IMPACTS OF NEEDLE AGE, TREE ARCHITECTURE, AND DROUGHT STRESS ON PHOTOSYNTHETIC PHYSIOLOGY OF FRASER FIR (*Abies fraseri*) CHRISTMAS TREES

A Thesis
by
ASHLEY MARIE HULL

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ABSTRACT

Impacts of Needle Age, Tree Architecture, and Drought Stress on Photosynthetic Physiology of Fraser fir (*Abies fraseri*) Christmas Trees

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Anthropogenic carbon dioxide emissions have already begun to increase average global temperatures and drought frequency in parts of the southeastern US, leaving many agricultural industries to question the future of their crops. To determine the effect of reduced precipitation on growth and harvest quality of Fraser fir (*Abies fraseri*) trees grown for the US Christmas tree industry, I exposed them to drought using rainfall exclusion channels on a choose-and-cut farm in the Southern Appalachian Mountains. I also studied aspects of their physiology and crown architecture. Although Fraser firs are only native above ~1,500 m, those for the Christmas tree industry are typically grown at lower elevations and trimmed annually to create a dense, cone-shaped canopy that is favored by customers. Little is known about how these changes in tree architecture affect carbon assimilation, nor how needles of differing age adjust, if at all, to lower light environments resulting from canopy extension. I studied light interception and photosynthetic characteristics of three needle age classes (current, 1, and 2 yr old) on a nearby set of trees at the same farm. I hypothesized that drought conditions would cause a decline in gas

exchange rates, reduced growth, and quicker release from dormancy and bud burst, while aging and a decline in light penetration would cause a decrease in gas exchange rates. For the drought study, I measured soil moisture biweekly, pre-dawn and mid-day water potentials, gas exchange, and growth parameters. For the needle age study, I measured light depletion at three canopy depths, gas exchange as a function of needle age, minimum stomatal conductances, epicuticular wax contents, and chlorophyll contents. An extremely wet winter of 2020-2021 combined with potential underground water movement thwarted my rain exclusion set-up, even with 74% channel coverage since July 2020. No notable differences in growth, soil moisture, water potentials, or photosynthetic physiology were found between the droughted and control trees. For the needle aging study, direct light intensity on a sunny day near noon in October was ~2000 $\mu mol\ m^{-2}\ s^{-1}$ for current year needles but decreased by 97% and 99% at typical interior canopy locations for 1 and 2 yr old needles, respectively. Gas exchange was measured for planar needles without self-shading and on twigs with needles in their natural orientation. Apparent quantum efficiency, maximum photosynthesis, and light saturation were lowest in 2 yr old planar needles, while dark respiration, maximum photosynthesis, and light saturation were lower in 1 yr old twigs. Needle density was also lowest in 2 yr old needles. Chlorophyll content was highest in 2 yr old needles, while minimum stomatal conductances and epicuticular wax content did not differ among needle age classes. Higher chlorophyll contents and lower Chl *a*:*b* ratios indicated some adjustments to the low light environment typical of older needles, but lower photosynthetic rates in 2 yr old needles indicated an aging effect independent of canopy light environment, possibly influenced by decreased needle density. Trimming increases canopy density, causing older needles to have low or even negative carbon budgets due to the low light in the interior

portion of the tree canopy. Why these trees maintain the oldest, heavily shaded needles is unknown and suggests the need for further research.

ACKNOWLEDGEMENTS

I would like to acknowledge my advisor, Dr. Howard Neufeld, for all of his wisdom, support, and assistance throughout this project. Thanks to Claire Martin for her assistance and support during the heavy lifting of this project and for always being willing to help. I would like to thank Will Kepley, Sam Lee, and Charles Martin for their help in constructing my drought experiment, and thanks to Dustin Murray for his help during deconstruction and calculating the covered area of my drought plot. Thanks to Drs. Cara Fiore and Ray Williams for allowing me to use their lab during epicuticular wax extraction, and to Dylan Fehl and Dr. Darren Seals for supplying liquid N_2 during chlorophyll extraction. This project would not have been possible without the cooperation of Joey Clawson from Clawson's Christmas Tree Farm, who allowed me to set up a drought experiment and perform my physiological measurements on his trees. Funding for this study was provided by the Cratis D. Williams Graduate School and Office of Student Research at Appalachian State University. Lastly, I would like to thank my close friends and family for their encouragement and listening ear when I needed support.

DEDICATION

To my cat, Wally, for more love, support, and snuggles than I could ever ask for.

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FOREWORD

References are in the style of the journal *Tree Physiology*.

INTRODUCTION: CH. 1 AND 2

One of the many repercussions of the rapid expanse of the human population includes the impact on our climate; fossil fuel consumption has led to a record high levels of global atmospheric carbon dioxide at 420.6 ppm as of April 2022 (since record-keeping began, Jones 2022), with an increase of ~10 ppm from 2019 and an increase of ~140 ppm from pre-industrial levels (Etheridge et al. 1996; Lindsey 2020; Thoning et al. 2020). This dramatic increase is believed to be responsible for ~90% of current global warming (Butler and Montzka 2020). Projected climate change scenarios include an increase of 2-4ºC in average temperature around the globe by the year 2050 (New et al. 2011).

Warming around the globe will likely be paired with more stochastic precipitation patterns, characterized by a "boom and bust" cycle of lengthy drought periods and infrequent, but intense thunderstorms (IPCC 2014; Coble et al. 2017). This phenomenon has already started to become noticeable; the North Carolina Climate Office reported that severe storms started early and lasted throughout the year of 2020, rather than occurring primarily in the spring. Additionally, extreme precipitation events with more than 3 to 4 inches being recorded in a single day are becoming more frequent (Davis and Dello 2021).

Approximately 35-40% of the US has already been affected by severe droughts in recent years, including a substantial area of the southeastern US (New et al. 2011; Wang et al. 2011). Although annual precipitation levels in the Southern Appalachian Mountains are expected to increase by up to 10% in the summer and fall, with larger increases in the winter and spring (Luce et al. 2016), this region is also predicted to have one of the largest percentage increases in

the number of days without precipitation, creating intermittent drought conditions (Knapp et al. 2008; Heisler-White et al. 2009; Coble et al. 2017).

Within the Western North Carolina region of the Southern Appalachian Mountains, the effects of climate change are only beginning to be detected (Meehl et al. 2012). The region is considered a "warming hole," because throughout the twentieth century it experienced less warming than did many other parts of the world. This anomaly is thought to be caused by cold-air advection in winter and low-level moisture convergence in summer (Meehl et al. 2012). Some researchers believe that increasing temperature trends since the 1960s have already deemed the term "warming hole" outdated (Shadbolt 2019), while some current projections anticipate its complete disappearance, driven primarily by increased nighttime warming, particularly in the winter months (Meehl et al. 2012; Luce et al. 2016). Warmer nights have already begun to be detectable in North Carolina; 2020 was the sixth consecutive year that minimum nighttime temperatures have broken previous North Carolina records (Davis and Dello 2021).

Increased rainfall stochasticity coupled with warmer temperatures will have large effects on plant physiological processes, most notably water relations and gas exchange. High temperatures and lack of rainfall will result in lower relative humidities and create high vapor pressure deficits (VPD) between the leaf and surrounding air (Anderson 1936), potentially decreasing stomatal apertures and reducing photosynthetic rates (Turner et al. 1984; Katul et al. 2009). Closing the stomata in response to a high VPD may result in greater water use efficiency (Turner et al. 1984; Katul et al. 2009), even though overall carbon gain is reduced. If the plant fails to close its stomata, transpiration could exceed root uptake and cause hydraulic failure, resulting in the loss of needles and branches downstream from the embolized xylem (McDowell et al. 2008; Anderegg et al. 2016). Even with stomatal closure, extreme soil water deficits due to

drought would eventually result in xylem disruption (Anderegg et al. 2016). While hydraulic failure is considered the main cause of mortality in drought-stressed plants, drought has also been shown to decrease non-structural carbohydrate storage. Some studies suggest that death could result not only from desiccation, but also from carbon starvation (McDowell et al. 2008; Sevanto et al. 2013; Choat et al. 2018), although other studies have found no direct evidence of depletion of carbon reserves prior to drought-induced mortality, and sometimes a reduction in water availability actually increases carbon stores (Körner et al. 2003; Sala and Hoch 2009).

Fraser fir (*Abies fraseri*) is a native conifer endemic to the highest elevations in the Southern Appalachian Mountains. It is the most commonly grown species in the Christmas tree industry due to its unmatched needle retention, strong branches, and pleasant aroma (Williams 1958). Christmas trees represent an important cash crop in Western North Carolina, contributing approximately $100 million to the region annually (USDA 2017). Unfortunately, Christmas tree farms are suffering declines in production because of diseases such as root rot and stem canker, lack of young growers becoming tree farmers, and pests like the Balsam twig aphid (*Mindarus abietinus*) and the introduced Balsam woolly adelgid (*Adelges piceae*) (Eagar 1978; Frampton et al. 2012). Additionally, the species is limited to just seven peaks in the high elevations of its native range in one of the most endangered ecosystems of the United States, the Southern Appalachian spruce-fir forests (White et al. 2012). It was designated as endangered by the IUCN Red List in 2013 because of its restricted distribution and the damage caused by the adelgid in the 1980s (Smith and Nicholas 2011; White et al. 2012; Farjon 2013).

The natural elevation range of Fraser firs is at or above 1,350 m, existing solely in high-elevation fir forests that are immersed in clouds for a majority of the year. In fact, the red spruce-Fraser fir ecosystem experiences cloud immersion on about 65% of all days annually (Reinhardt

and Smith 2008; 2010). Fraser fir trees rely on this frequent cloud cover to maintain vital physiological processes. For example, recent studies have shown that Fraser firs obtain up to 31% of their water from clouds in their native habitat (Berry et al. 2014). Photosynthesis in native Fraser firs is highly influenced by the response of stomata to VPD, which is relatively low when clouds are present, but which can increase substantially on sunny, warm days (Reinhardt and Smith 2008). Disappearance of the warming hole will lead to a heightened cloud ceiling, subjecting the species to fewer cloud events, lower humidities (i.e., higher VPD), higher temperatures, and more intense solar radiation (Reinhardt and Smith 2008; Berry and Smith 2013; Berry et al. 2014; Berry and Smith 2014), all of which may contribute to greater water stress for this species. Further, Fraser fir are restricted to these high-elevation peaks not only because of cloud immersion, but also because of competition with other tree species at lower elevations and higher evaporative demand (Stehn et al. 2011; Urza et al. 2020).

All Christmas tree farms are currently located below the lower natural elevation range of Fraser fir (1350+ m), and some operate successfully at elevations as low as 670 m. Farms lower than this most likely exceed the environmental limits of this species (Cory et al. 2017), whether ecological or physiological. Low elevation farmers harvest trees at the same time as high elevation farmers, but elevation (a surrogate for warming trends) affects the phenology and growth patterns of these trees. For example, bud burst occurs sooner, duration of shoot extension is shorter, and duration of diameter growth is lower at low elevation farms compared to high elevation farms (1,340 m), suggesting a strong response to temperature. Interestingly, trees at low elevations did not exhibit signs of heightened water stress (Wood 2016; Cory et al. 2017), which suggests there may be compensatory mechanisms at work to minimize water loss for warmer trees, although this idea has not been explored. Trees exceeding the lower elevation limit

may be exposed to higher evaporative demand, higher temperatures, standing water (i.e., Phytophthora root rot), or a higher frequency of pest invasion that would negatively impact tree health and reduce market quality.

Because of high annual rainfall in the Southern Appalachian Mountains, there have been few extensive droughts (Hursh and Haasis 1931; Vose and Swank 1994; Kloeppel et al. 2003), and little is known about how Fraser firs react to such events. Four-year-old *A. fraseri* seedlings planted in the ground in a semi-controlled environment (open-air hoop houses) and exposed to drought shed their oldest, interior needles and had significantly reduced relative height growth after one growing season (Kulac et al. 2012). Silver fir (*Abies alba*), a European tree that also has ideal Christmas tree qualities, had high defoliation rates, reduced growth, and heightened mortality in trees subjected to artificial drought (Toromani et al. 2011; Matias et al. 2016). These two studies are the only known drought studies on fir trees, so much remains to be discovered about how this genus will react to seasonal water deficits. Projections indicating warming and less predictable precipitation patterns suggest that farms at lower elevations may have to move their tree production to higher elevations to successfully bring their crop to market (Meehl et al. 2012, Cory et al. 2017).

Unlike most endemic conifer species, commercialized Fraser fir trees retain their needles for three to six growing seasons due to an intensive genetic breeding program aimed towards increasing needle retention (McKinley and Hazel 2019). However, the relative contribution of each needle age cohort to the overall carbon budget of a tree is currently unknown. In fact, the literature detailing how needle age affects the carbon budget of conifers remains relatively understudied. Freeland (1952) demonstrated that photosynthetic capacity significantly decreases with increasing age in six conifer species but did not adjust the results for differences in leaf

area– rather, he based his calculations off of 100 needles per sample. In a related study, Yuan et al. (2018) compared nitrogen and phosphorus resorption in different needle ages of white spruce (*Picea glauca*) and balsam fir (*Abies balsamea*) and found that concentrations of N and P significantly decreased with needle age, as did nutrient resorption efficiency. Other studies have been aimed at understanding how needles of varying ages are affected by air pollution (Karhu and Huttunen 1986; Turunen and Huttunen 1990; Langebartels et al. 1998; Hernandez-Clemente et al. 2011), but data are lacking for Fraser fir trees. To date, there is a poor understanding of the architecture of commercially-raised Fraser fir trees and how this architecture might affect the distribution of the variously-aged needle cohorts and their contribution to the whole tree carbon budget.

The purpose of my study was to determine the drought response and photosynthetic contribution of differently aged needles on 3-4-year-old commercialized Fraser fir trees. I hypothesized that:

(1) Prolonged drought conditions would result in lower gas exchange rates, reduced water potentials, and stunted growth phenology compared to control trees, thus contributing to lower market quality.

(2) Photosynthetic activity would decline with needle age due to (a) reduced stomatal conductance and/or (b) reduced biochemical activity.

(3) Older needles would also contribute less to the carbon budget of the tree because of (a) needle loss, (b) changes in needle morphology that affect gas exchange, and/or (c) lower photosynthetic rates due to their placement in the canopy interior where light levels would be low.

CHAPTER ONE

Impacts of Drought Stress on Photosynthetic Physiology of

Fraser fir (*Abies fraseri*) Christmas Trees

METHODOLOGY- CH. 1

Study Site

A Christmas tree farm located adjacent to the Meat Camp fire department in Watauga County, North Carolina (36.27ºN, 81.65ºW, 968 m) was selected for study. A small plot was chosen near the bottom of a westward-facing slope of the farm. The selected study plot contained trees that were approximately 3-4 years old and 1.5-2 m tall at the start of the study in August 2020. Workers at the study site were instructed to practice normal agricultural techniques of fertilization and spraying of pesticides but did not trim trees so as to not influence data collection (see Appendix Table 1).

A rain diversion set-up was constructed in June-July of 2020 on the selected plot of trees. Tan, plastic roofing material (~50 cm wide, ~9 m long) was placed between the columns of trees and held approximately 15 cm above the ground by wooden stakes. Given the wet seasons western North Carolina experiences, channels were also placed perpendicularly across the rows of trees to further increase the amount of ground area intercepting precipitation (Figure 1). Using ArcGIS Pro software (Esri, Redlands, CA), it was determined that 73.7% of ground area was covered by the plastic channels (65.3 m^2 of 88.6 m^2 total). A high percentage of coverage was used to try and ensure the imposition of soil drying in the study area because this region generally gets substantial rainfall throughout the year. I also needed to compensate for potential belowground water movement down the slope and drippage from the tree crowns, which would offset the diversion of rainfall by the channels. I was not able to insert belowground barriers to stop subsurface flow, because this would have caused water to pool upslope, and that can promote *Phytophthora* root rot, a devastating condition once established in the soils of a Christmas tree farm.

Figure 1. Aerial image of study site in Watauga County, North Carolina (36.27ºN, 81.65ºW, 968 m). The innermost 10 trees as shown in the red box were used for the drought study, and 10 trees within the yellow box were used as control trees.

Environmental Parameters

Weather conditions were monitored with a Davis Vantage Pro2 weather station (Davis Instruments, Hayward, CA), located approximately 2 m off the ground near the sample trees. Air temperature (ºC), relative humidity (%), precipitation (mm), wind speed (m/s), and solar radiation (W/m^2) were recorded every 2 seconds and archived in 30-minute averages from September 2020-October 2021.

Soil moisture was measured bi-weekly from January-October of 2021 using a Campbell Scientific HydroSense II soil moisture probe (Campbell Scientific Inc., Logan, UT). Readings were taken at a depth of ~20 cm on both the north and south sides of each sample tree in the control and droughted sections.

Growth and Phenology

Bud burst was measured biweekly using the 7-stage system developed by Dr. Jeff Owen of the North Carolina Cooperative Extension Service to determine if water stress affected the timing and progression of phenological events (see Appendix Table 2). The 7 phenological stages range from dormant, waxy buds (stage 1) to fully emerged green shoots (stage 7).

After the trees reached stage 7 of bud burst, shoot length was measured biweekly to determine if timing and duration of stem elongation was affected by drought conditions. Trunk diameter growth was measured biweekly by marking the trunk at chest height with a permanent marker and using a diameter tape. Height growth was also recorded biweekly from the ground to the tip of the main leader using a standard measuring tape.

Diurnal Gas Exchange

Diurnal patterns of gas exchange were measured on five droughted and five control trees every three hours (8 am to 6 pm) using the Li-Cor 6400 portable gas exchange system with the attached conifer chamber (6400-05) (Li-Cor, Inc., Lincoln, NE). Measurements were taken once monthly May-July 2021. Standard chamber conditions were: CO_2 at 415 ppm, flow rate at 750 $\mu mol\ s^{-1}$, and RH at ~50%. This chamber did not have temperature or light control, so measurements were highly dependent on ambient weather conditions. Because of this, diurnal measurements were restricted to clear, cloudless days. Measurements alternated between a droughted and a control tree to minimize confounding between treatment and hourly weather conditions. Due to the fragile stems of Y0 needles and the inaccessibility of the Y2 needles by the bulky Li-6400, only Y1 needles were used for the duration of this measurement. Water use efficiency (i.e., photosynthesis/stomatal conductance) was calculated for each tree.

Approximately a week prior to the first gas exchange measurements, a small number of needles were removed from two locations of the branches being measured to allow the branch to seal into the conifer chamber with minimal leakage problems. This allowed enough time for the tree to recover from removing the needles so as not to bias later measurements. At the end of the measurement period, branches were removed, and dry needle weights were obtained for each sample using the methodology previously described.

Water Potentials

Water potentials were measured using a Scholander Pressure Chamber (PMS, Inc., Corvallis, OR). Y1 twigs were cut from alternating drought and control trees and placed in the pressure chamber to determine the degree of water stress. Water potentials were measured May-July of 2021 on a monthly basis at both predawn (to assess maximum plant water status) and between 2-4 pm, when plants are often under maximum water stress in the hottest time of day. Because this was a destructive technique, frequency of sampling was minimalized by only conducting this measurement on a monthly basis.

Statistical Analysis

All statistical analyses were performed using Minitab statistical software (Minitab, State College PA), SigmaPlot 14.0, and Microsoft Excel 2019 (Microsoft, Redmond, WA). One-way analysis of variance (ANOVA) with needle age as the treatment effect was used for all measurements detailed above apart from drought study measurements. Two-sample t-tests of droughted vs. non-droughted trees were used for all drought study measurements apart from diurnal gas exchange. Since gas exchange was measured on the same trees over time, a repeated measures analysis of variance (RM-ANOVA) was used for this analysis. If assumptions for

parametric testing were not met, non-parametric alternatives (Kruskal-Wallis) were used. Significance for all analyses was assumed at $p \leq 0.05$.

RESULTS- CH. 1

Environmental Parameters

Precipitation

The period from mid-August 2020 to early November 2020 was unusually wet (301.8 mm total), followed by a month-long dry spell, and then rainfall resumed for the period of late December 2020 through early March 2021 (149.2 mm total; Figure 2). These precipitation patterns, presumably caused by strong La Niña events in late 2020, led to the winter of 2020-2021 being the 13th wettest winter on record for western NC (Davis 2021).

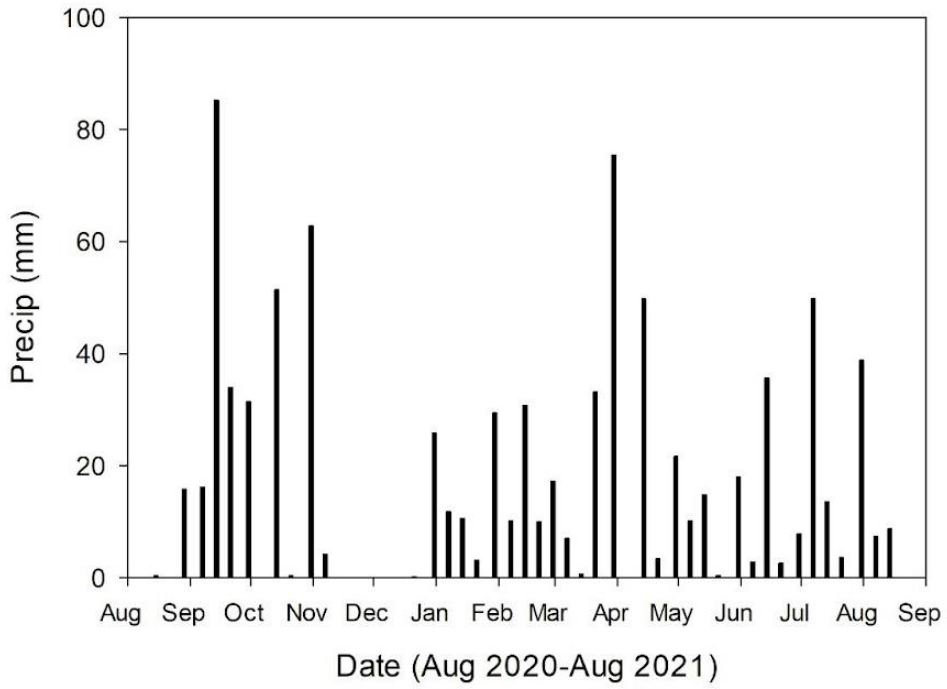

Figure 2. Accumulated weekly precipitation levels from August 2020-August 2021. Symbols represent weekly rainfall.

Soil Moisture

The control plot had significantly higher soil moisture than the drought plot on February 4, June 16, July 30, and August 13, 2021 (Figure 3). Otherwise, there were no statistical differences in percent soil moisture between the control and drought plots over the eight months after imposition of the drought treatment. Thus, for most of the time the experiment was conducted, there was no significant water deficit experienced by the trees.

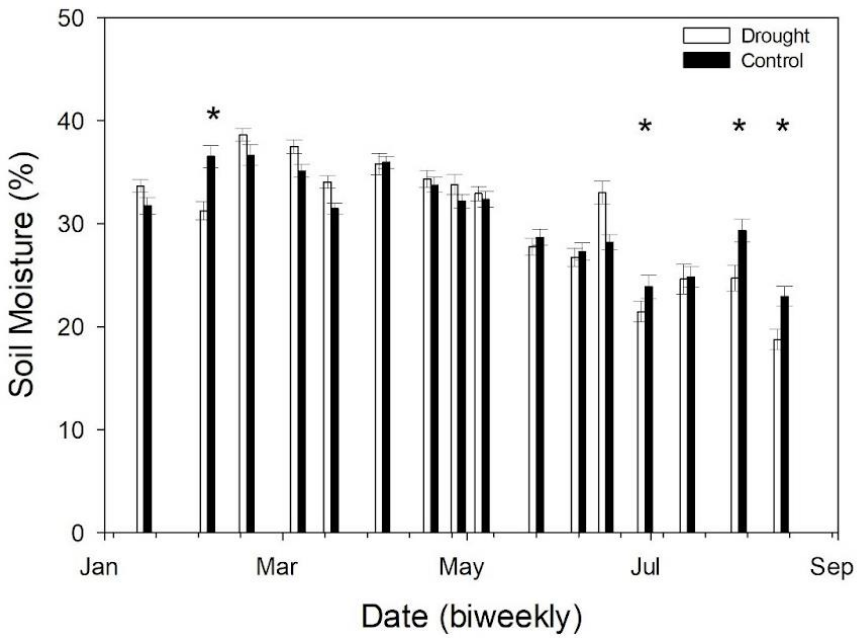

Figure 3. Percent soil moisture from January-August 2021 for drought and control study plots. Bars are mean ± se, n = 10. Asterisks indicate differences between treatments ($p \leq 0.05$).

Growth and Phenology

Trunk Diameter

Trunk diameter growth was evaluated as the percent change from May 27-September 6, 2021 (Figure 4). There were only sporadic differences between the droughted and control trees. For example, on July 6th, diameter growth was higher for droughted than control trees (3.3 ±

0.78% vs. 1.0 ± 0.46%; $p = 0.044$), while on August 20th, control trees grew more (9.4 ± 1.23% vs. 5.4 ± 0.87%; $p < 0.001$). On all other dates, trunk diameter growth did not differ between treatments ($p = 0.496$).

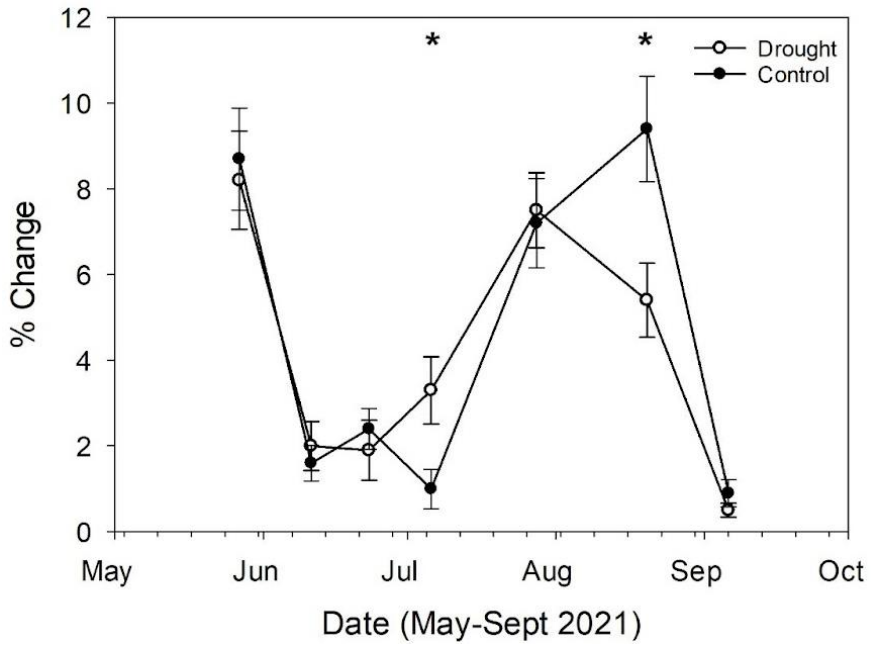

Figure 4. Average trunk diameter rate of change for *A. fraseri* drought and control plots from May-September 2021. Symbols represent mean ± se, n = 10. Asterisks indicate differences between treatments ($p \leq 0.05$).

Bud Burst

Progression through each phenological bud burst stage was monitored from March 29 to May 31, 2021 (Figure 5), by which time all trees had reached the final stage of 7. There were no differences between the control and droughted trees for the times to bud stage 3.5 (50% of final

stage), 6, or the final stage of 7 (p = 0.340, 0.478, 0.333, respectively).

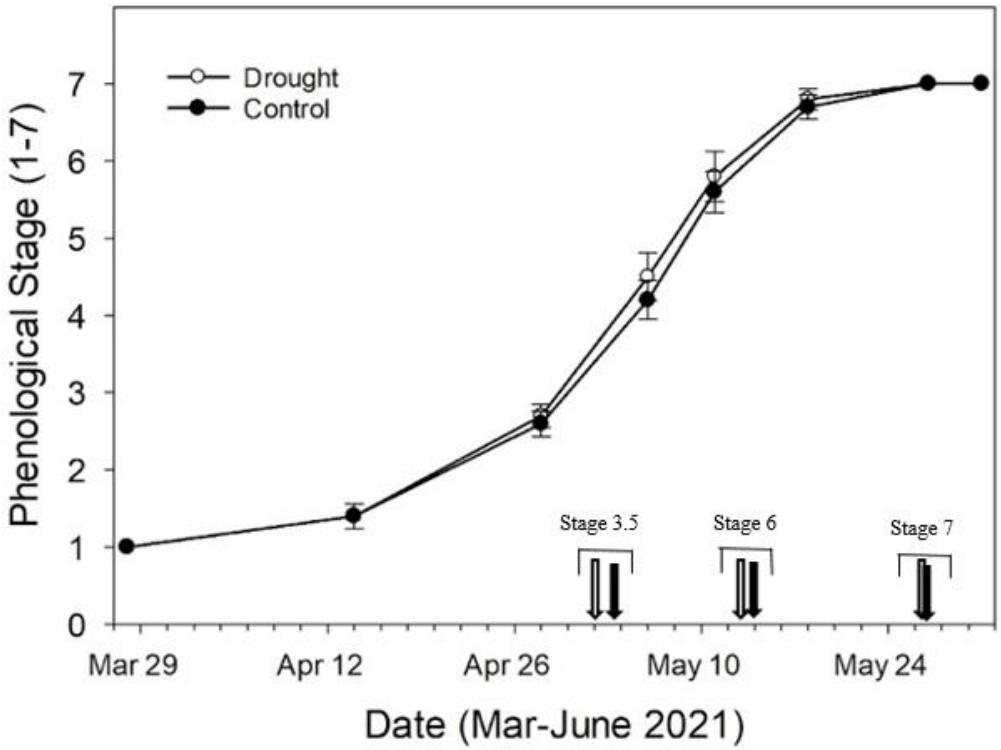

Figure 5. Bud burst progression through 7 phenological stages as detailed in Appendix Table 1. Symbols represent mean ± se, n = 20. Arrows indicate dates when buds reached stage 3, 6, and 7 for each treatment.

Tree Height

The percent change in height growth did not differ between control and droughted trees on any of the three measurement periods (Figure 6): May to June (p = 0.659), June-July (p = 0.554), or July-August (p = 0.881). Droughted and control trees also did not differ significantly in the total percent change in height once growth ceased (p = 0.909).

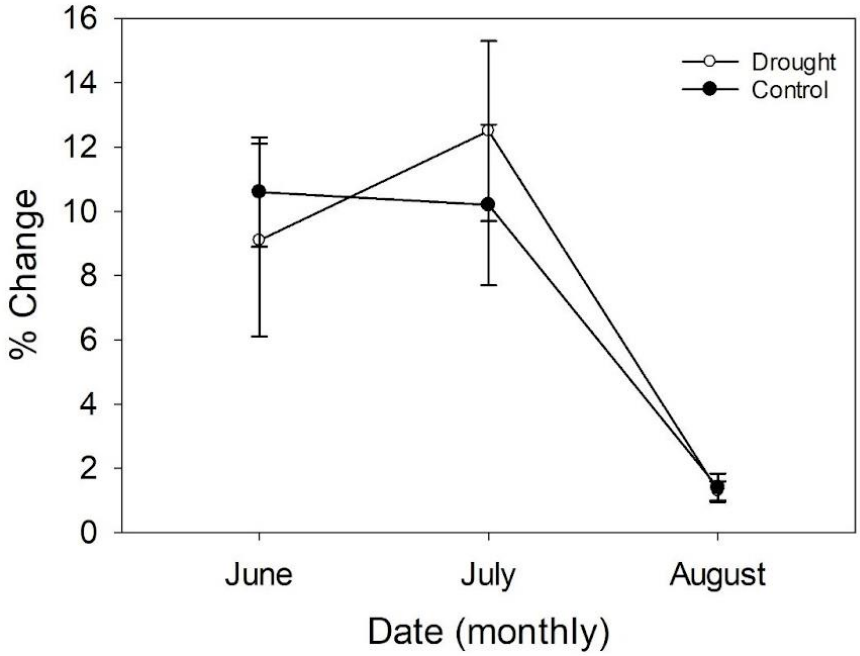

Figure 6. Tree height taken during peak growing season May-August 2021, expressed on a rate of change basis. Symbols represent mean ± se, n = 10.

Water Potentials

Over the period from April to August, there were no instances where pre-dawn water potentials differed between the control and droughted trees (p = 0.886; Figure 7). For mid-day water potentials, no differences were observed up to late July (p = 0.719). However, by August 3, droughted trees were showing slightly greater water stress than control trees (-1.41 ± 0.03 vs -1.23 ± 0.04 MPa; p = 0.004).

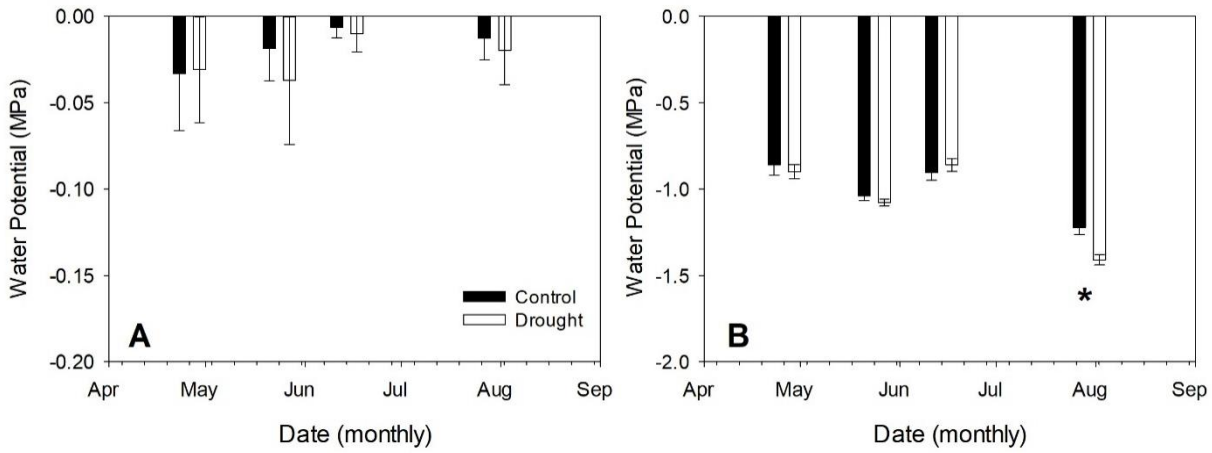

Figure 7. (**A**) Pre-dawn water potentials and (**B**) mid-day water potentials, taken between 1-3 pm in April-August 2021. Bars are mean ± se, n = 10. Asterisks indicate differences between treatments ($p \leq 0.05$). Note the difference in the scales for water potential between panels A and B.

Gas Exchange

Photosynthesis

There was no effect of time of day on photosynthetic rates, as control and droughted trees did not differ in photosynthetic rate at 8 am, 11 am, 2 pm, or 5 pm in May (p = 0.790, 0.118, 0.241, 0.142, respectively), June (p = 0.343, 0.366, 0.211, 0.132, respectively), or July (p = 0.548, 0.690, 0.421, respectively; Figure 8) 2021. The July 2021 5 pm measurements were excluded from results because of sudden dark cloud cover that caused a sharp decrease in photosynthetic rate halfway through measurements.

Stomatal Conductance

There was no effect of time of day on stomatal conductance, as control and droughted trees did not differ in stomatal conductance rates at 8 am, 11 am, 2 pm, or 5 pm in May (p = 0.824, 0.138, 0.548, 0.138, respectively), June (p = 0.268, 0.839, 0.818, 0.810, respectively), or

July (p = 0.548, 0.548, 0.548, respectively; Figure 8) 2021. The July 2021 5 pm measurements were excluded from results because of sudden dark cloud cover that caused a sharp decrease in stomatal conductance rates halfway through measurements.

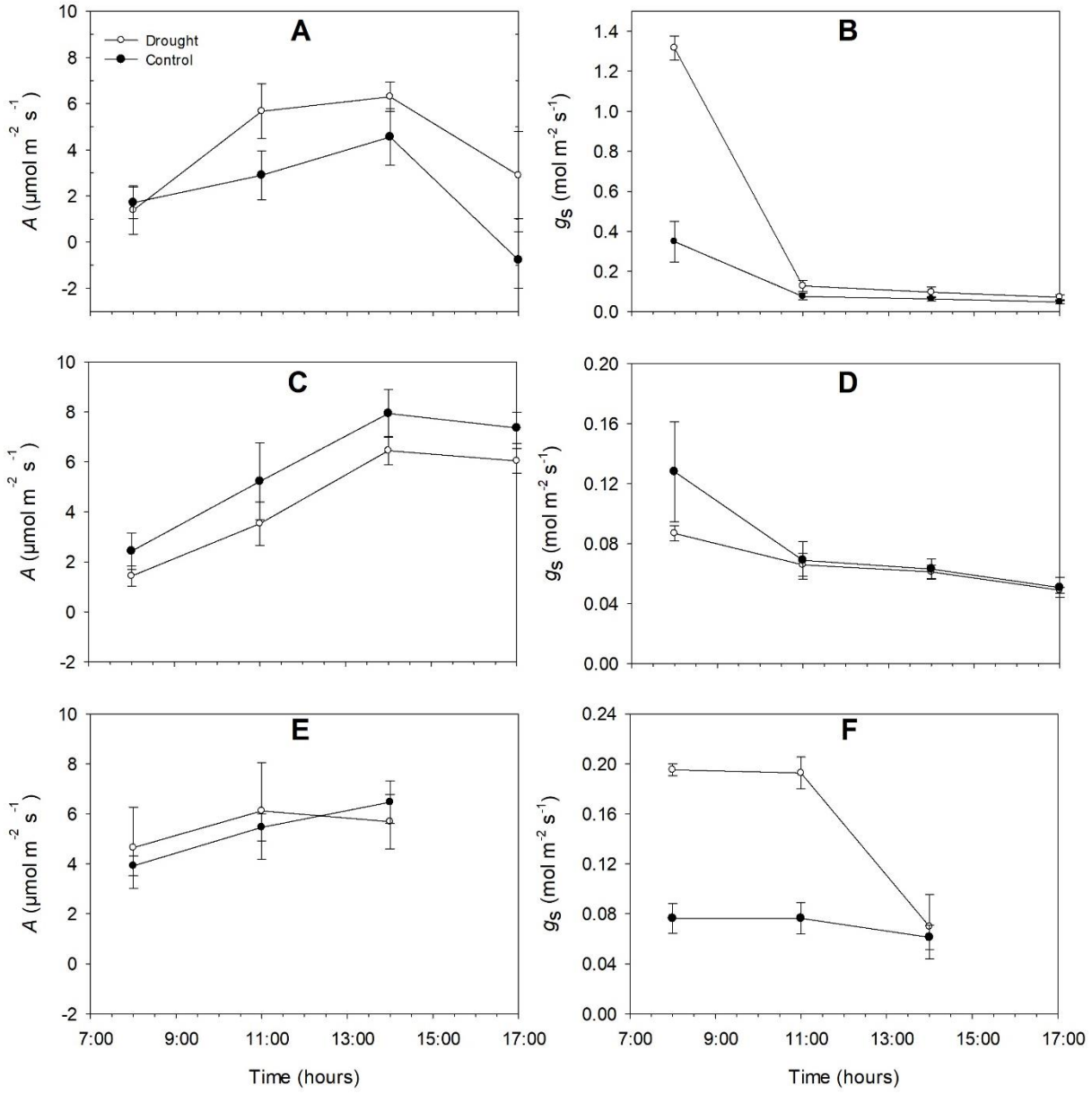

Figure 8, A-F. Photosynthesis and stomatal conductance rates in May (**A, B**), June (**C, D**), and July (**E, F**) 2021 of drought and control trees for Y1 needles. July 2021 5 pm measurements **(E, F)** were excluded due to poor weather conditions. Symbols represent mean ± se, n=5.

Water Use Efficiency

There was no effect of time of day in water use efficiency rates, as control and droughted trees did not differ in water use efficiency rates at 8 am, 11 am, 2 pm, or 5 pm in May (p = 0.548, 0.222, 0.934, 0.066, respectively), June (p = 0.886, 0.841, 0.589, 0.690, respectively) or July 2021 (p = 0.390, 0.212, 0.468, respectively; Figure 9).

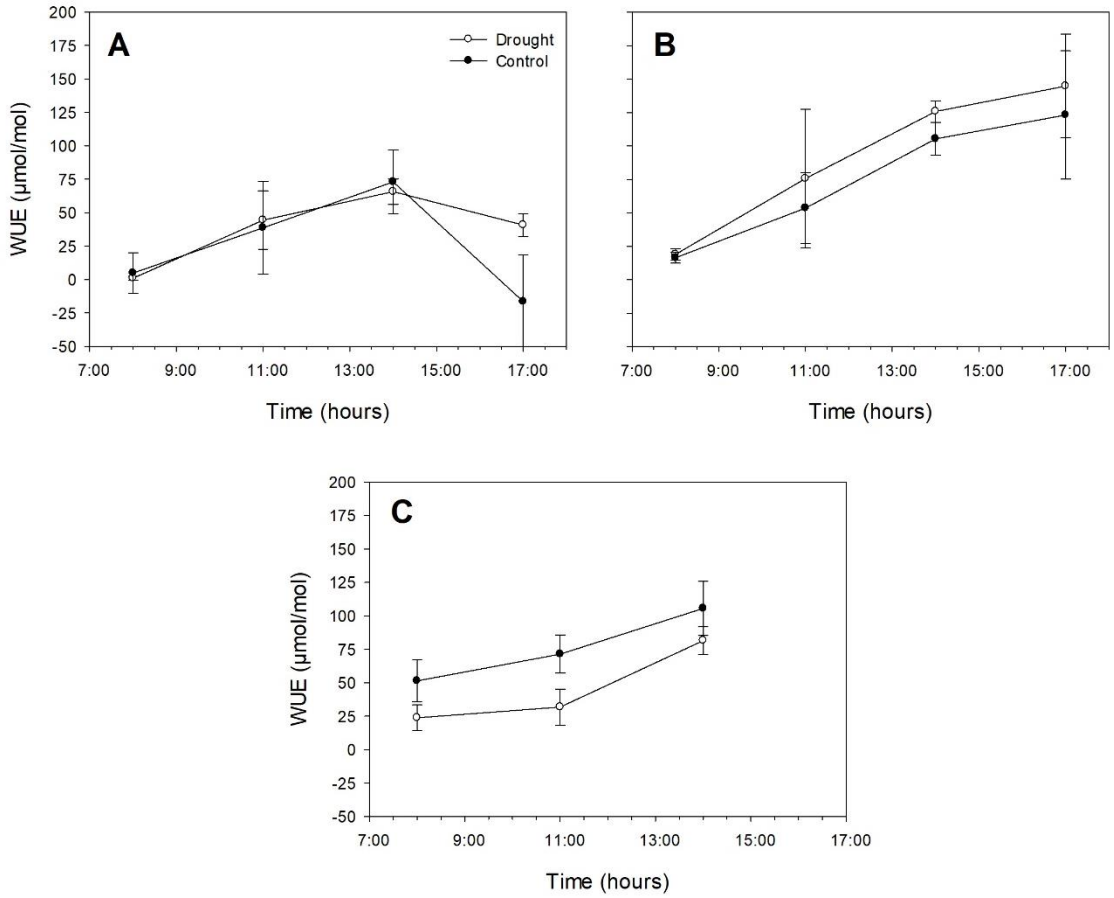

Figure 9. Water use efficiency (WUE) for May (A), June (B), and July (C) 2021. Symbols represent mean ± se, n=5.

DISCUSSION- CH. 1

I hypothesized that prolonged drought conditions would result in lower gas exchange rates, reduced water potentials, and stunted growth phenology compared to control trees, thus contributing to lower market quality. In several studies of evergreen and deciduous conifers, short-term drought conditions have resulted in variable differences in growth parameters like trunk diameter, tree height, new shoot length, and timing of bud burst from control trees (Swidrak et al. 2013; Lévesque et al. 2014). They have also found differences in photosynthetic rates, water potentials, and chlorophyll content (Turner et al. 1984; Katul et al. 2009). Decreased growth, gas exchange, and water potentials are thought to arise in droughted trees because of stomatal closure in response to hydraulic stress, or by incomplete stomatal closure causing transpiration to exceed root uptake, resulting in hydraulic failure (McDowell et al. 2008; Anderegg et al. 2016). However, the few significant results found in our drought study were likely due to stochastic weather conditions; droughted trees showed minor symptoms of drought stress during periods in the summer when there was reduced rainfall, but then heavy rainfall replenished the soil water, resulting in similar conditions for both drought and control trees by the time of the next measurement. For example, measurements taken in late July-early August 2021 provided a glimmer of potential drought conditions that may have led to significant results given more time; mid-day water potentials were significantly lower in drought trees compared to control trees, and this was reflected in the lower soil moisture measurement taken during that same week. However, the study ended in mid-August because heavy rainfall occurred soon after, and we did not observe substantial physiological effects indicative of moderate drought when comparing our two study plots. Although the rainfall diversion set-up covered over 73% of the soil surface area, it is most likely that there was substantial subsurface drainage downslope

which prevented the soils from drying out as much as was intended (Weyman 1973). However, as noted earlier, we were not able to install a barrier above the study plot because of the risk of *Phytophthora* root rot, which is favored in wetter soils.

If the climate continues to warm, and if precipitation stochasticity further increases, then drought may eventually become a factor that Christmas tree farmers will encounter; even if the total amount of precipitation remains near current levels, warming coupled with precipitation stochasticity will result in drier soils due to greater evaporative demand in the atmosphere. At some point, depending on the relative degree of change for each factor, commercially-grown Fraser fir trees will begin to suffer the effects of water stress. Tree ring studies have shown that diameter growth of Fraser firs and other tree species were reduced in the mid-1980s when there was a severe multi-year drought in the Southern Appalachian Mountains (Vose and Swank 1994; Kloeppel et al. 2003). However, aside from these tree-ring studies, no physiological follow-up work was ever done, so how commercially-grown, mature Fraser firs will respond to future climate change remains to be studied. I was unable to properly test hypothesis (1) during this experiment.

CONCLUSION- CH. 1

While field experiments are crucial to our understanding of plant physiology, they can also be difficult to conduct successfully because of many environmental factors that must be considered. I believe that underground water movement caused by the slope of our study plot is to blame for the lack of drought conditions on our study trees, especially considering the plot was 73.7% covered in rain diversion channels. However, the results suggest that, for the present, Christmas tree farmers in most locations will not have to worry about the effects of drought on the ability to bring their trees to market, since it is so difficult to implement drought severe enough to affect the physiology of these trees. While we know that late season drought, if severe enough, can result in the loss of older needles, drought at this time of year does not affect growth because that ceases earlier in the year. A decadal study of growth rings of trees that covered the 2017 late season drought period showed no apparent impacts on growth (unpublished data, Neufeld lab). In future drought studies on sloped plots, we recommend installation of a trench uphill from the research area to divert downhill runoff. Although this methodology was considered for the present study, fear of standing water causing *Phytophthora* root rot halted this idea (Joy and Phibbs 2015). Therefore, consideration should be given to the effects of standing water on the root systems of the study species before installation of a trench. The question remains as to how Fraser fir Christmas trees will respond to prolonged drought conditions in the field given projected climate change scenarios.

CHAPTER TWO

Impacts of Needle Age and Tree Architecture on Photosynthetic Physiology of Fraser fir (*Abies fraseri*) Christmas Trees

METHODOLOGY- CH. 2

Study Site

The same westward-facing plot on a Christmas tree farm located adjacent to the Meat Camp fire department in Watauga County, North Carolina (36.27ºN, 81.65ºW, 968 m) was used for this study. Control trees located adjacent to the droughted trees in the sister study were used so that data could be shared between both studies (Figure 10). Five trees were randomly selected from this plot and were positioned at least two rows away from the drought plot so as to not bias measurements.

Figure 10. Aerial image of study site in Watauga County, North Carolina (36.27ºN, 81.65ºW, 968 m). Ten trees shown in the yellow box were used for this study.

Canopy Architecture and Penetration of Photosynthetically Active Radiation

To determine the pattern of light depletion from the exterior to the interior of the tree canopies, the light intensity (photosynthetically active radiation, PAR; µmol m^{-2} s^{-1}) was measured at three locations in the crown at mid-height on each of five trees. Measurements were taken on both a sunny day (October 1, 2021), when most of the light was in the form of direct radiation, and on a cloudy day (October 5, 2021), when all of the radiation was diffuse, both during early morning (~8:30 am EST) and near solar noon, using Li-190s quantum sensors attached to a Li-1500 Quantum Sensor Logger (LI-COR Biosciences, Lincoln, NE).

Three sensors were placed ~25 cm apart on a metal pole to mimic the midpoint distribution of needles formed during the 2021 growing season (hereafter: Y0, located at the exterior of the crown), the 2020 growing season (Y1, located midway between the crown exterior and trunk), and in the 2019 growing season (Y2 and located closest to the trunk). For these young trees, a typical mid-height canopy branch ranged in length from 90 to 130 cm.

PAR was logged every 5 s for a total of 20 s. The average PAR of the 4 logs was calculated for each of the records upon analysis. This protocol was performed on the east and west side of each tree with the sensors facing the sun to measure direct radiation and then again with the sensors facing in the opposite direction to ascertain diffuse and reflected radiation penetration into the canopy. Solar elevation angle and azimuth at each measurement time were calculated using the *NOAA* ESRL Solar Calculator (NOAA 2021), and sensors were adjusted accordingly using a digital protractor and compass.

Needle Packing

Needle packing was calculated for an entire south-facing branch detached at chest-height from five sample trees. Needles on each twig were grouped by age class and dried to a constant weight. The number to weight ratio of a subset of needles was used to convert weight to total needle count. Stem length and diameter were also recorded for each twig and used to calculate needle packing, expressed as the number of needles per cm^2 surface area of the twigs.

Chlorophyll Concentrations

Chlorophyll concentrations were determined by dipping samples of 5 freshly plucked needles into liquid N_2 for 10 seconds, then breaking the needles apart by hand. Samples from each age category were then placed into small vials filled with 3 mL of N, N-dimethylformamide (DMF), and placed in a dark refrigerator for 72 hours at 5ºC. Absorbances were measured in quartz cuvettes using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan) and concentrations calculated according to the equations of Porra (2002). Analyses were done June 22-25, August 9-12, and September 3-6 of 2021.

Gas Exchange Physiology

Gas exchange measurements were obtained from five trees to determine if photosynthetic rates (A) and stomatal conductances (g_s) are affected by increasing needle age. Two sets of measurements were made on each tree: the first involved stripping off a piece of bark containing 7-15 needles in a planar row, with minimal to no self-shading (see Figure 11), while the other method utilized needles in their normal packing and orientation on intact twigs. For the detached planar needles, the Li-6800 portable gas exchange system (Li-Cor Biosciences, Lincoln, NE) equipped with the 6 cm^2 cuvette (6800-13) and LED light source (6800-03) were used. With this

technique, Y0, Y1, and Y2 needles were measured. Preliminary experiments showed that constant gas exchange rates could be maintained near their original values for approximately 15 minutes after detaching (Figure 12).

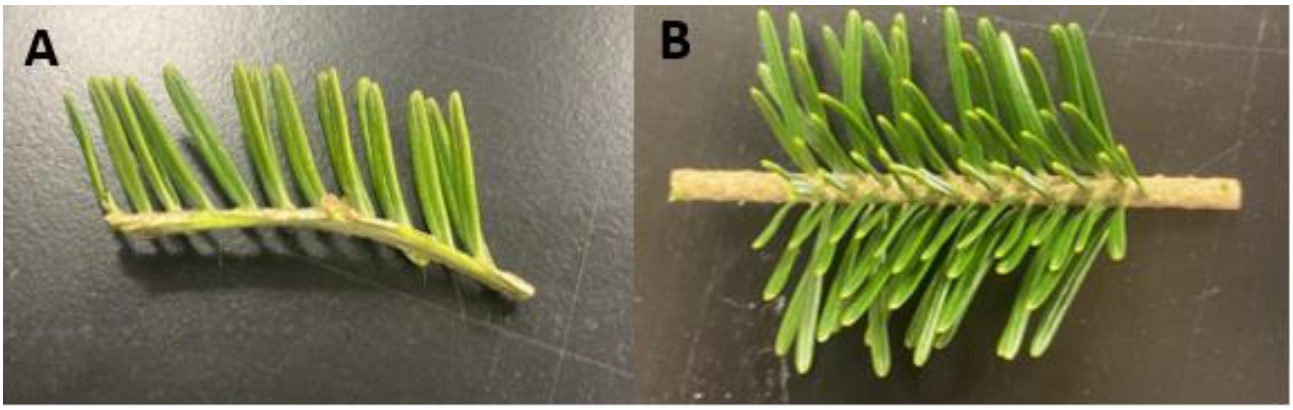

Figure 11. (**A**) Detached needle and (**B**) intact twig methodology.

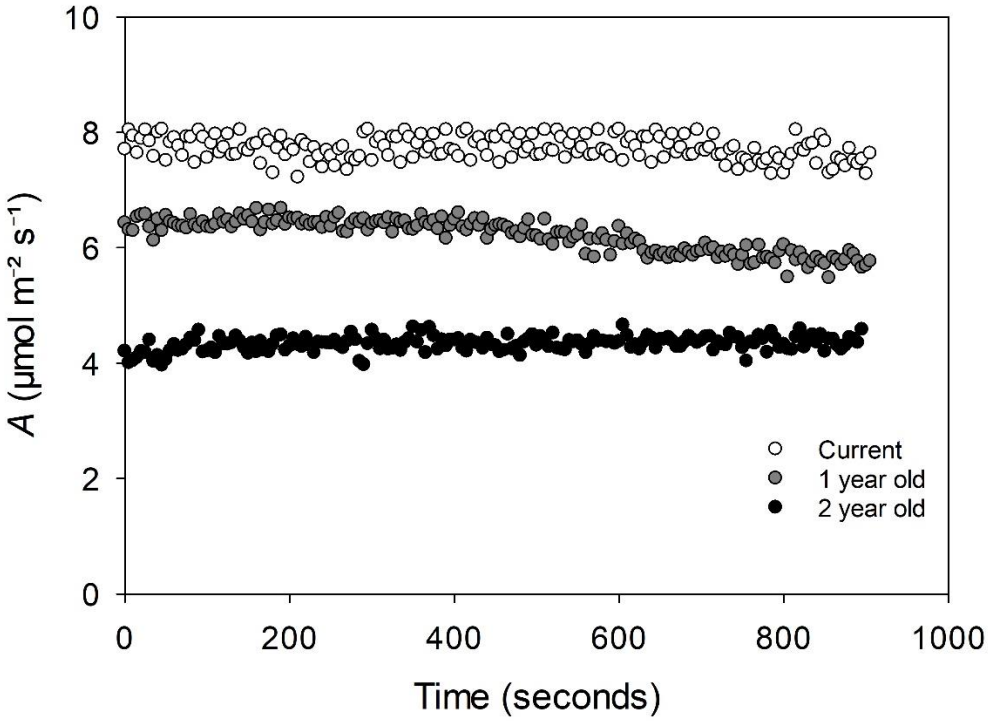

Figure 12. Representative tests of photosynthetic rate maintenance after detachment from the tree for Y0, Y1, and Y2 needles.

Y2 needle measurements were not made on the intact twigs because the diameters of the twigs were too large to seal into the chamber without leakage. Light (PAR) and rapid CO_2 (A/C_i) response curves were made for the needles attached to the bark strip, but only light response curves were made for the needles on intact twigs, because bark respiration and needle orientation would have complicated interpretation of the results. Each curve was completed in 15 minutes or less to avoid issues with detaching.

PAR curves were completed using the "Light Response" auto-program with values of 2000, 1000, 700, 500, 300, 100, 50, and 0 µmol m^{-2} s^{-1}, in that order, for the detached needle method. PAR values of 2000, 1500, 1000, 750, 500, 300, 200, 100, 50, 25, and 0 µmol s^{-1} m^{-2} were used for the intact twig method. The program waited 60-120 s to reach stability before logging data at each PAR level. Sample and reference CO_2 concentrations were matched after completion of three needle ages on one tree. Standard chamber conditions were CO_2 at 415 ppm, flow rate at 300 µmol s^{-1}, RH at 50%, and temperature set to ambient for the day that measurements were taken, which ranged from 20 to 25ºC.

A 3-parameter exponential rise to maximum function was used to model the response of A to PAR:

$$A = a + b \cdot e^{(c \cdot PAR)} \tag{1}$$

where a, b and c are estimated parameters (SigmaPlot 14.0, Systat Inc., San Jose, CA). From these curves the following estimates were extracted: Dark Respiration (R_d, net gas exchange when PAR = 0); Light Compensation Point (LCP, the PAR when net gas exchange is zero); Apparent Quantum Efficiency (AQE, the initial slope in the light limited portion of the curve); Light Saturation Point (LSP, the PAR where A reaches its maximum value); and finally, the

maximum rate of photosynthesis at light saturation (A_{max}). In some cases, curves did not saturate and 2000 µmol m^{-2} s^{-1} was used as a place setter for statistical purposes.

After the gas exchange measurements were completed, sample needles were removed from their strips of bark or intact twigs. Projected needle area was determined by placing the needles on a scanner (CanoScan 9000F Mark II scanner, Canon USA, Melville, NY), and then converting the jpg images to area (cm^2) using the shareware program BlackSpot (Varma and Osuri 2013). For the needles attached to the bark strip, only the needle portions inside the cuvette, as determined from the foam pad marks, shown in Figure 13, were scanned. After calculating the area, needles were placed in a drying oven at 65ºC for 48 hours and then weighed to determine the dry weight-to-area ratio.

Figure 13. The portion of needles inside the cuvette (as indicated by the white underside of needles) was used to calculate needle area.

Rapid A/C_i curves were constructed according to the protocols described in Stinziano et al. (2017). Measurements were made using the Auto Control program in conjunction with the Auto Log function. The Auto Control program was set to run for 10 minutes with a reference

CO_2 range that increased from 10 to 1010 µmol/mol, and the Auto Log function was set to log every 2 seconds. Sample and reference CO_2 were matched using the Auto Match function after every two needle ages. A/C_i curves were corrected using empty cuvette measurements (Lynch, 2020). However, estimates of stomatal conductance could not be accurately assessed, so the analysis was restricted to determining A_{max} at a standard C_a of 900 µmol/mol, which would allow an estimation of the CO_2 response with minimal influence from stomatal conductance.

Minimum Needle Conductance

I measured minimum conductance (G_{Smin}) on October 26th and November 1st, 2021, respectively, from five sample trees using the detached planar needle method described above for measuring gas exchange of Y0, Y1, and Y2 needles. Minimal conductance is a measure of mainly cuticular water loss, which might change with needle age due to changes in wax coverage and structure. Once removed from the tree, needle strips were placed into small plastic bags and stored over ice in a cooler. In the lab, excess needles were removed until only 18 remained, and a small amount of petroleum jelly was applied to the underside of the bark to eliminate water loss from anywhere but the needle surfaces. Each sample was placed into a pre-weighed aluminum tin and weighed to a resolution of 0.01 mg every 5 minutes for the first 30 minutes, and then every 15 minutes until the relative rate of change decreased to <5% for at least three consecutive measurements. At this point, it was assumed that stomata were fully closed, and any further water loss was likely cuticular (Kerstiens 1996; Riederer and Muller 2006; Fernández et al. 2017), although leaky stomata are possible and well-documented, especially in older needles (Mott and Buckley 2000; Grassi and Magnani 2005). Needles were then removed, and their dry weight obtained. A zip-tie was smeared with petroleum jelly, placed into a pre-weighed aluminum tin, and weighed at the same times as the needle samples to ensure mass loss was not

coming from the jelly. Gs_{min} was calculated using Equation 2 and a barometric pressure (BP) of 88.8 kPa. VPD was calculated by assuming needles were at room temperature and by using the relative humidity and room temperature to determine VP_{sat} and VP_{air}. Humidity and temperature of the room varied from 23-31% and 21-23°C, respectively, and the last three transpiration measurements were averaged and divided by needle area to convert transpiration to mmol m^{-2} s^{-1}. Previous weight/area relationships were used to determine needle area. Gs_{min} was calculated as:

$$GS_{min} = E/VPD \quad (2)$$

where Gs_{min} is minimum needle conductance (mmol m^{-2} s^{-1}), E is water loss per unit time, with the same units as Gs_{min}, and VPD is the calculated vapor pressure deficit (kPa), corrected for the barometric pressure.

Epicuticular Wax Content

Total epicuticular wax content was determined for all three needle ages on November 8th, 2021, in accordance with the protocol of Hutcherson (1993). A set of 30 needles per age class per tree was placed into a 125 mL Erlenmeyer flask and washed with 5 mL of HPLC grade chloroform for 20 seconds. After washing, the chloroform was poured through filter paper into a 50 mL beaker and the process was repeated three times. After the final washing, the 125 mL flask was washed with another 5 mL of chloroform to remove any remaining wax residue and poured through the filter paper into the beaker. The beaker was then emptied into a small, pre-weighed aluminum tin, and the chloroform allowed to evaporate, leaving only the epicuticular wax behind. The aluminum tins were left to dry for 5 hours and then weighed on a microbalance with a resolution of 0.01 mg. The needles were placed into small envelopes, dried at 60ºC for 48 hours, weighed, and total wax content was expressed as mg wax/gdw of needle.

RESULTS- CH. 2

Canopy Architecture and Penetration of Photosynthetically Active Radiation

Light sensor data were organized into a diffusion group (light sensors pointing away from direction of the sun) and a sun-facing group (sensors aimed in direction of the sun) to reflect the distribution of light within the canopy of the trees, and reflecting the influence of branch architecture, needle orientation, and needle packing. Note that measurements were taken in early October, so the position of the sun at solar noon was not directly above the trees, but rather, shifted to the east.

Sun-facing group

Current year needles (Y0) on the east side of trees received the highest light levels (2081 ± 38 µmol m^{-2} s^{-1}; Figure 14) on a sunny day, and these were significantly higher than on a cloudy day (201 ± 26 µmol m^{-2} s^{-1}; $p < 0.001$). In Y1 needles on the east side of the tree, which averaged about 25 cm interior to the Y0 needles, PAR (photosynthetically active radiation) on a sunny day dropped dramatically to 64 ± 21 µmol m^{-2} s^{-1}, a 97% decrease, and did not differ from a cloudy day (50 ± 15 µmol m^{-2} s^{-1}, $p = 0.622$). In Y2 needles on the east side of the tree, which averaged 51 cm from the exterior, PAR on a sunny day continued to drop to 18 ± 11 µmol m^{-2} s^{-1}, a 99% decrease from light levels seen in Y0 needles. Further, light levels on Y2 needles did not differ from a cloudy day (10 ± 8 µmol m^{-2} s^{-1}, $p = 0.841$).

On the west side of the tree, light intensities did not differ between sunny and cloudy days for Y0 needles (17 ± 7 µmol m^{-2} s^{-1} vs. 20 ± 6, respectively; $p = 0.920$; Figure 14). The same trend was found for west-facing Y1 needles (3 ± 1 µmol m^{-2} s^{-1} vs. 4 ± 1, respectively; $p = 0.554$), and west-facing Y2 needles (12 ± 8 µmol m^{-2} s^{-1} vs. 1 ± 0.4, respectively; $p = 0.421$). Light intensities on the west side were lower than the east side on both a sunny and cloudy day

for Y0 needles ($p < 0.001$, 0.001, respectively) and Y1 needles ($p = 0.002$, 0.008, respectively), but were not different for Y2 needles ($p = 0.690$; $p = 0.222$, respectively).

Diffusion group

I also analyzed reflected light impinging on the needles from a western direction on both sunny and cloudy days (Figure 14). On the east side of the tree, light intensity averaged 428 ± 383 µmol m^{-2} s^{-1} for Y0 needles on a sunny day and was significantly higher than on a cloudy day (10 ± 1 µmol m^{-2} s^{-1}; $p < 0.001$). For Y1 needles on the east side of the tree, reflected light intensity averaged 4 ± 2 µmol m^{-2} s^{-1} on a sunny day, and was significantly higher than on a cloudy day (1 ± 0.3 µmol m^{-2} s^{-1}; $p = 0.032$). There was no difference between sunny and cloudy days for Y2 needles on the east side of the tree (1 ± 0.6 µmol m^{-2} s^{-1} vs. 1 ± 0.5, respectively; $p = 1.00$).

On the west side of the tree, there was no difference between sunny and cloudy days for Y0 needles (41 ± 6 µmol m^{-2} s^{-1} vs. 39 ± 3 µmol m^{-2} s^{-1}, respectively; $p = 0.575$; Figure 14). The same pattern was seen for Y1 needles (14 ± 5 µmol m^{-2} s^{-1} vs. 11 ± 3 µmol m^{-2} s^{-1}, respectively; $p = 0.608$) and Y2 needles (4 ± 0.8 µmol m^{-2} s^{-1} vs. 5 ± 0.7 µmol m^{-2} s^{-1}, respectively; $p = 0.744$) on the west side of the tree.

For Y0 needles, light intensities were lower on the east side of the tree than the west side on a cloudy day ($p < 0.001$; Figure 14) but did not differ on a sunny day ($p = 0.727$). The same pattern was seen in Y1 needles on a cloudy day ($p = 0.009$) and sunny day ($p = 0.076$). Light intensity on Y2 needles was higher on the west side of the trees than the east side on both a sunny and cloudy day ($p = 0.009$, 0.015).

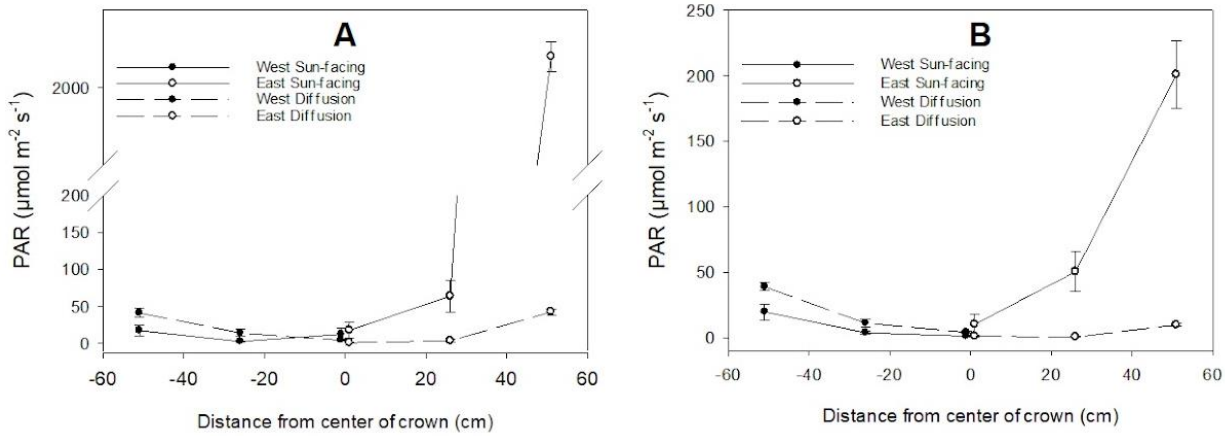

Figure 14. Light intensity on sunny (**A**) and cloudy (**B**) days. Open symbols indicate east side of tree with diffusion and sun-facing groups on the same graph. Negative values on the horizontal axis indicate distance west from the center of the crown. Symbols represent the mean ± se, n = 5.

Needle Packing

I calculated needle packing as a function of age class by determining the total number of needles per cm² of stem surface since average stem diameters increased from Y0 to Y2 needles (1.03 ± 0.12 cm vs. 1.82 ± 0.20 cm; $p = 0.011$). There were significantly more Y0 than Y2 needles (2123 ± 585 vs 722 ± 111, respectively; $p = 0.039$; Figure 15a) on the entire branch, but the number of Y1 needles did not differ from either Y0 or Y2 needles. The same pattern with respect to age was also found for the packing density of Y0 vs Y2 needles (0.478 ± 0.078 needles/cm² vs. 0.233 ± 0.048 needles/cm², respectively; $p = 0.029$; Figure 15b), and the Y1 needles with respect to Y0 and Y2 needles.

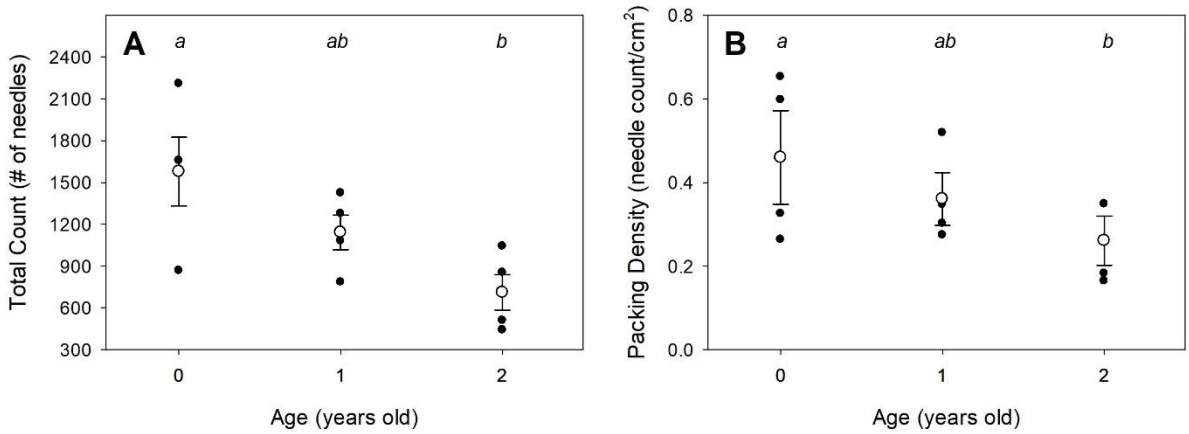

Figure 15. (**A**) Total number of needles on one branch and (**B**) packing density of needles (# per cm²), for Y0, Y1, and Y2 needles. Open symbols represent the mean ± se, n= 4. Closed symbols are values for each branch, n= 4.

Leaf Mass/Area (LMA)

In August 2021, Y0 needles had a significantly lower LMA than Y1 and Y2 needles (0.012 ± 0.001 g/cm^2 vs. 0.019 ± 0.001 g/cm^2 vs. 0.018 ± 0.001 g/cm^2, respectively; $p < 0.001$; Figure 16).

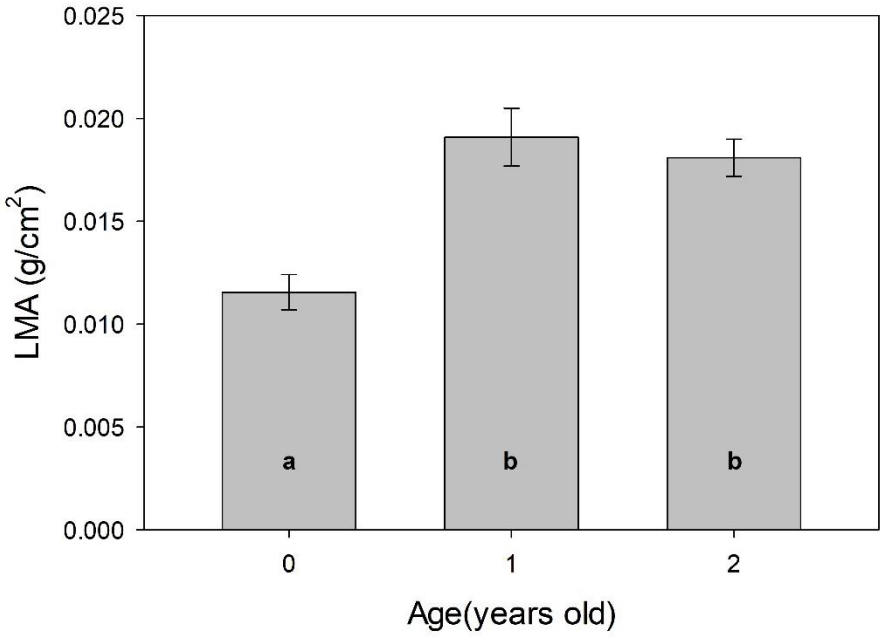

Figure 16. Leaf mass per unit area for Y0, Y1, and Y2 needles. Bars represent mean ± se, n = 5.

Chlorophyll Amounts and Ratio

Both needle age and time had a strong impact on chlorophyll *a* concentration ($p = 0.006$, 0.028), although only needle age had an impact on chlorophyll *b* ($p = 0.005$, 0.466), total chlorophyll concentrations ($p = 0.003$, 0.096), and chlorophyll *a/b* ratio ($p < 0.001$; $p = 0.983$; Figure 17). There was no significant age x time interaction effect for chl *a,* chl *b*, total chl, or chl *a/b* ratio ($p = 0.304$, 0.790, 0.543, 0.312, respectively). In June and July, when needles were still developing, Y0 needles had significantly lower levels of chlorophyll *a, b*, and total chlorophyll content than Y1 needles. Once Y0 needles were fully developed in September, only chlorophyll *b* and total chlorophyll levels were significantly higher in Y1 needles. Y2 needles had similar levels of chlorophyll *a*, *b*, and total chlorophyll content to Y1 needles throughout the season. The

ratio of chl *a/b* was higher in Y0 needles than Y2 needles in June, July, and September, primarily due to the higher levels of chl *a* in Y0 needles.

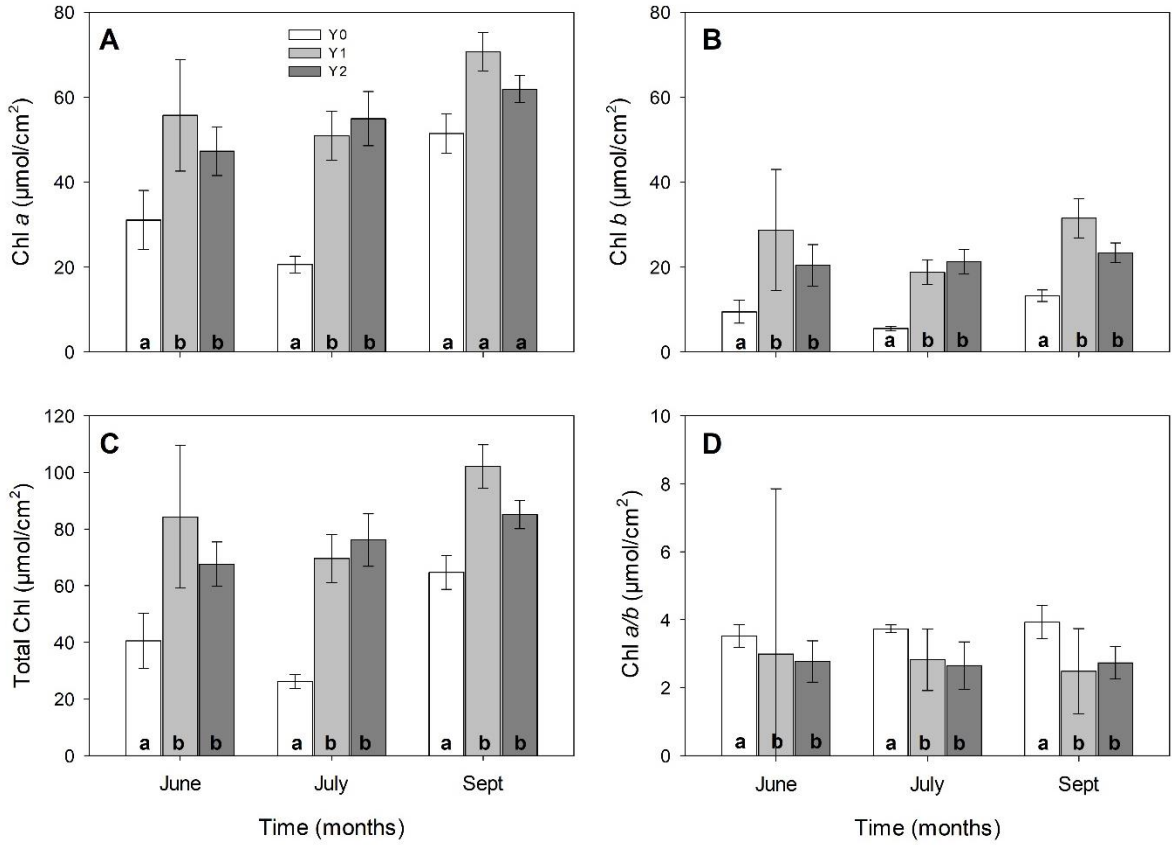

Figure 17. (A) Chlorophyll *a*, (**B**) *b*, (**C**) total, and (**D**) Chl *a*:*b* ratio for Y0, Y1, and Y2 needles. Bars represent mean ± se, n = 5, averaged across June, July, and September 2021.

Gas Exchange Physiology

Light Response Curves- Detached Planar Needles

Neither dark respiration nor LCP varied among needles of differing ages ($p = 0.613$, $p = 0.681$, respectively; Table 1). AQE was highest in Y0 needles and lowest in Y2 needles ($p = 0.018$). A similar result was found for the LSP in regard to Y0 needles and Y2 needles ($p = 0.022$). Y0 needles and two of the Y1 samples did not photosynthetically saturate at the highest PAR employed, so they were arbitrarily assigned a value of 2000 µmol m^{-2} s^{-1} for the LSP.

Photosynthetic rates at LSP were highest in Y0 needles and lowest in Y2 needles (p = 0.002; Figure 18).

Table 1. Light response parameters of both detached planar needles and intact twigs: DR is dark respiration rate, LCP is light compensation point, AQE is apparent quantum efficiency, A_{max} is photosynthesis at saturating light, and LSP is the light saturation point. Numbers are mean ± se, n = 5.

Needle Age	DR (µmol m^{-2} s^{-1})	LCP (µmol m^{-2} s^{-1})	AQE (µmol CO_2/µmol photons)	A_{max} (µmol m^{-2} s^{-1})	LSP (µmol m^{-2} s^{-1})
Detached Planar Needles					
Current year (Y0)	-0.8 ± 0.32	27 ± 10.7	0.026 ± 0.002	12.6 ± 1.37	+2000 ± 0
1 year old (Y1)	-0.7 ± 0.09	34 ± 3.0	0.019 ± 0.002	6.2 ± 0.41	1950 ± 50
2 year old (Y2)	-0.6 ± 0.11	34 ± 5.0	0.016 ± 0.001	3.2 ± 0.68	1542 ± 148
Attached Twigs					
Current year (Y0)	-0.5 ± 0.10	26 ± 4.7	0.020 ± 0.001	10.7 ± 0.56	+2000 ± 0
1 year old (Y1)	-0.3 ± 0.03	16 ± 2.4	0.018 ± 0.002	6.6 ± 0.71	1924 ± 20

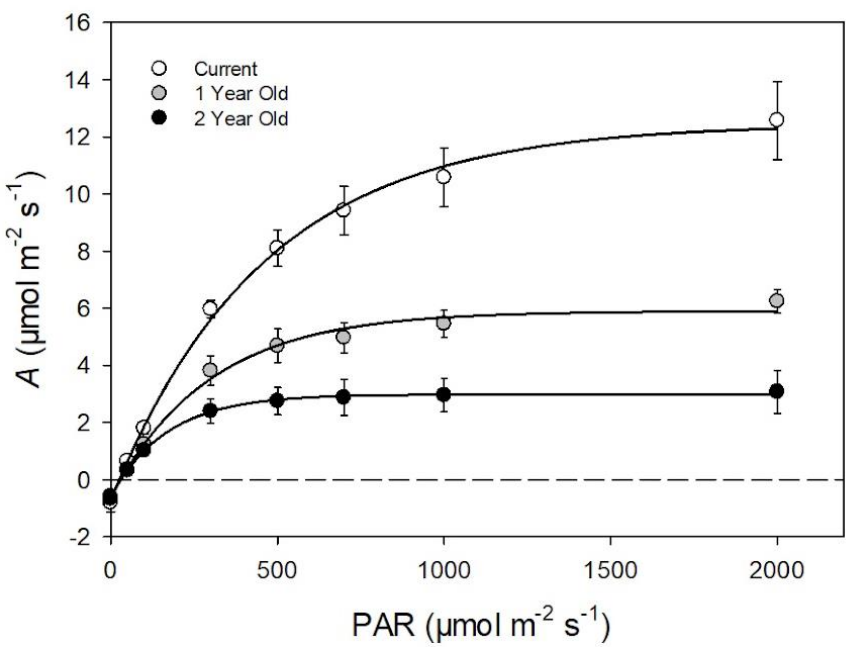

Figure 18. Light response curves of Y0, Y1, and Y2 *A. fraseri* needles using the detached planar needle method (see text). Symbols are mean ± se, n = 5.

Light Response Curves- Intact Twigs

Only Y0 and Y1 needles were measured because the diameter of the Y2 twigs were too large to seal into the Li-6800 chamber without leakage. LCP did not vary between Y0 and Y1 branches, nor did AQE (p = 0.082 and 0.473, respectively; Table 1). Dark respiration rates were slightly lower in Y0 needles than Y1 needles (p = 0.036). A_{max} and the LSP were higher in Y0 needles than Y1 needles (p = 0.002; 0.037, respectively; Figure 19). None of the Y0 samples saturated at the highest PAR, while only Y1 sample failed to saturate.

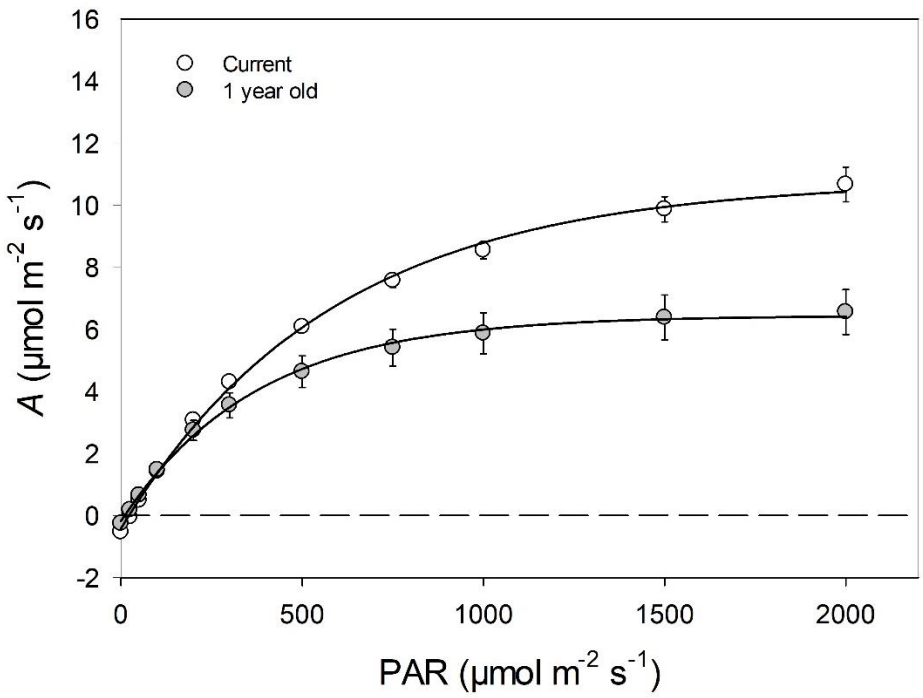

Figure 19. Light response curves of Y0 and Y1 *A. fraseri* needles using the intact twig method. Symbols are mean ± se, n = 5.

Stomatal Conductance- Detached Needles

At a standard PAR of 1000 µmol m^{-2} s^{-1}, Y0 needles had significantly higher stomatal conductances than Y1 and Y2 needles (0.152 mol m^{-2} s^{-1} ± 0.022 vs. 0.074 mol m^{-2} s^{-1} ± 0.006 and 0.037 mol m^{-2} s^{-1} ± 0.010, respectively; $p < 0.001$; Figure 20). Y0 needles on the intact twigs

had significantly higher stomatal conductances than Y1 needles (0.116 mol m^{-2} s^{-1} ± 0.012 vs. 0.063 mol m^{-2} s^{-1} ± 0.008, p = 0.005).

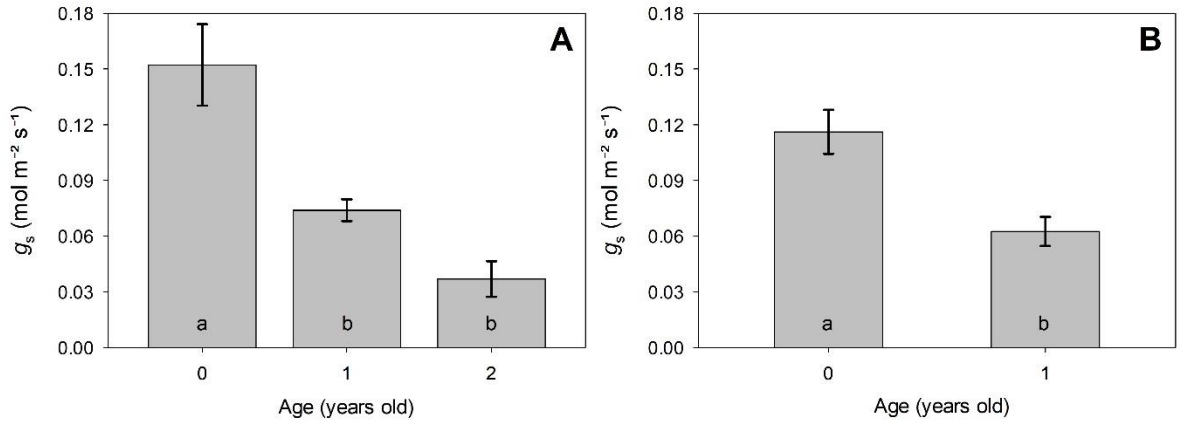

Figure 20. Stomatal conductance using (**A**) detached needle methodology and (**B**) intact twig methodology at a standard PAR of 1000 µmol m^{-2} s^{-1}. Bars are mean ± se, n = 5.

Rapid A/C_i *Curves*

There were no differences in A_{max} among the variously aged needles using detached needle methodology (p = 0.642; Figure 21).

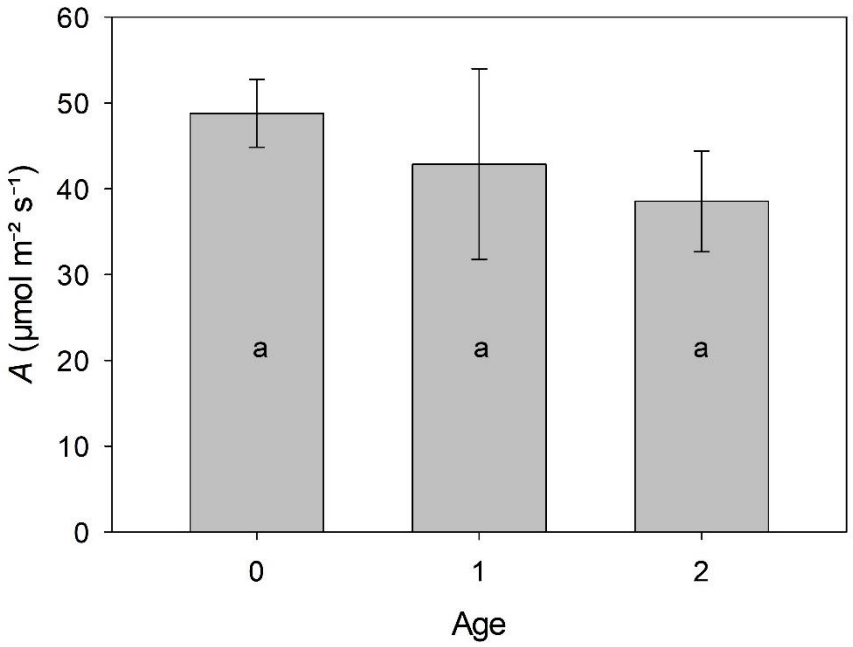

Figure 21. Photosynthetic rates at a standard C_a of 900 µmol/mol using detached needle methodology. Bars are mean ± se, n = 5.

Minimum Needle Conductance and Epicuticular Wax Content

There were no differences in epicuticular wax content among the variously aged needles (p = 0.305; Figure 22a). There were also no differences in minimum needle conductance (p = 0.454; Figure 22b).

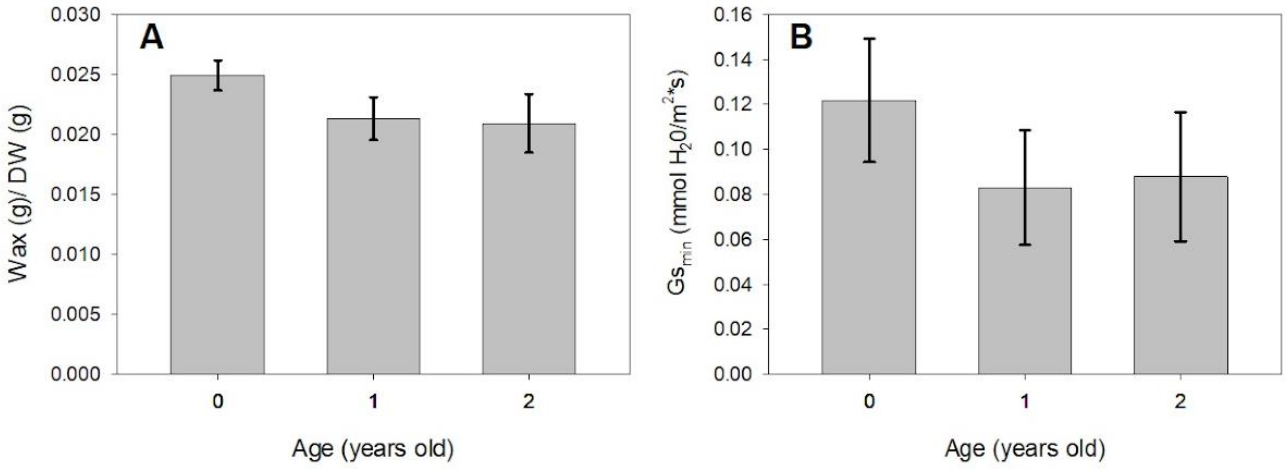

Figure 22. (**A**) *A. fraseri* minimum stomatal conductance and (**B**) epicuticular wax content by needle age. Bars are mean ± se, n = 5.

DISCUSSION- CH. 2

Canopy Architecture and its Physiological Implications

Fraser fir trees consist of endemic populations that exist primarily in high-elevation fir forests, where they consistently experience cloud immersion, less intense sunlight, increased diffuse radiation, and higher humidity levels (Gu et al. 2002; Berry and Smith 2013). In contrast, commercially-grown Christmas trees exist entirely below the lower natural elevation range of 1350 m, and experience frequent intense solar radiation, lower humidities (i.e., higher VPD), and higher temperatures (Berry and Smith 2013). This species has evolved to effectively utilize the frequent diffuse light in its natural habitat by having a 3-dimensional radial needle arrangement that allows interception of radiation from multiple directions, thereby reducing the effects of self-shading and orientation (Carter and Smith 1985). Trees growing in the open also have a unique shoot architecture (Berry and Smith 2013), in which older needles remain relatively exposed and can intercept incoming radiation despite being located in the interior of the canopy.

Commercial growers take advantage of this architecture to create densely-shaded canopies by trimming trees annually, which allows them to sculpt the classic Christmas tree form (Hinesley and Derby 2004). Pruning also may result in thicker branches (personal observations), which functions better to hold ornaments. In this study, I found that pruning results in dramatic decreases in light intensity over very short distances into the canopy (~25 cm to the location of Y1 needles); on sunny days, PAR is reduced by up to 97% and 99% for the older Y1 and Y2 needles compared to Y0 needles on the exterior of the canopy.

When light penetration measurements were taken in October, the west side of the trees were shaded by the dense canopy, and even when the sun was at its zenith near solar noon, no direct light reached that side of the tree. As a consequence, PAR was extremely low, even for the

Y0 needles located on the exterior of the canopy. Values were around 50 µmol m^{-2} s^{-1} while Y0 needles on the east side were being exposed to over 2,000 µmol m^{-2} s^{-1} of light. Based on my light response curves, needles exposed to such low light (50 µmol m^{-2} s^{-1}) would have greatly reduced photosynthetic rates at these times. These results show that there is a huge disparity in photosynthetic potential among needles of different ages and for needles located in different sectors of the canopy.

On cloudy days, when diffuse radiation is impinging on the trees from essentially all directions, there is the potential for greater penetration into the crown (Berry and Smith 2013), but on such days, the maximum PAR is reduced by about 90% compared to a sunny day. So even though radiation may penetrate farther into the crown, the levels are so low as to be of little influence on photosynthetic carbon gain. The one difference in the pattern of light penetration that I observed on a cloudy day was that the Y0 needles on the west side of the tree had a significant increase in PAR, whereas this was not observed on a sunny day.

The implications of dense canopy architecture are severalfold. First, older needles located in the canopy interior are subject to extremely low light often below the LCP, which means these needles are rarely, if ever, contributing in a positive manner to the carbon budget of these trees. Only if these needles are exposed to a sun-fleck might they have rates of photosynthesis high enough to gain carbon (Hardy et al. 2004). Second, these widely varying rates across the canopy would result in spatial differences in carbohydrate production, storage, and phloem transport. How this affects the distribution of carbon, nutrients, and water within the tree crown is not well understood and deserves further attention. Finally, these patterns would shift temporally across the canopy over the course of a day with unknown implications.

Light penetration most likely varies across the season as the direction and zenith of the sun changes. It is known that conifers make extensive use of side light as well as direct overhead irradiation (Hardy et al. 2004). For example, the sun reaches its highest zenith on June 21, and at the latitude of my study site, permits direct radiation to reach the western side of the trees. Thus, in mid-summer, these trees would have fundamentally different patterns of radiation interception and carbon gain. Because my study was initiated in late August, I was unable to make measurements of light penetration when the sun was highest, but I plan to do so next summer.

The large decreases in light intensity are correlated with the physiological capacity of the oldest needles. For example, the Y1 and Y2 needles, which are heavily shaded, had lower quantum efficiencies, lower maximum photosynthetic rates, and photosynthetically saturated at lower light levels than the Y0 needles on the exterior of the canopy. At the same time, Y1 needles on intact twigs had lower respiration rates, lower maximum photosynthetic rates, and photosynthetically saturated at lower light levels than Y0 needles on intact twigs. Some of the differences between the detached, planar needles and those in their original orientation on intact twigs probably results from the effects of mutual shading and variable needle orientation in the latter, as well as from the additional respiratory carbon loss from the twig itself. In general, the physiological patterns were similar for detached needles and those in their natural placement on a twig, differing mainly in degree.

Hypothesis (2) stated that photosynthetic activity would decline with needle age due to reduced stomatal conductance and/or reduced biochemical activity. A decrease in photosynthetic capacity and stomatal conductance with increasing needle age is well-documented in the literature (Bond et al. 1999; Niinemets 2002; Ethier et al. 2006; Warren 2006). Further, Freeland (1952) found that in several conifer species, maximum photosynthetic capacity occurs

immediately following needle maturation during the first growing season. In the present study, the photosynthetic capacity of commercially-grown Fraser fir has been shown to decrease with age. Hypothesis (2) is thus supported because a reduction in stomatal conductance and biochemical activity was found. From complete darkness to full sunlight, Y0 needles had the highest average *A*, AQE, and LSP, while Y1 and Y2 needles had significantly lower values in all categories.

Hypothesis (3) stated that older needles would contribute less to the carbon budget of the tree because of needle loss, changes in needle morphology that affect gas exchange, and/or lower photosynthetic rates due to their placement in the dense canopy interior. Hypothesis (3) is thus supported because decreased Y2 needle packing, interior light levels below the LCP, and decreased Y2 photosynthetic ability were found during this study.

Adaptation to the light environment, both morphological and biochemical, occurs during and just after expansion of young needles. From a morphological standpoint, once a sun needle is formed, it cannot revert back to a shade needle due to outward expansion of the canopy. Biochemically, Y0 needles can continue to adjust to a changing light climate, even after the needles have completed expansion. For example, immature Y0 conifer needles saturate and are susceptible to photo-inhibition at lower light intensities than mature needles (Hellinga 1974), yet they also receive the highest light intensity regardless of current weather conditions, and they continue to receive frequent saturating light for at least their entire first year. Moreover, chlorophyll levels continue to increase as needles mature (note how light of green Y0 needles are when still expanding compared to fully-grown older needles, which are much darker green).

It is difficult to separate the effects of needle age and needle light environment on the physiology and morphology. Needle aging occurs alongside a sharply decreasing light gradient,

and these parallel changes result in statistical confounding. From a morphological point of view, there is also the need to consider how needles cope with ever increasing twig diameter growth, and how this may affect their ability to maintain their vascular connection. Needles are connected to the cambial layer which expands and moves outward as the twig adds new woody growth. If this expansion disrupts the vascular connections to the needles, it could alter the movement of water, nutrients, and carbon between needle and twig, and if deleterious in any way, could eventually lead to needle senescence. More work on changes in the vasculature of differently-aged needles would help resolve these questions.

The pattern whereby needles that were once on the exterior of the canopy when the tree was younger and are now located deep within the shaded interior of the canopy as the tree has grown older, has received relatively little study by physiologists. In most plants, formation of a sun leaf predisposes that leaf to performing poorly in dimly lit environments, mainly because sun leaves have a variety of characteristics that adapt them to a high light environment (Boardman 1977). Morphological changes may include multiple palisade mesophyll layers, reduced internal airspaces, higher chlorophyll amounts on a unit area basis, as well as altered stomatal densities (Abrams and Kubiske 1989; Jordan and Smith 1993; Lichtenhaler et al. 2007; Wyka et al. 2008; Greenwood et al. 2009). Rates of photosynthesis are higher in full sunlight, while respiration rates are higher in the dark (Leverenz and Jarvis 1980; Carter and Smith 1985; Bond et al. 1999). Therefore, the compensation point, the light level where gross photosynthesis matches dark respiration, is higher for sun than shade leaves (Bond et al. 1999; Wyka et al. 2008). Conversely, shade leaves are often larger in area, thinner due to having only a single palisade mesophyll layer, have greater internal airspaces, and have lower chlorophyll *a:b* ratios, indicating

adaptation to a low light environment (Bond et al. 1999; Lichtenhaler et al. 2007; Metslaid et al. 2007; Wyka et al. 2008; Greenwood et al. 2009).

Once needle morphology is set in place, additional adjustments to low light must come primarily from anatomical changes, such as in chloroplast number and size, changes in internal cellular density (i.e., thicker cell walls), and alterations in the biochemical machinery of the needle, like electron transport capacity and the amount and activation state of RUBISCO (Lichtenhaler et al. 2007; Wyka et al. 2008; Greenwood et al. 2009). These irreversible morphological changes reduce CO_2 diffusion within the needle, which in turn, could limit photosynthesis. In my study, LMA increased with needle age, consistent with the findings of earlier researchers and further supporting the above argument about the potential for adjustment to low light.

Large-scale changes in irradiation occur in densely grown crops and forest-grown trees, where the increasing height of a stand causes the bottom-most leaves to become intensely shaded. In these studies, plants often respond by senescing the shaded leaves and moving their nutrients, particularly nitrogen, into the upper, more active leaves where light levels are much higher (Fukai and Hammer 1987; Hikosaka et al. 1994; Hikosaka and Terashima 1995; Ravi and Indira 1999).

Leaf economics theory has demonstrated that leaf (or needle) lifespans are related to their ability to pay back their construction costs (Chabot and Hicks 1982; Givnish 1988; Kikuzawa 1991). When needles are in high light and have high rates of photosynthesis, they can pay back those costs more quickly than in shaded needles. Thus, needle lifespans can be negatively related to the light environment in which they exist. This suggests that if it takes more than one year to pay back the construction costs for a needle, that the tree may retain those needles for more than

one year. If needles get progressively more shaded with age, this may delay the timing of senescence, as it would take longer in lower light to pay back those construction costs and yield any profit (i.e., exportable carbon). This may be one reason why Fraser firs retain their older needles, even though they are in low light environments. However, at some point, the annual carbon budget for these needles will become negative, and then the needles will be a liability for the tree. At that point, economic theory predicts that they should be senesced (Givnish 1988; Kikuzawa 1991).

Despite the drastic change in environment as a sun needle becomes a shade needle, commercially-grown mature Fraser fir are known to retain their needles for up to six years (McKinley and Hazel 2014). Given the very low light environment of older needles, there remains the question of what these needles contribute to the tree and why they are retained for so long. I speculate that commercially-grown trees may retain their oldest needles because of a genetic pre-disposition; native-grown trees have a less dense canopy, and therefore, older needles may contribute more significantly to the carbon budget through better access to diffuse radiation and sun-flecks.

Needle Packing and its Implications for the Carbon Budget

Fraser fir Christmas trees are trimmed annually to create the dense, inverted-cone shape that is sought out by consumers each year. Trimming a branch disrupts the sink-source relations of the tree by removing many buds (sinks) and their main source of carbohydrates (needles). Therefore, increasing the flow of carbohydrates from other intact branches to the pruned branch will increase radial growth and decrease needle density of a stem over time (Långström and Hellqvist 1991; Hinesley and Derby 2004).

In one study of several conifer species (*Picea engelmannii, Abies lasiocarpa,* and *Pinus contorta*), higher needle packing density increased photosynthetic rates per shoot, but decreased photosynthetic rates per needle because of self-shading (Smith and Carter 1988). In the present study, photosynthetic rates using the detached needle methodology (i.e., decreasing needle packing/self-shading) were higher than when using the intact twig methodology across needle ages, indicating that needle packing, self-shading, and twig respiration have significant impacts on intact twig photosynthesis (Carter and Smith 1985; Stenberg et al. 1998; Metslaid et al. 2007). This has several implications; because needle packing increases self-shading, twigs would have a higher compensation point than if needles were arrayed in a plane. Thus, twigs that become located farther into the interior of the crown as the tree increases in size will find it more difficult to maintain a positive carbon balance as PAR decreases. However, a decrease in needle packing density with increasing age would partially compensate for the lower PAR by reducing self-shading. Accordingly, I did find less needle packing with increasing age.

For intact twigs, photosynthetic rates are also influenced by bark physiology. Fraser fir twigs have a significant green tissue layer just below the outer bark, and it is known that a large number of plants carry out stem photosynthesis. While that was not the focus of my study, it does suggest that in addition to what the needles are doing, one must also consider bark activity. Under high light conditions, there may be significant carbon assimilation, most likely from cellular respiration within the twig (Postuka 1968), or from CO_2 carried in the transpiration stream from the roots and other parts of the tree to the needles (Teskey et al. 2008). Again, there are implications for whole-tree carbon gain when one considers the differently-aged needles; twigs with Y0 needles may also sequester carbon from high rates of stem photosynthesis, whereas older needles like the Y1 and Y2 needles may find that PAR is so low that stem

photosynthesis is not possible, and hence this carbon would be lost due to respiratory processes (Teskey et al. 2008). Only a fraction of the light incident on a stem penetrates far enough to elicit carbon uptake through photosynthesis, so stems located in low light environments are most likely respiring more than photosynthesizing.

Chlorophyll and its Physiological Implications

The type and amount of chlorophyll in chloroplasts has been shown to change with age and influence of environmental factors, like shading (Brooks et al. 1994; Pettigrew and Vaughn 1998; Bond et al. 1999; Kivimäenpää and Sutinen 2007; Deligöz et al. 2018). Chlorophyll is also a major sink for nitrogen, and levels are often correlated with amounts of RUBISCO, the enzyme that fixes CO_2 into sugars (Lu et al. 2020). I did not measure RUBISCO or nitrogen levels in my trees, but I did determine chlorophyll levels, which can act as a surrogate for the status of the photosynthetic machinery in needles as they age. New conifer needles typically have lower total chlorophyll levels than older needles until they achieve maturity late in the season (Deligöz et al. 2018; Apple et al. 2002). Chlorophyll *b* is often in higher abundance with increased age (as indicated by a decrease in the chl *a:b* ratio). Older, shaded needles can enhance light capture by having a greater abundance of PSII light harvesting complexes. PSII complexes have primarily chl *b*, which is why the ratio of chl *a:b* decreases in low light environments (Pettigrew and Vaughn 1998; Valladares and Niinemets 2008). This is consistent with the findings in my study, in which the ratio of chl *a:b* decreases from Y0 to Y1 and Y2 needles. In addition, Y1 and Y2 needles contained similar levels of chlorophyll *a*, *b*, total chlorophyll content, and had similar chlorophyll *a:b* ratios, which is consistent with the intense shade gradient that occurs after one year of growth. These findings suggest that there is some capacity, albeit limited, for needles to

adjust their pigment concentrations to the prevailing PAR, which would help them adapt to their new, more dimly lit environment within the canopy of these trees.

Because age and location within the canopy are confounded, it is still not possible to attribute changes among the needle cohorts solely to aging or environmental causes. The way to eliminate confounding would be to manipulate branches on young trees so that as the canopy extends outward, older needles are exposed to nearly the same PAR as those on the exterior of the canopy. If this is done for several years, most likely by doing some creative pruning and branch manipulations, then one could have Y2 needles that are still in a light environment comparable to Y0 needles. From studies of the carbon economy of leaves that develop in different light environments (Chabot and Hicks 1982, Neufeld and Young 2014), one could hypothesize that this might reduce older needle lifespans because such needles would be able to pay back their construction costs more quickly. Alternatively, one could also hypothesize that older needles might be retained for longer because they would be contributing more carbon to the tree given the higher light levels. It would be interesting to carry out such an experiment in the future.

Older needles may also retain their original N content, at least on a mass basis (Bond et al. 1999; Wyka et al. 2008), which when combined with decreased photosynthetic rates, would result in a lower photosynthetic nitrogen use efficiency (PNUE; Warren 2006; Wyka et al. 2008). In my study, chlorophyll amounts were maintained in the two older needle classes, which would suggest that these needles have a lower PNUE. Older, more shaded needles may also down-regulate electron transport capacity and reduce the amount and activation state of RUBISCO (Ethier et al. 2006; Robakowski and Bielinis 2017), all of which would contribute to a decreased photosynthetic capacity.

A/C_i Curves

In my study, when needles were exposed to high ambient CO_2 (~900 µmol/mol), there were no statistically significant differences in the rate of *A*, which is somewhat puzzling given the results of the light curve responses, where *A* declined at saturating, or near saturating PAR with age. Elevating CO_2 to 900 µmol/mol should have reduced the stomatal limitations on diffusion, allowing the biochemical potential for *A* to be expressed. If so, then these paradoxical results suggest that the older needles still retain the capacity to carry out high rates of photosynthesis, but do not in nature because of stomatal limitations caused by low PAR at ambient levels of CO_2.

Minimum Needle Conductance and Epicuticular Wax Content

Epicuticular waxes minimize cuticular water loss from needles, and in some cases may protect needles from excessive high light, either by absorbing UV radiation or by increasing needle reflectance (Holmes and Keiller 2002; Pfündel et al. 2006). Waxes also permit conifer needles to survive in unfavorable conditions, such as frost, drought, high irradiance, and wind. During winter, when soils and possibly stems are frozen, access to water is limited. If there has been degradation of the waxes due to aging and/or abrasion, cuticular water loss, which is normally low when the waxes are intact, could be large enough to cause needle water stress, and even hydraulic failure (Baig and Tranquillini 1980; Duursma et al. 2018). Cuticular water loss is difficult to measure because separating it from stomatal water loss is technically difficult (Márquez et al. 2021). However, if a needle is water-stressed to induce stomatal closure, and one assumes that there is little damage to the cuticle from shrinkage, then measuring water loss in this state could be taken as a best estimate of cuticular water loss. Since stomata may not shut completely in these conditions, water loss is more properly termed minimal water loss, and the

assumption is that most of the water that is lost is derived from cuticular transpiration (Mohammadian et al. 2007, Márquez et al. 2021). If one divides the minimum water loss by the vapor pressure deficit (after correcting for the barometric pressure) then one can calculate a minimal needle conductance (Gs_{min}), and this parameter has been found to increases with needle age in some conifers (Heinsoo and Koppel 1998; Anfodillo et al. 2002), while not changing in others (Anfodillo et al. 2002; Warren 2006).

It is also subject to variation due to the environment in which a needle exists. For example, epicuticular wax content can increase under high irradiance and decrease with age in some species. If this occurs in Fraser fir, then older and more shaded needles may have a different Gs_{min} (Giese 1975; Prügel et al. 1994; Heinsoo and Koppel 1998; Anfodillo et al. 2001; Shepherd and Griffiths 2006). However, I found no difference in Gs_{min} or epicuticular wax contents due to needle age, and by implication, with PAR. The Gs_{min} portion of our study relied on the methods outlined by Heinsoo and Koppel (1998), with the exception of using petroleum jelly instead of latex glue to prevent evaporation from the sliced bark. In addition, measurements ceased after approximately 4 hours in my study, while the aforementioned study continued to record stomatal conductance for an additional 10 hours. It is possible that difference might have shown up had I extended my observations for more time, but in other studies, authors reported stable Gs_{min} over the same time intervals that I used (Barnard and Bauerle 2013). Since my rates of water loss approached a steady-state near the end of measurements, I am confident that it does represent a true estimate of Gs_{min}. Only additional studies conducted over a longer time period would settle this conundrum.

CONCLUSION- CH. 2

The results of my study indicate that there is a decline in the photosynthetic capacity with increasing needle age of 3-4 year old Fraser fir Christmas trees. Photosynthetic rates consistently decreased with age regardless of light level, and there were significantly fewer needles per cm^2 with increasing age as well. Needles become intensely shaded inside the dense canopy throughout ontogeny, and chlorophyll *a*/*b* ratios declined with needle age, indicating perhaps a partial acclimation to low light in these needles. These results are consistent with hypotheses (2) and (3), suggesting older needles contribute little to the carbon budget of the tree. Future research efforts could investigate why the oldest needles are retained by measuring differences in soluble carbohydrate concentration across needle ages, comparing photosynthetic contribution and light levels of the endemic Fraser fir and the commercially-grown Fraser fir, or by conducting a multi-year study to observe how gas exchange and light levels change with increasing shade and age following one newly-formed branch. In addition, analyzing composition changes in epicuticular wax using gas chromatography and mass spectrometry, and tracking re-translocation of nitrogen and other nutrients from old to young needles would improve the understanding of physiological changes occurring throughout Fraser fir needle ontogeny.

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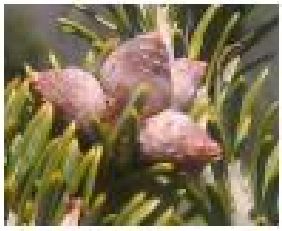
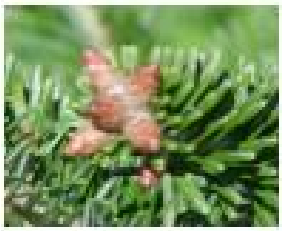
APPENDIX

Appendix Table 1. Timeline of farm management practices at Clawson's Choose-and-Cut Christmas tree farm in Boone, NC.

Date	***Action***
Early April	Plant seedlings
Mid-April	Fertilize soils: Urea 46-0-0 150-200 lbs/acre
Early May	Herbicide: Glyphosate "Round-Up" 8 oz/acre
Mid-May	Insecticide: High-pressure spray of Asana and Dimethoate Asana: 4 oz/acre. Dimethoate: 8 oz/acre.
Late June	Herbicide: Glyphosate "Round-Up" 8 oz/acre
Mid-July	Insecticide: High-pressure spray of Asana and Dimethoate Rotated with Bifenthrin
Mid-July	Annual trimming begins, continues through September
Early August	Herbicide: Glyphosate "Round-Up" 8 oz/acre
Early September	Insecticide: High-pressure spray of Asana and Dimethoate Rotated with Bifenthrin
Late September	Herbicide: Glyphosate "Round-Up" 8 oz/acre

Appendix Table 2. The seven-stage system of *A. fraseri* bud burst provided by Dr. Jeff Owen of the North Carolina Cooperative Extension Service.

Description	Image	Description	Image
Stage 1 Dormant buds with thick waxy coats and blunt tips, ranging from white to dull shades of red, brown, and purple.		**Stage 5** Bud scales begin to separate at tips and turn white.	
Stage 2 Tips begin elongating, appearing wet and sticky. Base and sides still waxy and dull.		**Stage 6** Green shoots visible beneath white scales, but have not yet emerged.	
Stage 3 Base begins to swell. Waxy coats become shiny or translucent, and bud scales become darker brown.		**Stage 7** Complete bud-burst. Green shoots emerging.	
Stage 4 Tips elongate and become pointy, and become deep brown or red.			

Vita

Ashley Marie Hull was born in Tampa, Florida in 1997. She attended Western Carolina University from 2015-2018 and was awarded a Bachelor of Science degree in Biology in December 2018. Immediately following her time at WCU, she planned on pursuing veterinary school. It wasn't until she was accepted into North Carolina State University's veterinary program that she realized her love for plants outweighed her desire to heal animals. She declined her acceptance to NCSU and applied to Appalachian State University to pursue a Master of Science degree in Biology with Howard Neufeld as her advisor. From 2020 to 2022, Ashley was a graduate student in the Department of Biology at ASU. Following graduation from ASU, she was actively applying for career positions with agricultural crop research facilities.