, and “NA” meaning that no discernable difference in growth compared to the control was occurring.

Species	Season	Treatment		
		Spring	Summer	Fall
A. rubrum	Spring	NA	NA	NA
	Summer	NA	NA	NA
	Fall	NA	NA	NA
B. papyrifera	Spring	Decrease	NA	NA
	Summer	Stop	Decrease	NA
	Fall	Stop	Stop	NA
Pr. serotina	Spring	NA	NA	NA
	Summer	Stop	Stop	NA
	Fall	Increase	NA	NA
J. virginiana	Spring	NA	NA	NA
	Summer	NA	NA	NA
	Fall	NA	NA	NA
Pi. strobus	Spring	Stop	NA	NA
	Summer	Increase	NA	NA
	Fall	NA	Stop	NA
T. occidentalis	Spring	NA	NA	NA
	Summer	NA	Decrease	NA
	Fall	NA	Increase	NA

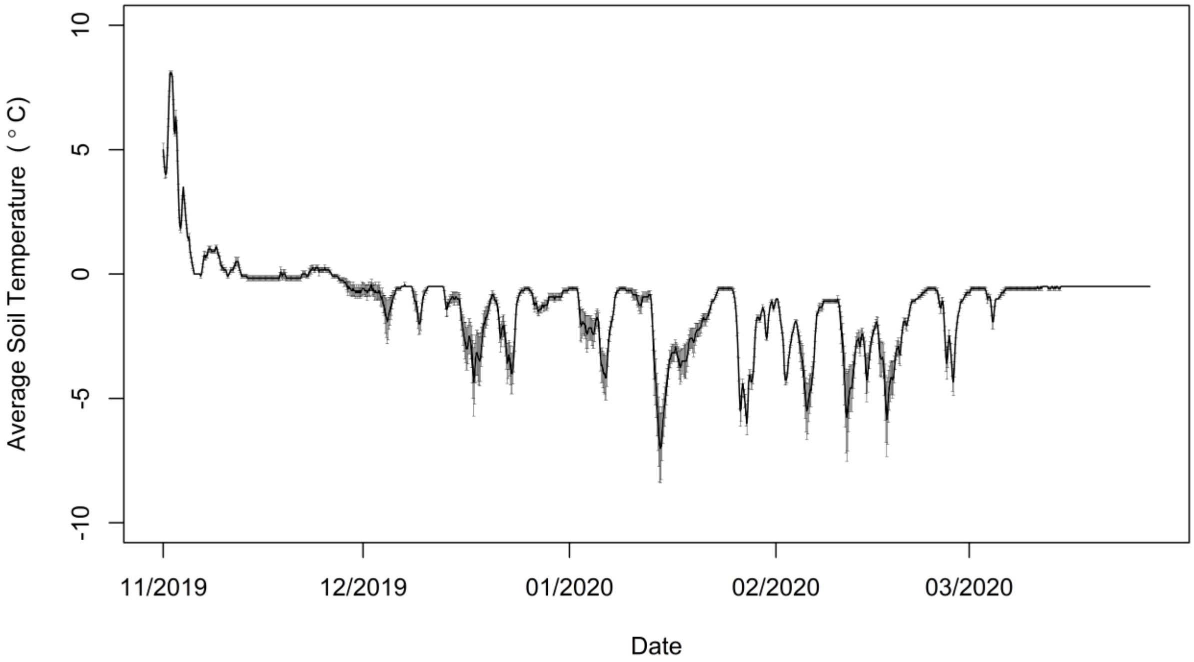

Figure A1.1. Mean ± SE soil temperature from November 2019 to late-March 2020from six iButton temperature loggers buried at 10 cm in six randomly selected containers. The black line is the mean of six measurements of soil temperature logged every six hours with gray SE bars above and below this line. These results suggest that plants rarely experienced soil temperatures low enough to induce root morality. Fine roots of saplings have been shown to survive winter soil temperatures down to -5°C (Tierney et al. 2001). The soil temperature in this study declined below -5°C approximately five times over the winter of 2019-2020.

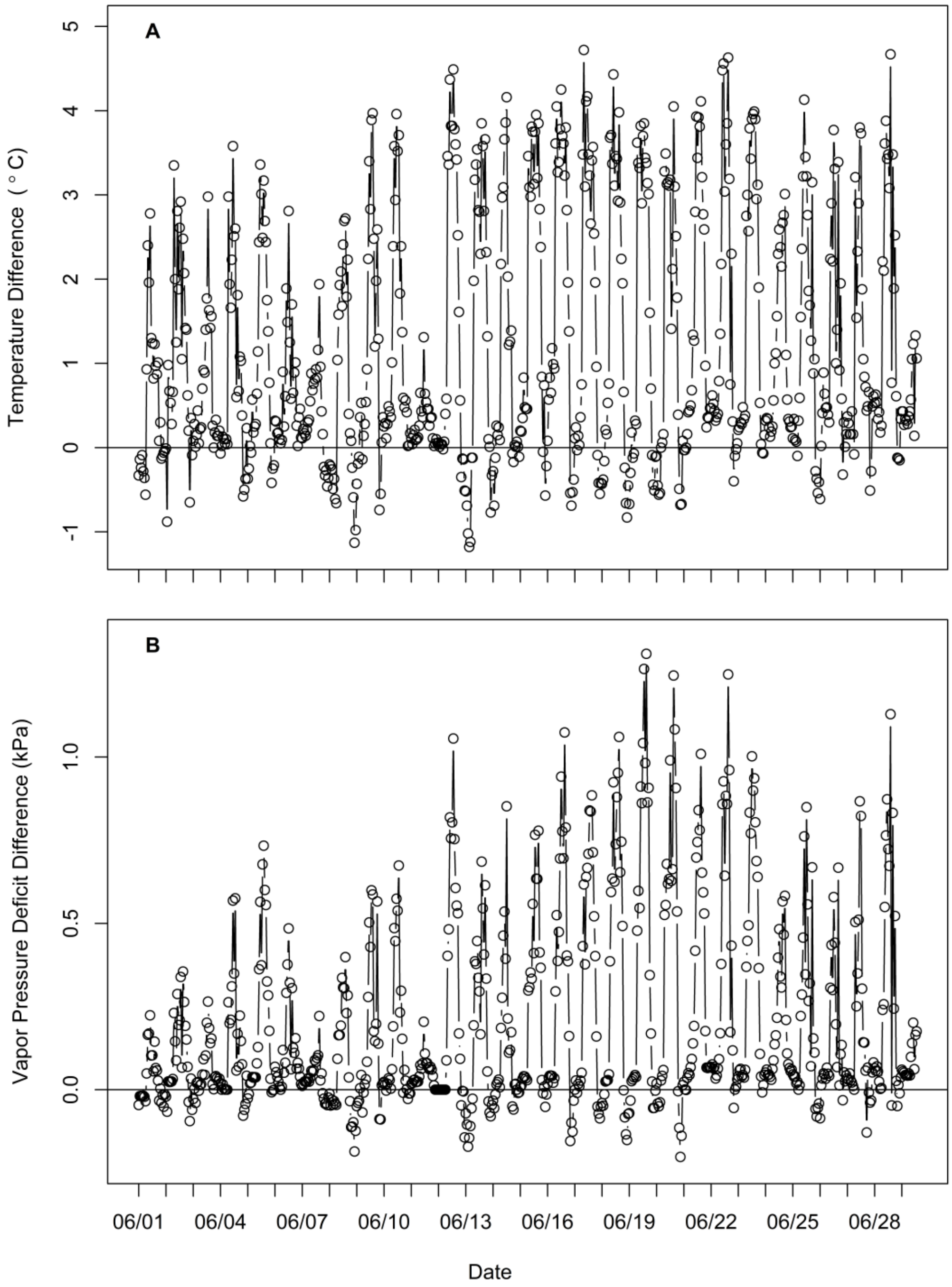

Figure A1.2. Hourly differences in temperature (°C; A) and vapor pressure deficit (kPa, B) in the greenhouse versus outside of the greenhouse during June 2020. In June, the average hourly temperature inside the greenhouse ranged from -1.18° to 4.72°C warmer than ambient, and hourly vapor pressure deficit inside the greenhouse ranged from -0.2 kPa to 1.3 kPa higher than ambient.

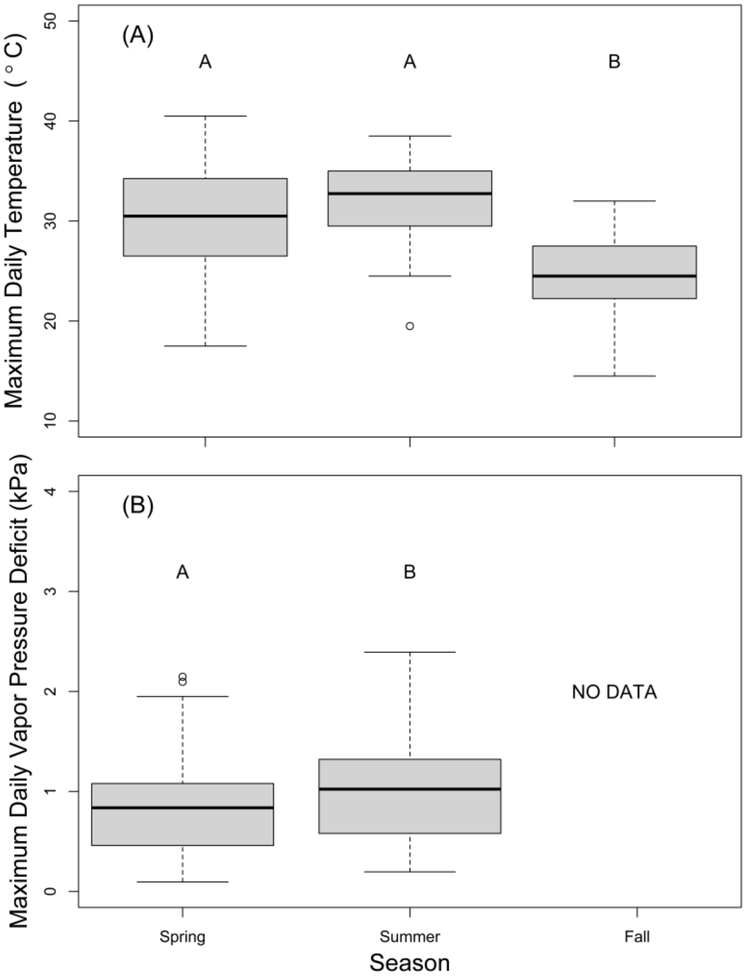

Figure A1.3. Boxplots of maximum daily temperatures (°C; A, measured with iButtons) and maximum daily vapor pressure deficit (kPa, B, measured with HOBO datalogger) inside the greenhouse during the spring, summer, and fall of 2020. Differences between the treatments at $p < 0.001$ (ANOVA with Tukey HSD) are denoted with asterisks. There is no VPD data for fall 2020 due to the malfunctioning of the HOBO datalogger. The spring and summer drought had similar maximum daily temperatures, while the fall was lower; the summer maximum daily VPD was higher in the summer than in the spring.

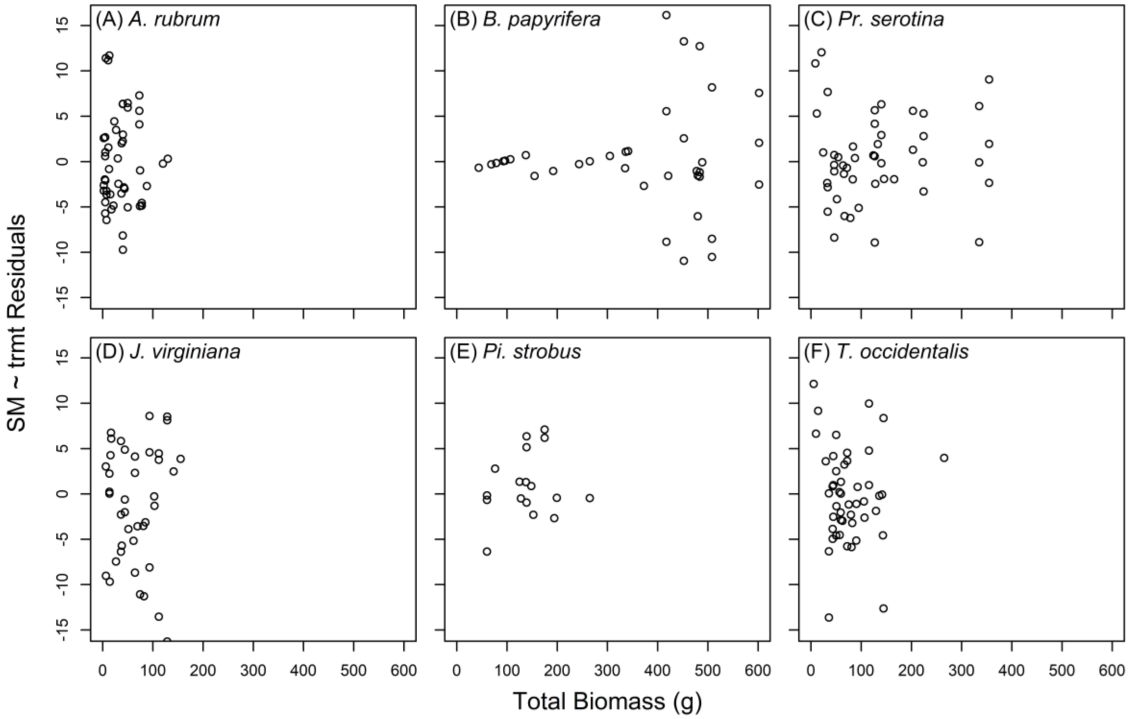

Figure A1.4. Residuals from the soil moisture ~ treatment ANOVA as a function of total biomass for each study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)). Larger trees lead to lower final soil moisture for *A. rubrum*, *Pr. serotina*, and *J. virginiana* but not the other three species.

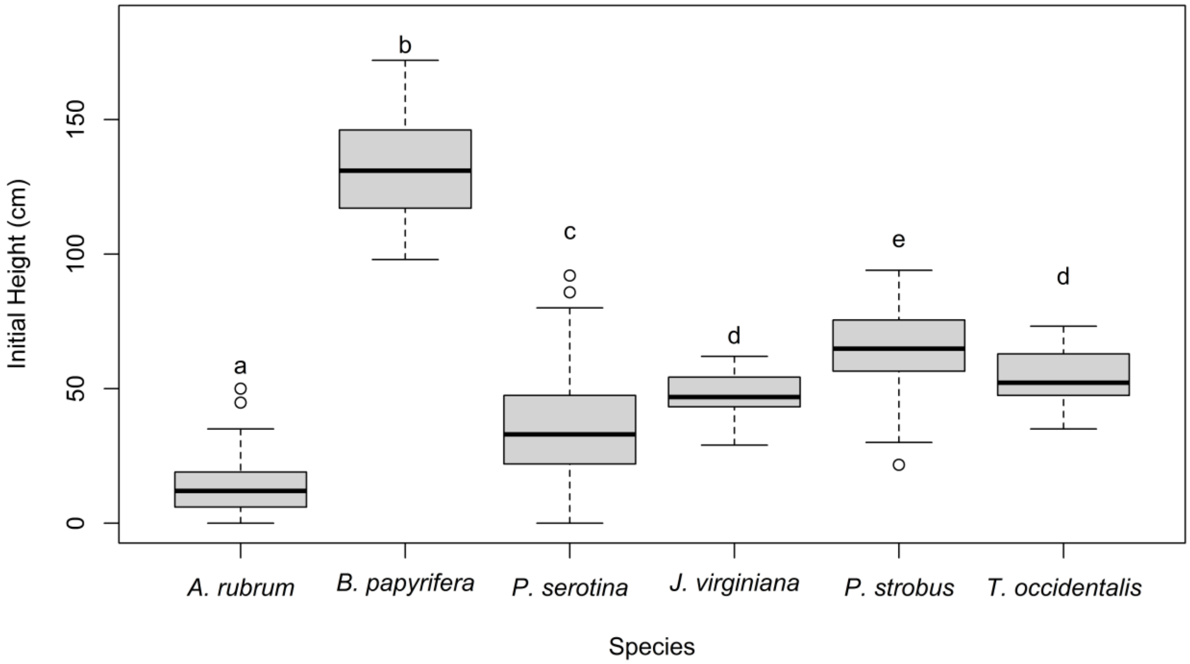

Figure A1.5. Boxplots of initial height in May 2020 (cm) for each of the study species (deciduous broad-leaved trees: *Acer rubrum*, *Betula papyrifera*, and *Prunus serotina*; and evergreen coniferous trees: *Juniperus Virginiana*, *Pinus strobus*, and *Thuja occidentalis* (F)). Differences between species are denoted with letters (ANOVA with Tukey HSD, $p < 0.05$). There was large intra- and extra-species variation in initial height in May 2020.

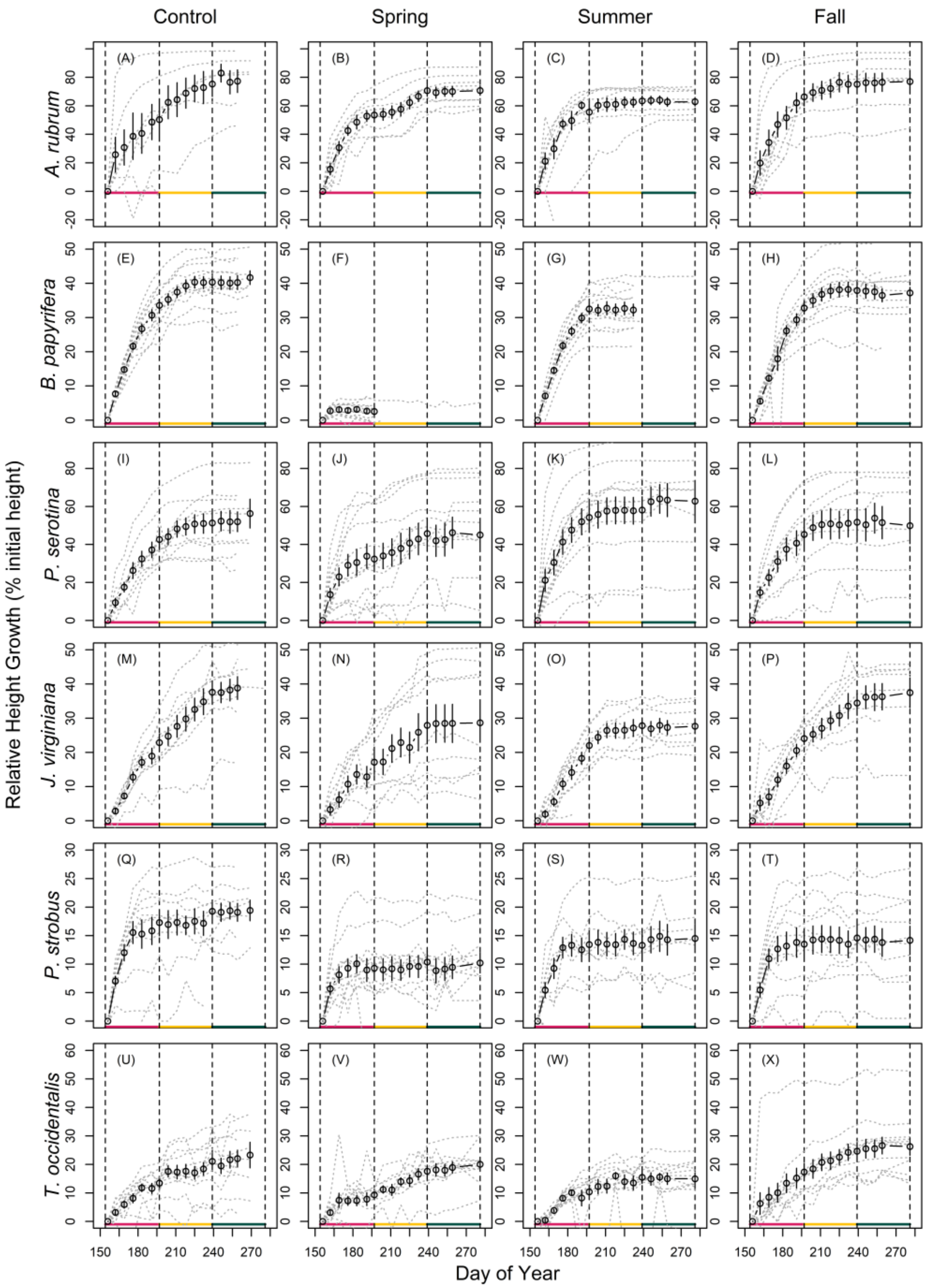

Figure A1.6. Relative height growth rate (% initial height) for each species and treatment over the course of the growing season. Each panel represents growth for each species and treatment combination, and contains light gray dashed lines for each sapling, with open black circles representing the mean ± SE of each sapling. The four vertical dashed lines represent the beginnings and ends of each of the three drought treatments, spring, summer, and fall. First column contains growth rates for controls, second column the spring, third column the summer, and fourth column the fall. Each row has one species (row 1: *Acer rubrum*, row 2: *Betula papyrifera*, row 3: *Prunus serotina*, row 4: *Juniperus virginiana*, row 5: *Pinus strobus*, row 6: *Thuja occidentalis*).

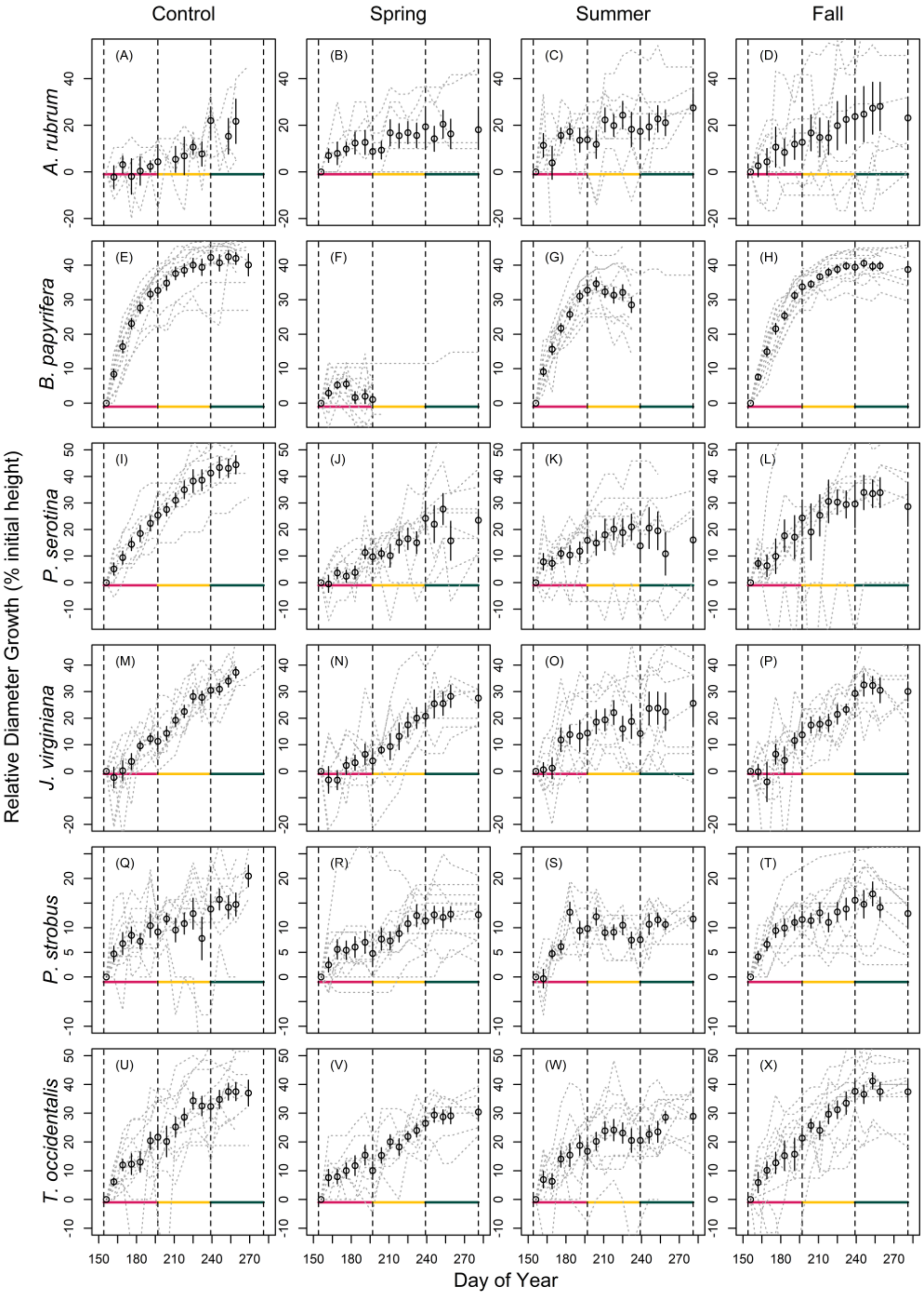

Figure A1.7. Relative diameter growth rate (% initial diameter) for each species and treatment over the course of the growing season. Each panel represents growth for each species and treatment combination, and contains light gray dashed lines for each sapling, with open black circles representing the mean ± SE of each sapling. The four vertical dashed lines represent the beginnings and ends of each of the three drought treatments, spring, summer, and fall. First column contains growth rates for controls, second column the spring, third column the summer, and fourth column the fall. Each row has one species (row 1: Acer rubrum, row 2: Betula papyrifera, row 3: Prunus serotina, row 4: Juniperus virginiana, row 5: Pinus strobus, row 6: Thuja occidentalis).

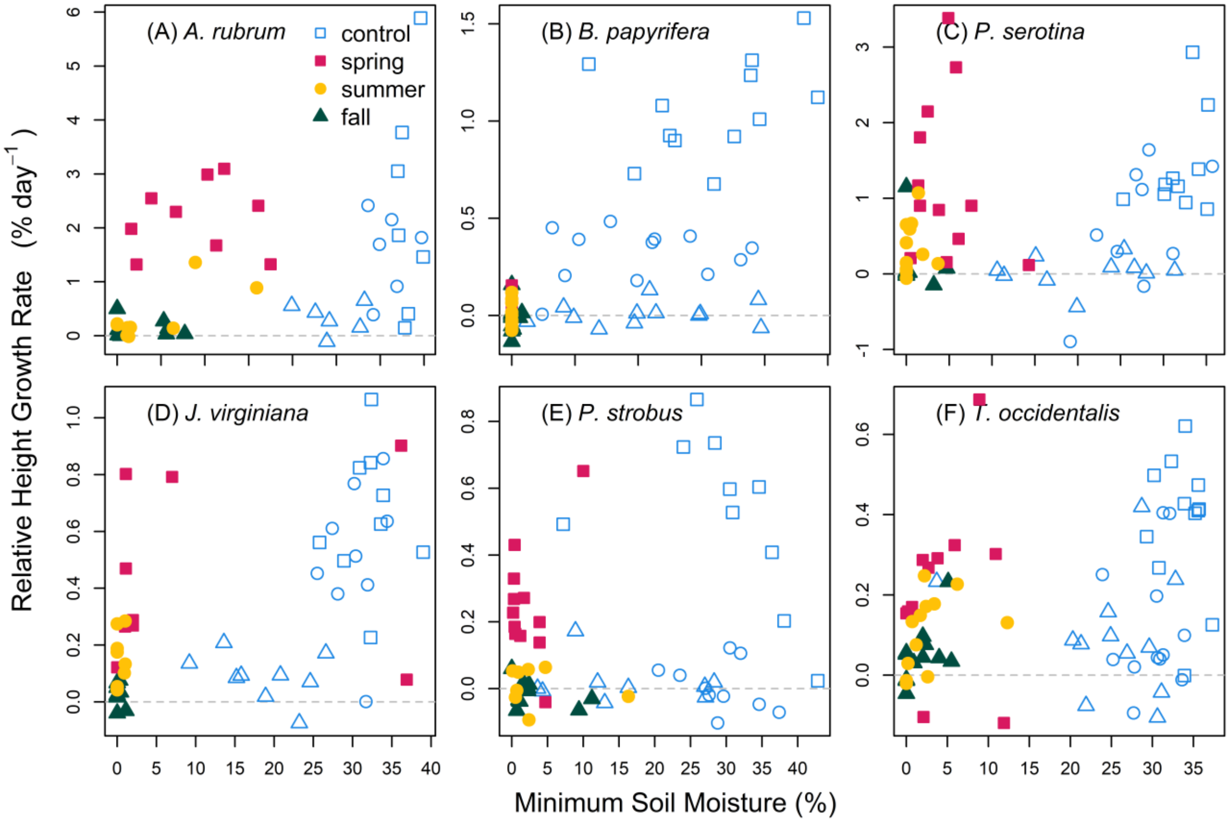

Figure A1.8. Relative height growth rate (% of initial height per day) as a function of minimum soil moisture experienced (%) during the drought. Each panel represents one of the study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)). Treatments are represented with colored symbols (blue empty symbols = control, red squares = spring, yellow circles = summer, and green rhombi = fall). Each filled treatment symbol has an empty blue symbol corresponding to the control measurement during that treatment. A gray dashed line is at zero height growth to facilitate comparison. Generally, lower minimum soil moisture values within a treatment led to lower height growth rates.

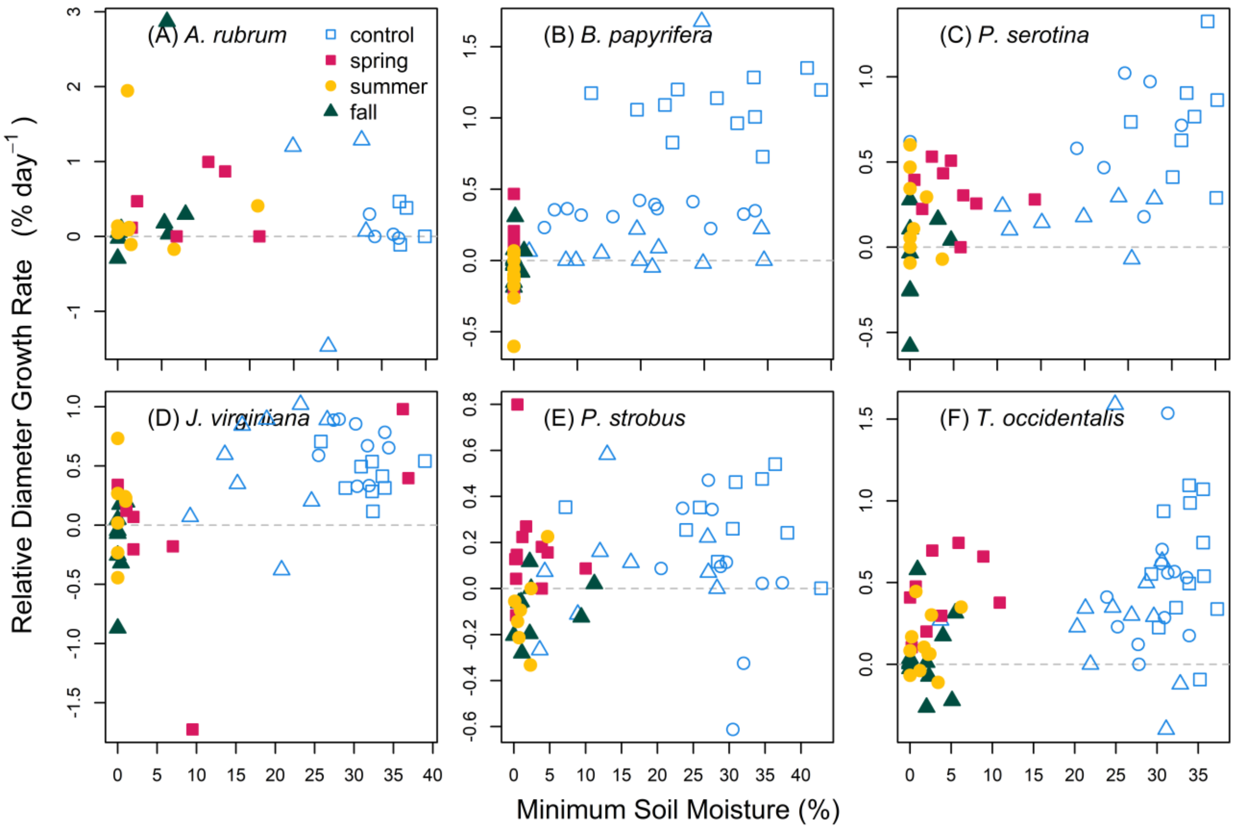

Figure A1.9. Relative diameter growth rate (% of initial diameter per day) as a function of minimum soil moisture experienced (%) during the drought. Each panel represents one of the study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)). Treatments are represented with colored symbols (blue empty symbols = control, red squares = spring, yellow circles = summer, and green rhombi = fall). Each filled treatment symbol has an empty blue symbol corresponding to the control measurement during that treatment. A gray dashed line is at zero height growth to facilitate comparison. Generally, lower minimum soil moisture values within a treatment led to lower diameter growth rates.

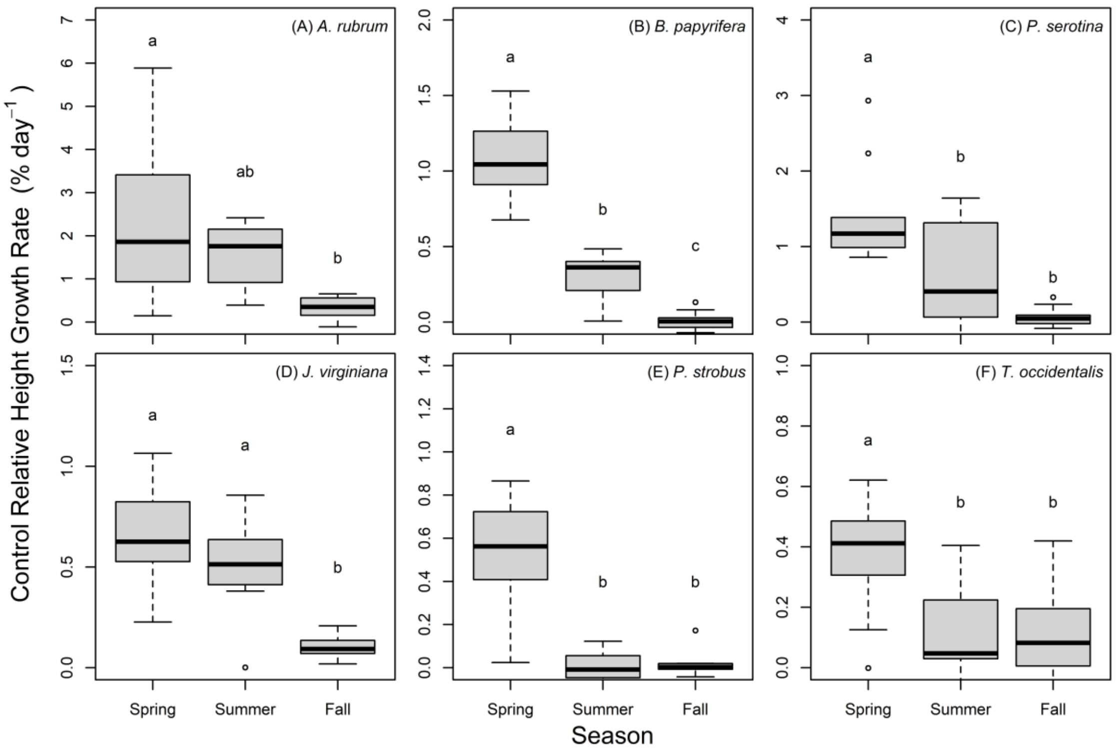

Figure A1.10. Boxplots of relative height growth rate (% per day) for the control saplings during each season for each of the study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)). Differences between seasons are denoted by letters of significance (Species-level ANOVA with Tukey HSD, $p < 0.05$). Species generally had the most height growth during the spring.

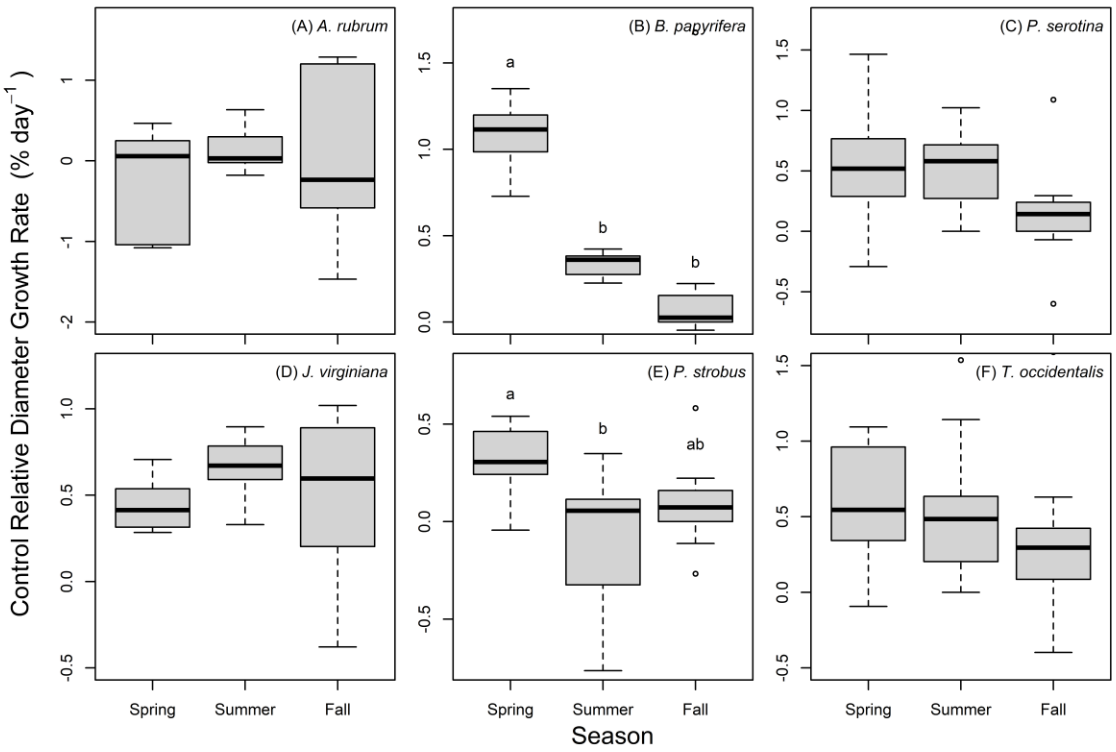

Figure A1.11. Boxplots of relative diameter growth rate (% per day) for the control saplings during each season for each of the study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)). Differences between seasons are denoted by letters of significance (Species-level ANOVA with Tukey HSD, $p < 0.05$). We did not observe consistent differences in maximum relative diameter growth rates among species and seasons.

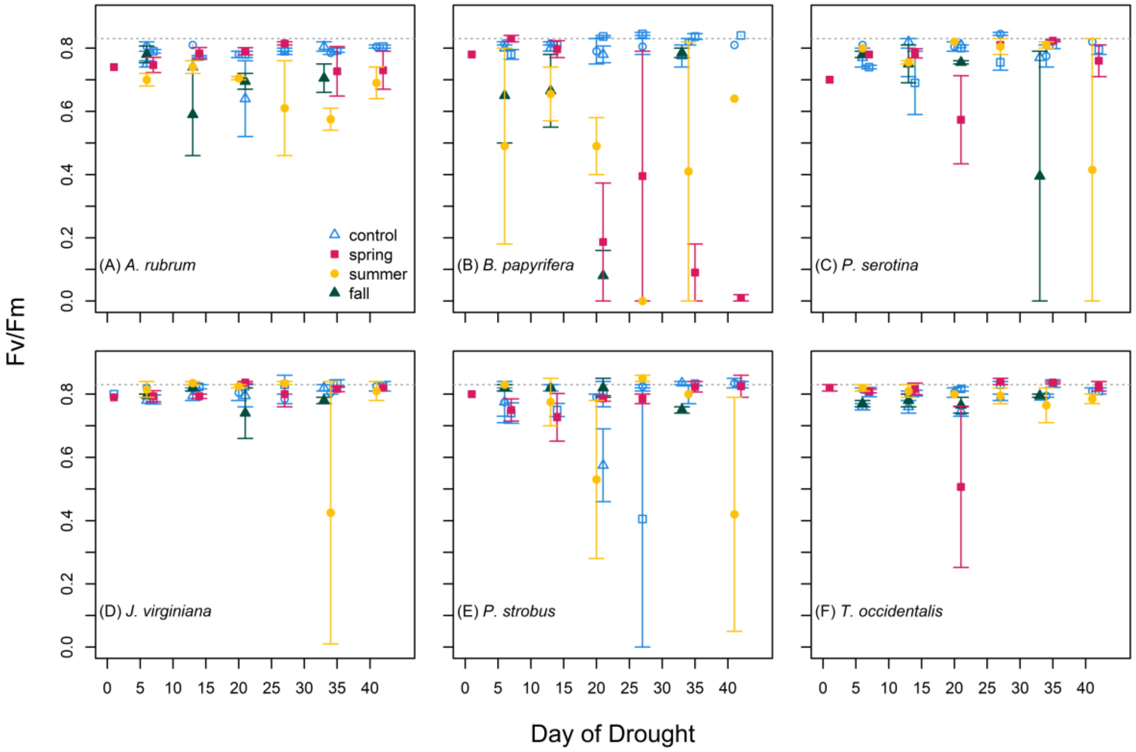

Figure A1.12. Mean ± SE predawn quantum yield (F_v/F_m) over the course of each seasonal drought, standardized to day of drought to facilitate comparisons between treatments. Each panel represents one of the study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C) and evergreen coniferous trees: *Juniperus Virginiana* (D), *Pinus strobus* (E), and *Thuja occidentalis* (F)). Treatments are represented with colored symbols (blue empty symbols = control, red squares = spring, yellow circles = summer, and green rhombi = fall). Each filled treatment symbol has an empty blue symbol corresponding to the control measurement during that treatment. A gray dotted line is at 0.83, which is a widely accepted F_v/F_m value for healthy leaves. We observed some declines in F_v/F_m among species and treatments; with *B. papyrifera* showing the most consistent declines. Very low control values in *Pi. strobus* may be due to measurement error or variation in the health of individual saplings.

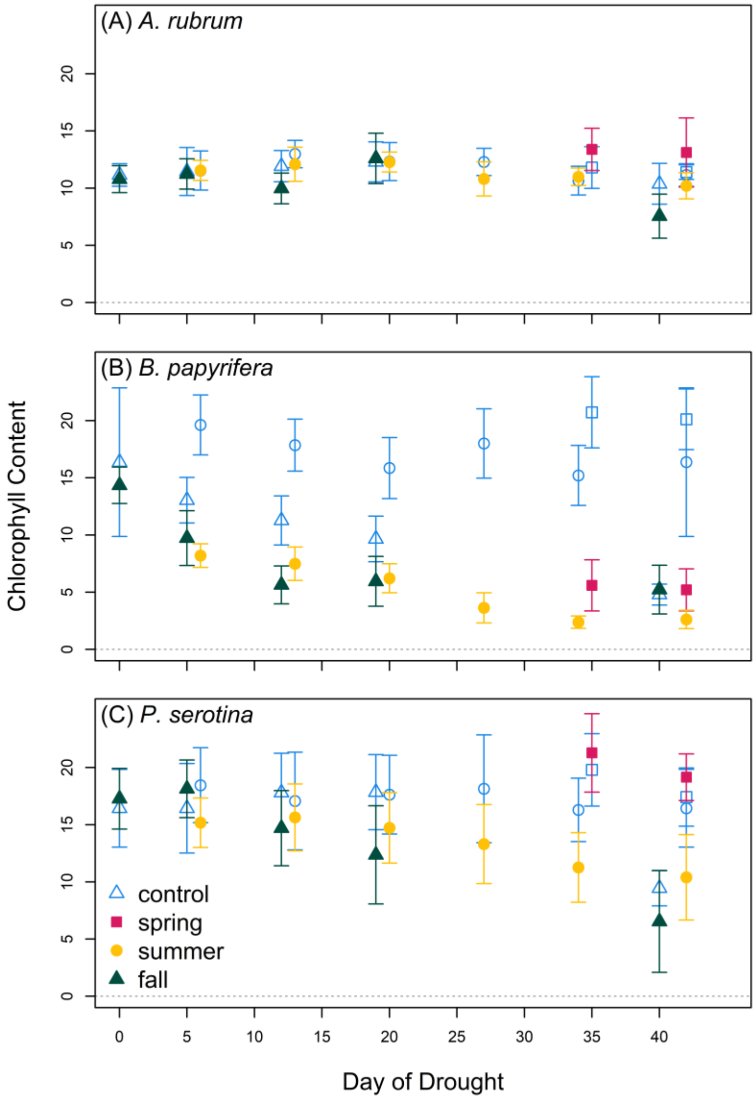

Figure A1.13. Mean ± 2SE chlorophyll content over the course of each seasonal drought, standardized to day of drought to facilitate comparisons between treatments. Each panel represents one of the study species (deciduous broad-leaved trees: *Acer rubrum* (A), *Betula papyrifera* (B), *Prunus serotina* (C)). Treatments are represented with colored symbols (blue empty symbols = control, red squares = spring, yellow circles = summer, and green rhombi = fall). Each filled treatment symbol has an empty blue symbol corresponding to the control measurement during that treatment. A gray dotted line is at 0. Chlorophyll content declined over the course of the spring, summer, fall droughts in *B. papyrifera*.

APPENDIX 2.

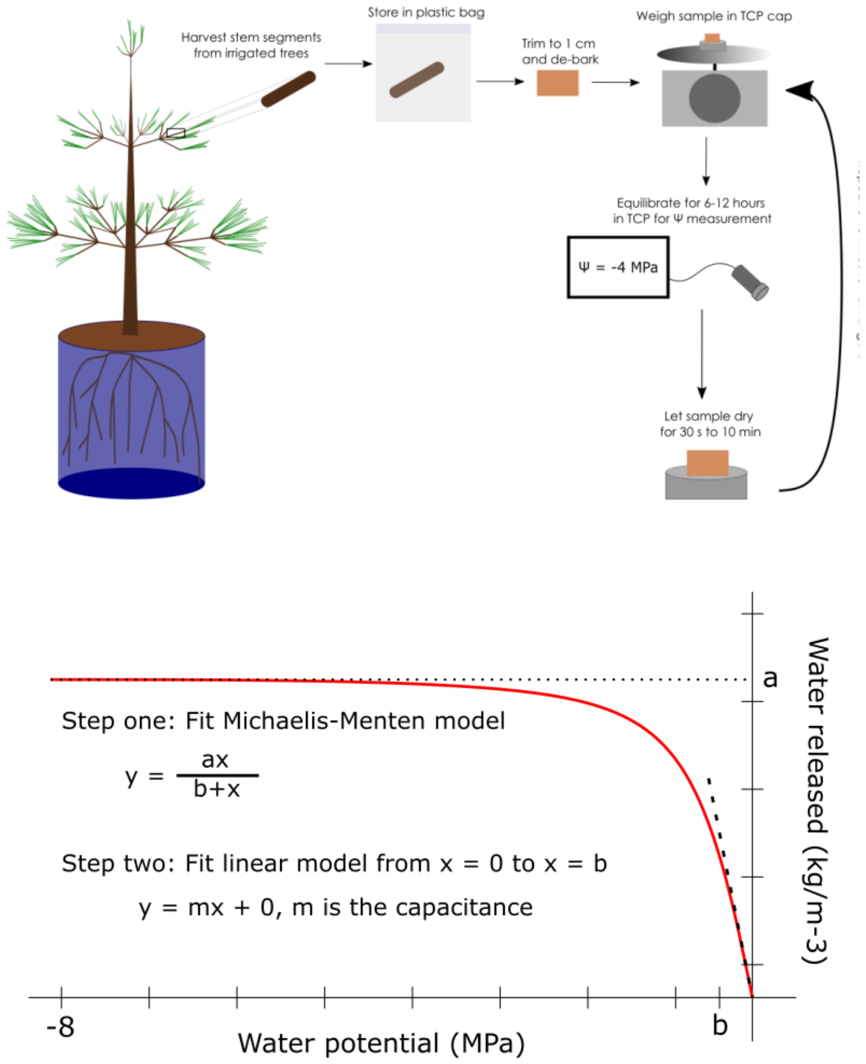

Figure A2.1. Schematic diagram of water release curve experiment. A) One-year old xylem segments were harvested from irrigated saplings and immediately stored and frozen in plastic bags. Within two weeks, samples were thawed, trimmed to one cm, debarked, and vacuum infiltrated overnight. Samples were then weighed in a TCP cap, equilibrated for 6-12 hours in the TCPs until water potential measurements stabilized, and then dried on the bench for 30s-10min. This process was repeated 6-10 times to obtain a range of water potential measurements. B) Following Meinzer (2003), we estimated the maximum water storage of the stem segment, a; and using the inflection point *b* we calculated the slope *m* of the line from 0 MPa to *b* MPa, which was the estimate of capacitance.

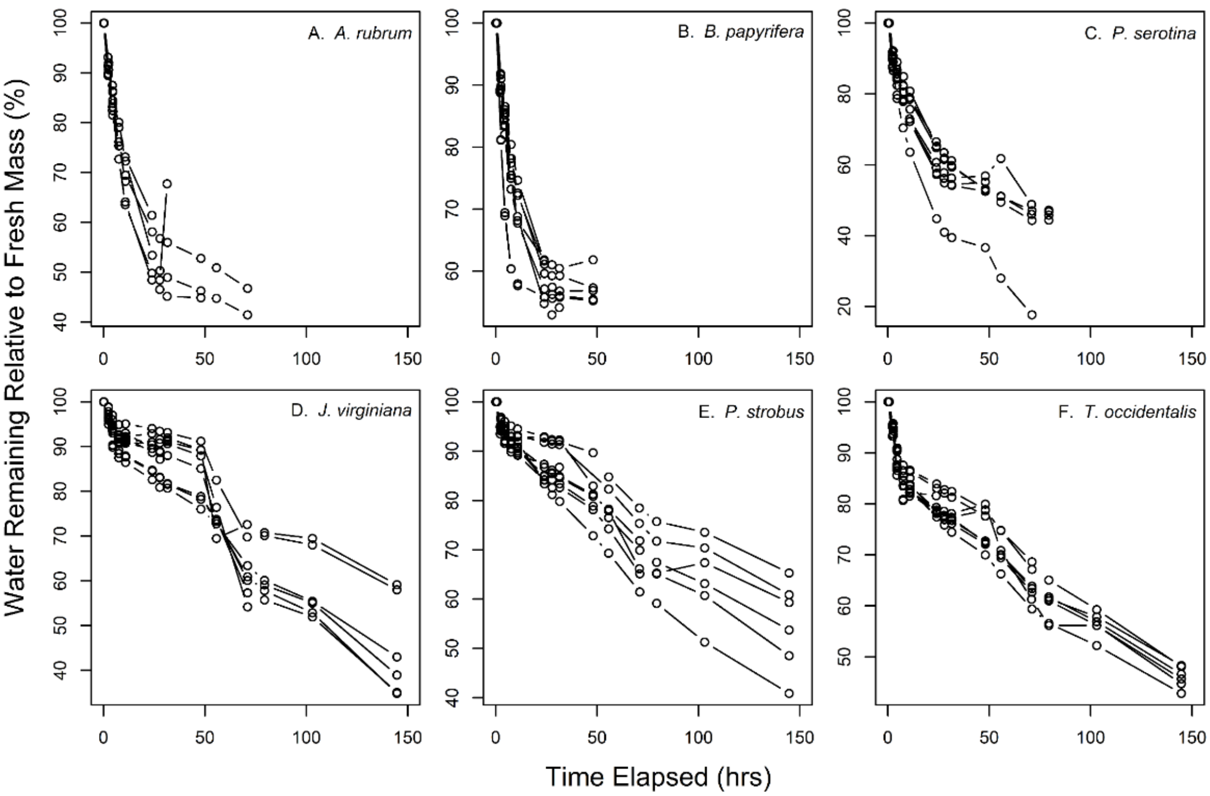

Figure A2.2. Water remaining relative to fresh shoot mass (%) as a function of time elapsed (hrs) from the shoot dry down experiment for each of the study species (A-F, *A. rubrum, B. papyrifera, Pr. serotina, J. virigiana, Pi. strobus,* and *T. occidentalis*). The entire experiment lasted approximately 150 hours; all shoots began at the same time and at the first, last, and 3-6 systematically chosen measurements in between, shoots from each species were destructively sampled to estimate shoot water potential (Ψ_{shoot}), stem relative water content (RWC_{stem}), and leaf relative water content (RWC_{leaf}) in order to understand the dynamics of shoot, stem, and leaf water storage relative to declines in shoot water potential (Ψ_{shoot}).

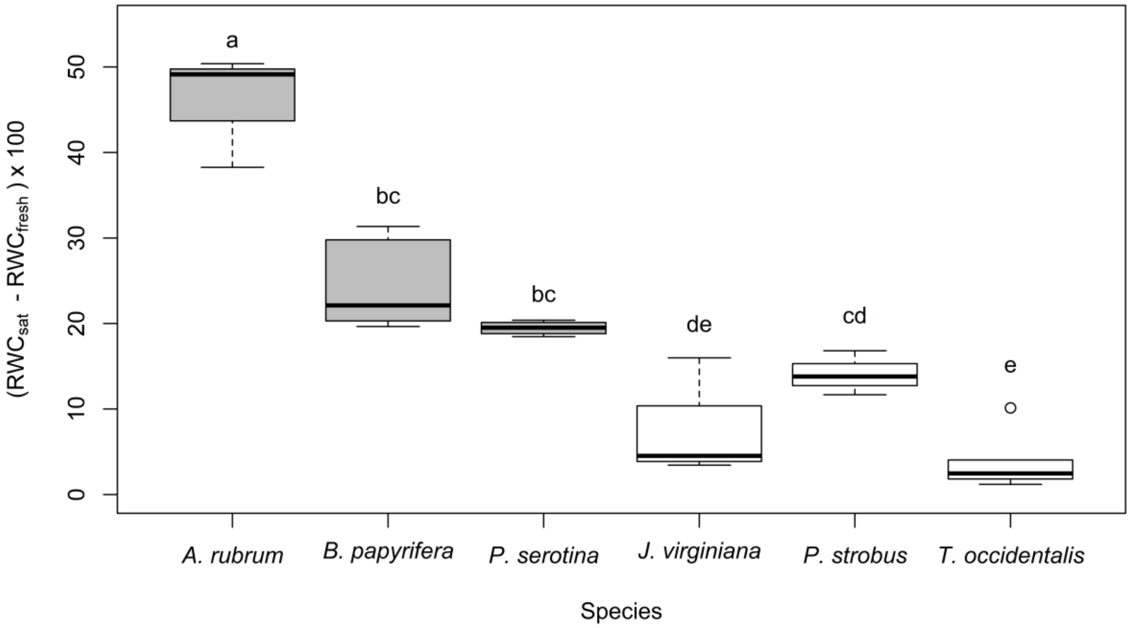

Figure A2.3. Percent difference in vacuum saturated RWC$_{stem}$ and fresh RWC$_{stem}$ for ~1 cm de-barked xylem segments for each of the study species. Broadleaved species are gray-filled boxplots and conifers are empty; post-hoc significance is denoted by letters. *A. rubrum* showed the largest difference between saturated and fresh RWC, while *T. occidentalis* showed the smallest difference.

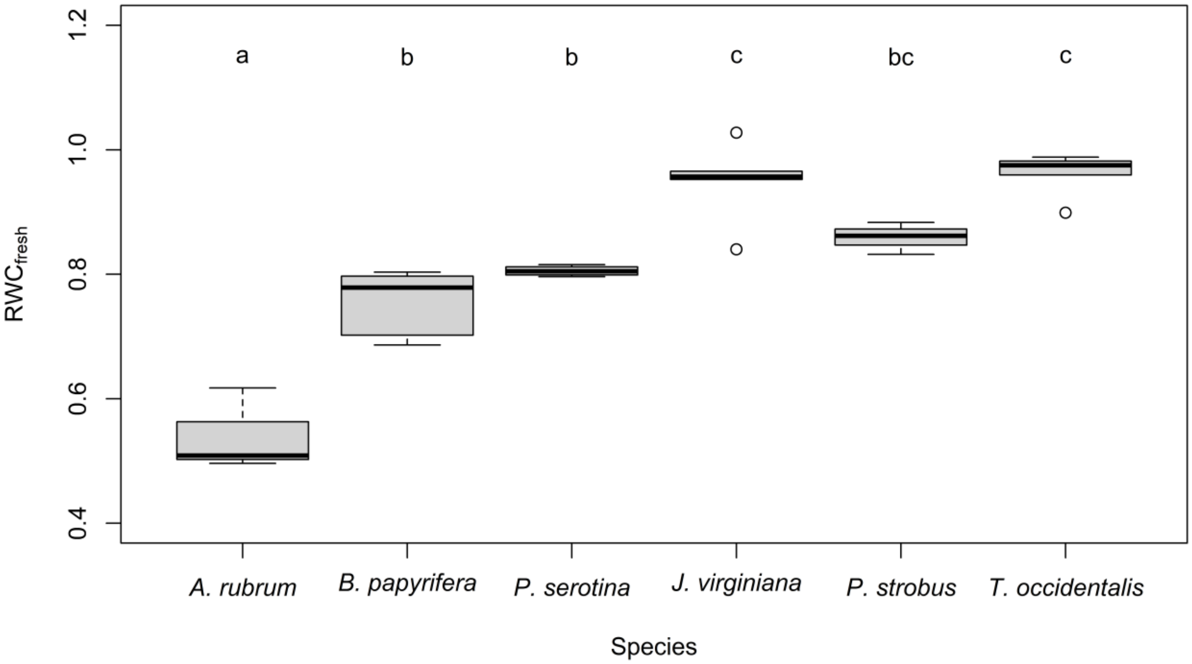

Figure A2.4. Boxplots of RWC$_{fresh}$ for ~1 cm de-barked xylem segments for each of the study species prior to beginning the water release curve experiment. *A. rubrum* showed the lowest RWC$_{fresh}$.

BIOGRAPHY OF THE AUTHOR

Ruth van Kampen has lived in Maine for nine years and has been visiting Maine their entire life. Born in Issaquah, Washington, and having lived in California, Virginia, and Ohio in addition to Maine, they grew up with a deep appreciation and fondness for forests of all sorts when their dad taught them about new growth on conifers, and their mom taught them about fungi. They quickly became enamored with trees during college when they took a course titled “Plants and Human Affairs,” taught by their future academic advisor, Dr. Brett Huggett, which taught Ruth they could study trees as a discipline.

After graduating from Bates College with a B.S. in biology and doing their undergraduate thesis on the effects of drought and defoliation on New England forest trees, they moved to Orono to begin their M.S. in Forest Resources at the University of Maine with Dr. Jay Wason. Ruth is a candidate for the Master of Science degree in Forest Resources from the University of Maine in August 2021.