ECOPHYSIOLOGICAL EXPLANATIONS FOR SPATIAL AND TEMPORAL VARIATIONS IN AUTUMNAL COLORATION WITHIN THE CANOPIES OF ORNAMENTAL RED MAPLE (*ACER RUBRUM* 'ARMSTRONG') AND FREEMAN MAPLE (*ACER X. FREEMANII*) TREES

A Thesis
by
CLAIRE MARIE MARTIN

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Abstract

ECOPHYSIOLOGICAL EXPLANATIONS FOR SPATIAL AND TEMPORAL VARIATIONS IN AUTUMNAL COLORATION WITHIN THE CANOPIES OF ORNAMENTAL RED MAPLE (*ACER RUBRUM* 'ARMSTRONG') AND FREEMAN MAPLE (*ACER X FREEMANII*) TREES

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Some urban red maples (Freeman maples, *Acer x freemanii*) turn red at the top of the canopy before the bottom, while others turn simultaneously all over (*A. rubrum* 'Armstrong'). My research investigated the ecophysiological mechanisms governing spatial and temporal variation in autumnal red coloration in both tree varieties. We compared leaves from the top (8-9 m) and bottom (2 m) of the canopy to see if coloration differences arise from environmental or physiological differences, or both. We used a bucket truck to reach leaves and weather stations to measure microclimates at the two heights. Wind speed and solar energy were the only environmental variables that differed between upper and lower leaves. Wind speed was higher in the upper canopies of both species. Solar energy input was higher in the upper canopy of the Freeman maples, but not the Armstrong maples. Lower leaves of Freeman maples leafed out 7 days earlier and persisted ~25 days longer into the fall than those from the top, resulting in longer leaf lifespans by ~32 days. Mid-summer chlorophyll content in Freeman maples was higher in lower leaves, and in fall, anthocyanins accumulated earlier and to greater amounts in upper leaves, whereas no such differences occurred in Armstrong maples. Photosynthetic rates at saturating PAR and nitrogen contents were higher in lower leaves of Freeman maples but did not differ in Armstrong maples. Earlier and greater accumulation of anthocyanins in upper leaves

correlated with their lower nitrogen content, a factor known to elevate leaf anthocyanin content. Lower nitrogen in upper leaves could be the proximate driver for early anthocyanin synthesis in upper leaves, and a bet-hedging strategy to avoid high light-cold temperature photoinhibition in early fall, but we do not know the ecophysiological reasons for why this occurs in Freeman, and not in Armstrong maples. The results of this study provide insight into intra-canopy variation in leaf ecophysiology of open-grown trees in an urban environment.

Acknowledgements

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FOREWORD

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

Table of Contents

Introduction

Red maple (*Acer rubrum)* is an extremely abundant and adaptable hardwood species that occupies a wide geographical range from Southern Canada to Florida and as far west as Minnesota and Texas (Crowder 2015). This species is well known for its striking red fall foliage and several varieties have been developed specifically for their brilliant autumn displays (USDA 2012), which are due to the abundant production of anthocyanins, the main pigment responsible for the red coloring (Chalker-Scott 1999; Field et al. 2001). Mature red maples can reach an average height of 28 m, with a trunk diameter of 46-76 cm, and are moderately shade tolerant. They are common in a variety of ecosystems, including, but not limited to, mountainous regions with good drainage, moist swamps, riparian zones, and urban areas (Hutnik and Yawney 1961; Crowder 2015). This species can also withstand a wide range of soil types, including those with high pH (DeForest and McCarthy 2011). Their hardiness and striking red color make them popular ornamental trees for urban environments.

Freeman maple (*Acer x freemanii)* is a hybrid of silver (*A. saccharinum)* and red maple (*A. rubrum*) that is often planted in urban areas. Freeman maples are typically categorized as red maples; however, they are botanically recognized as *Acer x freemanii* (Sibley et al. 1995). They are bred for the hardiness and coloration derived from the red maple progenitor and the fast growth rate of the silver maple progenitor (McNamara et al. 2005) and are generally marketed as stress resistant (Bachtell 1989). The first controlled hybridization of red and silver maples was conducted by Freeman (1941) in 1933, but there are now many naturally occurring hybridizations that are considered Freeman maples.

Phenological Patterns

Red maples are one of the first trees to flower in the spring (USDA 2000). After flowering, red leaf buds open, and in forests, the leaves usually unfold from the top of the canopy

down once flowering is completed (Koike et al. 2001). The timing and duration of fall leaf color in red maples can vary widely among varieties. In the Southeastern US, some varieties of red maples display fall coloration for a longer period of time than Freeman maples; for example, the *Acer rubrum* 'October Glory' can retain leaves and red coloration into November (Sibley et al. 1995). Freeman maples are known to reach peak color earlier than other maple species (Sibley et al. 1995). In the southeast US, maples typically start early, followed by sourwoods, blackgums, and then oaks (Neufeld, personal observations).

New leaves are usually red in the early stages of development, turn green when they reach maturity, and then return to red during senescence in the fall (Hutnik and Yawney 1961; Hughes et al. 2007). Spring reddening is related to the developmental stage of the leaf. When the leaf is young, and the cells are densely packed, the diffusion of CO_2 within the leaf is restricted, which may limit rates of fixation in the chloroplast, and coupled with cool temperatures and high light, lead to photoinhibition and damage, especially to the D1 protein of Photosystem II. Once leaves expand, and airspaces are created, primarily in the spongy mesophyll, the diffusion of CO_2 is enhanced and it is no longer as limiting to the chloroplasts (Hughes et al. 2007).

The proximate causes of reddening in the fall may be similar to those in the spring (Lev-Yadun et al. 2012), with the exception that in the fall, leaves are fully mature with adequate airspaces for the diffusion of CO_2. Yet, the same selective pressures may be at work at this time of the year, because at this time of the year, low temperatures can be coupled with high light, and this results in reduced stomatal apertures that in turn limit the diffusion of CO_2 into the leaf, and they limit the activity of the Calvin Cycle, resulting in an imbalance between electron transport and carbon fixation. And as in the spring, this can result in the production of reactive oxygen species, membrane damage, and destruction of the D1 protein, similar to the conditions that led

to photoinhibition in the spring (Agati et al. 2021). However, there are probably subtle differences in the inciting factors between spring and autumn, such as daylength, the degree and frequency of cold snaps when leaves are present, and the physiological capabilities of immature vs mature leaves, that result in different induction cues. For example, if a species has red leaves in autumn, it almost always has red leaves in spring, whereas only about half of those whose leaves are red in spring also have red leaves in autumn (Yev-Ladun et al. 2012).

Anthocyanins may also serve to protect leaves from photooxidative damage so they can reabsorb essential compounds like nitrogen, manganese, and iron back into the stem (Dickson and Shive 1982; Feild et al. 2001; Hoch et al. 2001; Brant and Chen 2015), although this theory has recently been challenged (Pena-Novas and Archetti 2021; Agati et al. 2021). Anthocyanins may act as honest signals of leaf quality to deter potential herbivores (Archetti 2000, Archetti and Brown 2004), although the evidence for this is quite limited. Finally, it is also possible that both sets of theories could be true, as they are not mutually exclusive (Archetti et al. 2009).

Intra-canopy Variations in Leaf Attributes - Morphology

Leaf physical attributes, including area and leaf mass per area (LMA), as well as phenological and physiological attributes such as coloration and growth patterns, can vary not only among species, but within the canopy of individual trees (Koike et al. 2001). There are numerous reasons for this. For example, leaf size may be related to intra-canopy variations in water potential, which may affect leaf expansion. Upper leaves are often subject to lower potentials due to their greater exposure to light and potentially higher evapotranspiration rates, but also because the gravitational component of water potential would be greater than for lower leaves. Under both conditions, upper leaves would have lower turgor pressures and hence less leaf expansion (Hsiao 1973; Coble and Cavaleri 2014).

Studies show that LMA, the ratio of leaf weight to area, is a function of both leaf thickness and density (Poorter et al. 2009) and tends to increase with height in the canopy of many species (Hutchison et al. 1986; Marshal and Monserud 2003, Coble and Cavaleri 2014, Paz-Dyderska et al. 2020). This may be due to that fact that upper leaves receive more sunlight and thus develop a thicker palisade mesophyll (Nobel et al. 1975), or, it may result from lower water potentials and reduced cell expansion, both of which contribute to a greater density (Villar et al. 2013).

There can also be variations in stomatal density and size within the crown of trees (Lei 1998; Eensalu et al 2008). Red maples have increased stomatal density on shaded leaves compared to those in higher light (Lei 1998). In other plants though, leaves in higher light have greater stomatal densities, which may seem paradoxical, as one might expect it always results in higher transpiration rates, and therefore more water stress. But when stomata are positioned close to each other, their diffusion shells overlap, which reduces the vapor pressure deficit above the stomatal pores, lowers stomatal conductances, and can actually decrease transpiration rates (Lehmann and Or 2015; Papanatsiou et al. 2017). Plants with larger stomata, but at lower densities, tend to have higher water use efficiencies, sometimes at the expense of lower photosynthetic rates, compared to plants whose leaves have more numerous, but smaller stomata (Drake et al. 2013). Maximum photosynthetic rates (A_{max}) can also vary depending on stomatal density, with leaves that have more stomata associated with an increased photosynthetic capacity (Tanaka et al. 2013).

Intra-canopy Variations in Leaf Attributes – Hormones and Nutrients

In addition to morphological differences among leaves in different portions of the tree canopy, there can also be intra-canopy differences in nutrients and hormone levels and these may

alter the physiology of leaves. For example, researchers have found higher concentrations of cytokinins in upper than lower leaves of sugar maples, and these levels were also positively correlated with leaf gas exchange rates (Held et al. 2005; Reeves et al. 2007; Reeves and Emery 2007).

Nitrogen from the soil is transported from the roots to the rest of the tree crown in the xylem (Pate 1973; Tischner 2000). Nitrogen availability can be a limiting factor in tree carbon gain (Chapin et al. 1987), so trees have evolved to allocate nitrogen in ways that maximize carbon uptake and growth (Feild 1983). Where there is substantial within-canopy shading, such as happens in closed forest canopies, or in dense stands of perennial old-field herbs, nitrogen is re-allocated to those leaves that receive more light and are therefore more physiologically active, especially with regards to photosynthetic carbon gain (Hirose and Werger 1987; Terashima and Evans 1989). More recently, Peltoniemi et al. (2012) suggest that with respect to light, nitrogen is only optimally allocated if hydraulic conductance to water is also. Their reasoning is that leaves in high light can only take advantage of the high nitrogen to carry on high rates of photosynthesis when they are optimally supplied with water, because that allows them to maintain open stomata and for CO_2 to enter the leaf. Again, such variations within the crown could be related to color variations found in the fall, since low nitrogen is associated with greater anthocyanin production (Schaberg et al. 2003)

Autumn Coloration and its Functional Significance

Autumn in temperate regions around the world is characterized by the change in leaf color of deciduous trees from green to orange, yellow, brown, and red. Oranges and yellows appear during chlorophyll degradation and are due to carotenoids (Krautler et al. 1991; Hortensteiner 2006) which are always present in the leaf, but are overshadowed in the summer

by the more abundant chlorophyll (Matile et al. 1992). Only when the chlorophyll is degraded do these compounds become visible to the eye. Red pigmentation, on the other hand, results from the synthesis of anthocyanins, which are water soluble flavonoid compounds found in the vacuoles of plants (Lee et al. 1979; Gould et al., 2000; Harborne and Williams 2000; Hughes et al. 2007). Anthocyanins absorb UV wavelengths of light (280-320 nm; Radyukina et al. 2019) as well as green-yellow light (centered on 530 nm) in the visible spectrum (Ferreyra et al. 2021). Plants at high elevations, where UV radiation levels are higher, often accumulate anthocyanins in their adaxial epidermal cells (Miret and Munne-Bosch 2015) and it is thought that this serves a protective function, most likely by minimizing damage to DNA, RNA, proteins and membranes, and by reducing the production of toxic reactive oxygen species (Ferreyra et al. 2021).

In the visible spectrum, anthocyanins function to protect the photosynthetic apparatus from those conditions that can create an imbalance between electron transport and carbon fixation, such as when leaves are young and dense cell packing restricts CO_2 diffusion (Hughes et al. 2007), or when mature leaves are exposed to the combination of high light and cold, which can induce similar stress (Chalker-Scott 1999). Light is necessary for photosynthesis, but excessive amounts, especially when temperatures are cooler, can adversely affect the photosynthetic apparatus and lead to membrane damage, production of reactive oxygen species, and ultimately, photoinhibition, resulting in a decline in quantum yield of photosynthesis, and eventually cell death (Adir et al. 2003). Plants that produce anthocyanins experience reduced rates of photosynthesis and suffer less photoinhibition (Nielsen and Simonsen 2011).

Another explanation is that the anthocyanin pigments protect the underlying cells from high light by absorbing the blue-green wavelengths that can be damaging to spongy mesophyll cells (Chalker-Scott 1999; Hughes et al. 2007). Anthocyanins may also be honest signals of

defense against herbivores (Archetti 2000). That is, brightly colored trees are better protected chemically against potential herbivores and bright fall colors would serve as a warning to ward off attack (Archetti 2000; Gould 2002; Lee and Gould 2007; Karageorgou and Manetas 2006; Schaefer and Rolshausen 2005) Finally, it is quite possible that anthocyanins serve both photoprotective and anti-herbivore roles and the debate on this is ongoing (Chen et al. 2021; Hughes et al. 2022).

Fall Coloration and Intra-Canopy Variations

Leaves of late-successional stage trees turn color first on the outside of the canopy, and then later toward the inside where light levels are lower, whereas early-successional trees progress in the opposite direction, from the inner to the outer canopy (Koike 1990). Leaves at the top of the canopy or at the ends of branches are subject to higher radiation than leaves located at lower or more interior locations in the crown and therefore might synthesize more anthocyanins for photoprotection (Choinski et al. 2003). Koike et al. (2001), working in a mixed species deciduous forest in Japan, also found that photosynthesis in the upper canopy of the maple *Acer mono*, in mid-summer, was lower than that in the lower canopy, possibly due to photoinhibition. However, upper leaves had higher maximum rates of photosynthesis (A_{max}) at high light than lower, shade-grown leaves in this same species. They also found that ambient CO_2 was generally higher closer to the ground, and leaves senesced earlier on the top of the canopy in maples, which suggested a correlation between CO_2 and delayed senescence.

A_{max} can vary depending on the location of the leaf in the canopy (Bassow and Bazzaz 1997) and is usually greater in leaves at the ends of branches or at the top of the canopy, where light levels are higher, compared to shaded, interior leaves. Upper canopy leaves are also known to have more chloroplasts (Crous and Ellsworth 2004). However, in the dry season, as stomata

close and transpiration rates decrease, A_{max} in the upper canopy may decline as leaves become more water stressed (Sendall et al. 2009).

Photoinhibition occurs when leaves absorb excess light (more than what is necessary for photosynthesis), resulting in decreased photosynthetic capacity (Öoquist et al. 1992; Long and Humphries 1994). This can occur when there is a combination of drought, low temperatures, and high light in the fall (Beadle and Sands 2004) and may be one reason why upper canopy leaves in some *Acer* species senesce before those in the lower canopy (Koike et al. 2001).

It is also possible that leaves in the upper canopy of maple trees may experience greater water stress and reduced nitrogen content, which might promote the formation of anthocyanins (Cobbina and Miller 1987; Nozzolillo et al., 1990; Balakumar et al. 1993). For example, in sugar maples (*A. saccharum*), anthocyanin production in leaves is higher in leaves with low N and further stimulated when combined with cold temperatures (Schaberg et al. 2003). Osmond et al. (1980) found that shaded leaves have a higher ratio of nitrogen to chlorophyll which enables higher rates of photosynthesis, presumably because of higher RUBISCO amounts. If leaves in the upper canopy are more exposed than those in the lower canopy, and also subject to the resource limitations discussed above, then this might explain why they turn red sooner in the fall. There could also be interacting factors that might affect the phenology of upper and lower canopy leaves. For example, in a study of olive trees, south-facing leaves received more sunlight and had more N than north-facing leaves, indicating that light exposure and N content were interrelated. This could make the intra-canopy pattern of leaf reddening in the fall spatially more complicated than previously thought (Perica 2006).

Microclimate of Urban Trees

Urban tree species are subject to unique microclimates, depending on where they are planted (Kjelgren and Clark 1992). Trees themselves may alter the microclimate by intercepting light and cooling that portion of the canopy and the surrounding air that becomes shaded (Gill et al. 2007; Rahman et al. 2019). When trees are planted singly, often in rows adjacent to roads, and are highly exposed on all sides, they can develop fairly dense canopies that become effective at both temperature stratification and attenuating light along the vertical gradient of the crown (Armson et al. 2013, Rahman et al. 2015).

The effects of wind on the leaves of urban trees can vary depending on their height above the ground. Wind speeds tend to be lower closer to the ground (Daudet et al. 1999; Geiger et al. 2003), while temperatures and VPD (vapor pressure deficit) are less variable (Daudet et al. 1999) and such microclimatic differences may differentially influence leaf development. If wind speeds vary throughout the tree canopy, they can also alter the leaf boundary layer conductance, thereby influencing leaf gas and energy exchange rates (Daudet et al. 1999; Aphalo and Jarvis 1993; Telewski 1995). Furthermore, high wind velocities can induce mechanical strain and abrasion which can induce stomatal closure and lower photosynthetic rates, and in some cases, result in reduced leaf expansion and smaller leaf sizes (Telewski 1995).

Implications of Study

Urban grown trees experience a different growing environment from those in forests because they grow in more open locations, where even the leaves at the lower portion of the canopy can still receive high light and high wind levels. They are also less subject to vertical stratification of atmospheric CO_2 and temperature. The intra-canopy patterns of fall coloration and their adaptive significance of reaching senescence at different times throughout the canopy

can provide insight into urban tree growth and perhaps help foresters manage trees in metropolitan areas.

Objectives

Although there is some research on intra-canopy variation of trees growing within mixed deciduous forests, and some for agricultural crops (Perica 2006), there are no physiological investigations into why leaves on some ornamental maples turn red first at the top of the tree, while others turn red all over at nearly the same time. My objectives in this study were to monitor red maple leaf development, phenology, and ecophysiology, over the growing season, for two maple varieties, each growing on different sites in Boone, NC. One variety, the Freeman maple (FM), has leaves turn red first in the upper, outer canopy, then later in the lower canopy, while in the other variety, Armstrong maple (AM), all of the outer leaves turn red at about the same time.

To determine why there were differences in the pattern of autumn coloring between the two maple varieties, I tested the following three hypotheses:

Hypothesis 1: Differences in microclimate between upper and lower leaves is correlated with the pattern of fall coloration.

Hypothesis 2: Differences in physiology between upper and lower leaves are responsible for the observed patterns of fall coloration.

Hypothesis 3: Both microclimate and physiology interact to determine the patterns of fall coloration.

Methods and Materials

Study Site and Maple Varieties

I sampled 10 maple trees in Boone, NC during the growing season of 2021. Five of them (*Acer rubrum* 'Armstrong'; hereafter AM) were located on Appalachian State University (ASU) property adjacent to the State Farm Parking Lot, while the other five (*Acer x freemanii* 'Freeman'; hereafter FM) were located along Rt. 105 at the Bubbles Car Wash at the south end of town. The FM trees change color at the top of the tree first, and the Armstrong change at the same time throughout (Figure 1). Both sets of trees were approximately 10 m tall. I rented a bucket truck with operators from either the ASU Physical Plant or New River Light and Power, in order to access both the upper and lower leaves for my project (Figure 2). Red maples are known to produce long-shoots and short-shoots (Critchfield 1960; Wilson 1966), which differ morphologically (Niklas and Cobb 2010). Because of the differences, all measurements were confined to short-shoots rather than long-shoots.

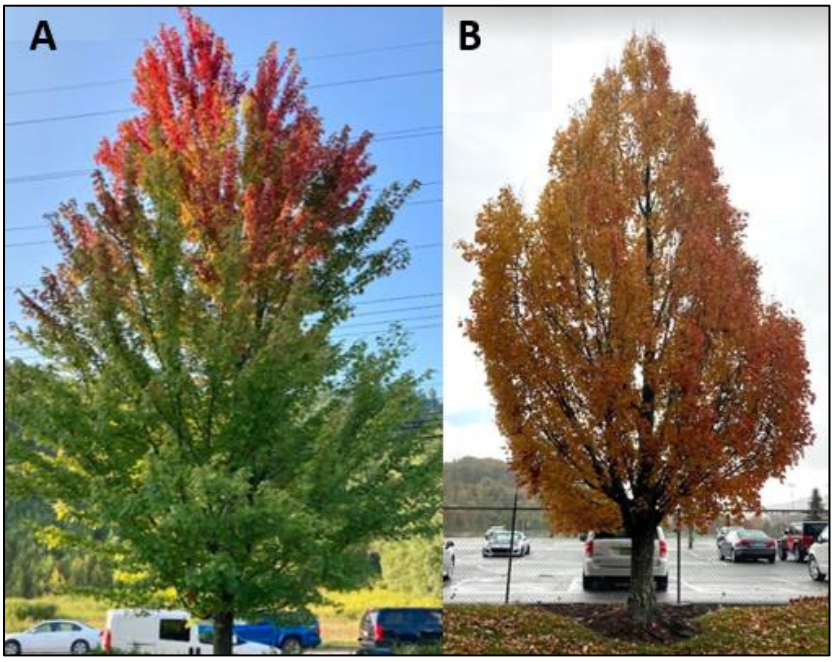

Figure 1. Freeman maple (A) in September and Armstrong maple (B) in October.

Figure 2. Taking measurements in October using the bucket to reach the upper leaves of Freeman maples at the Bubbles Carwash. Height of the bucket is approximately 8 m. Note how the upper leaves have turned red before the lower leaves in this maple variety.

Study Area

Boone (36°12′41″, -81°40′7″) is located in the Blue Ridge Mountains of Western North Carolina and has an elevation of ~1016 m. Boone features a subtropical highland climate, and has an average total precipitation of 1,500 mm annually. The average winter temperature is −0.3 °C and the average summer temperature is 20.2 °C (NOAA 2021).

Microclimate Measurements

To determine if differences in microclimatic conditions between the upper and lower canopy locations might be correlated with differences in leaf phenology, physiology, and coloration, I placed weather stations at two heights (2 m and 8 m) on poles located adjacent to my sample trees at both sites (Figure 3). Data were recorded at 5-minute intervals and downloaded periodically to a laptop computer. Due to technical difficulties at the lower State Farm site, the meteorological data from that site is incomplete. Meteorological variables measured included temperature (ºC), wind speed (m/s) and direction, humidity (%), and solar

radiation (W/m^2) from July through October of 2021 using Davis Vantage Pro2 weather stations (Davis Instruments, Hayward, CA).

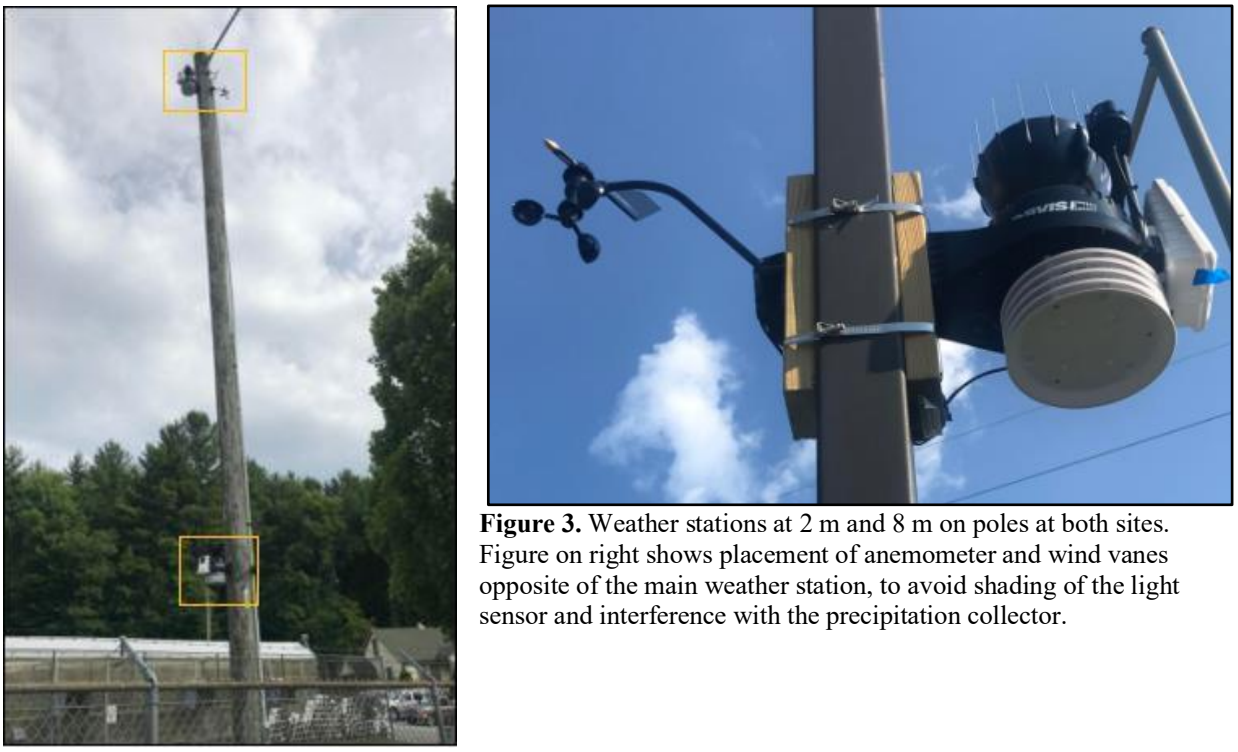

Figure 3. Weather stations at 2 m and 8 m on poles at both sites. Figure on right shows placement of anemometer and wind vanes opposite of the main weather station, to avoid shading of the light sensor and interference with the precipitation collector.

Phenology

I was interested in whether patterns of coloration in the fall could have resulted from differences in the phenology of leaves located in the upper and lower portions of the canopies of my study trees. To assess phenological patterns, I began monitoring the FM in March of 2021 for leaf development using binoculars to view the upper canopy. Three branches from the upper canopy and three from the lower canopy were selected and bud development was followed using a rating scale of 1-7, following the protocol of Skinner and Parker (1994; Figure 4). Because the AM were added to the study later in the season, I was not able to obtain bud break or leaf out data for this variety.

In the fall, I began monitoring leaf survivorship through to total senescence. I selected and flagged three branches from upper and lower canopies of each of my trees. Each week, I counted how many leaves remained on the branch, starting at the 10th pair of leaves and counting towards the distal end of the branch.

Figure 4. Bud development followed a rating scale of 1-7. (A) Bud with a rating of "3", (B) leaves on left with a rating of "7", while unfolding leaves on the right are rated a "6 ".

In order to monitor how color change progressed throughout the canopy, I took photographs of the trees at both sites throughout the season until the time when no leaves were left in late fall. I divided the tree canopy into four sectors: upper, mid-upper, mid-lower, and lower (Figure 5), and estimated the proportion of each canopy sector that was green using a scale that ranged from 0% to 100% green.

Leaves on my sample trees at the State Farm site began to exhibit marginal scorching in late August (Figure 6). Over the next few weeks, the condition of these trees continued to deteriorate. After discussing this with physical plant personnel, it was determined that this might be salt injury, since the road adjacent to these trees was routinely salted in winter. Because of this, and the fact that by 3 weeks later the trees had completely defoliated (Figure 6), I shifted my sampling to another set of 5 trees farther down the road where no salting had been done. These

trees, which were planted at the same time as the others and were growing similarly, appeared healthy for the rest of the season, and were used in all subsequent sampling at this site.

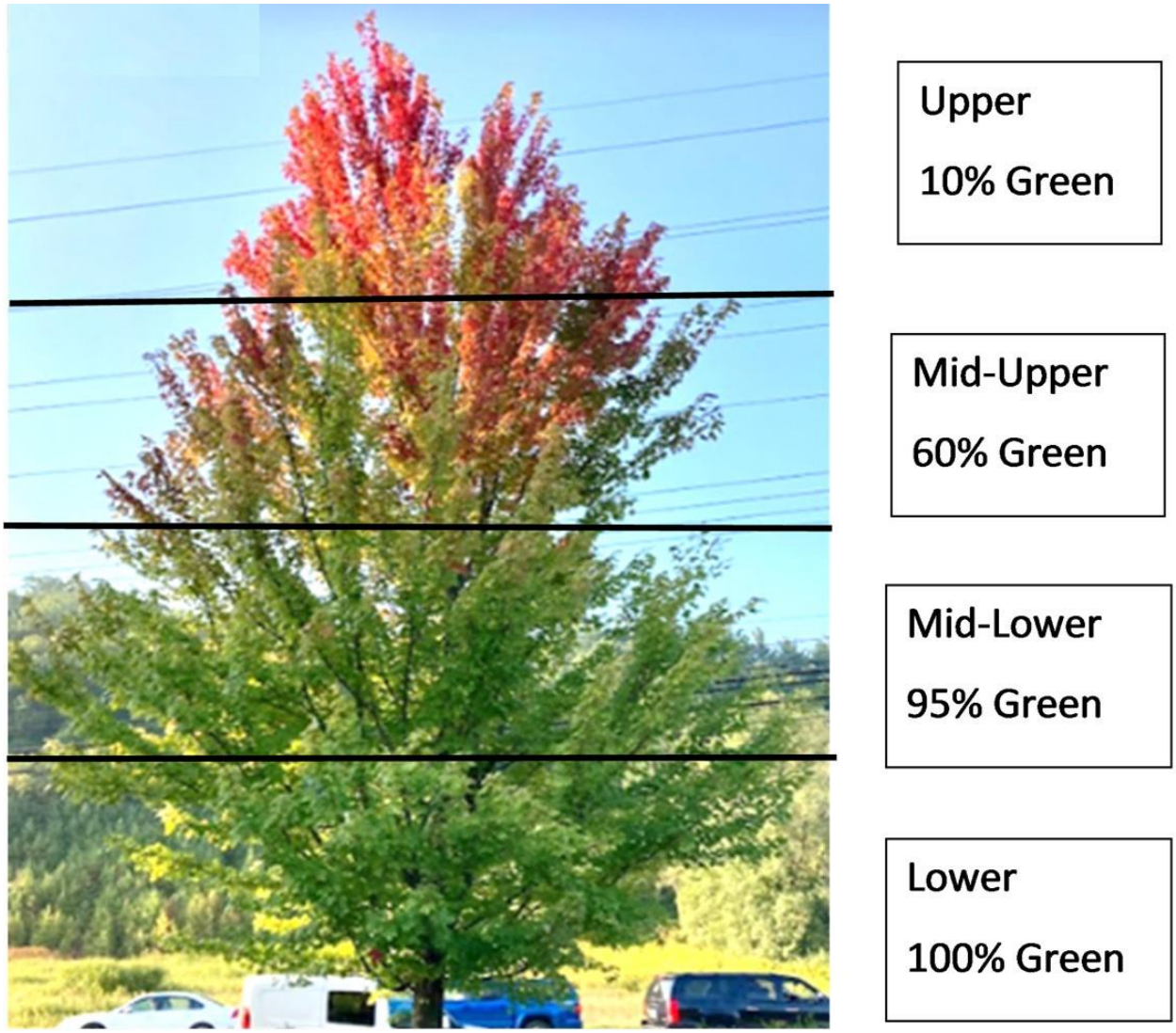

Figure 5. An example of the canopy locations on Freeman maple trees used for estimating the percent of leaves still green.

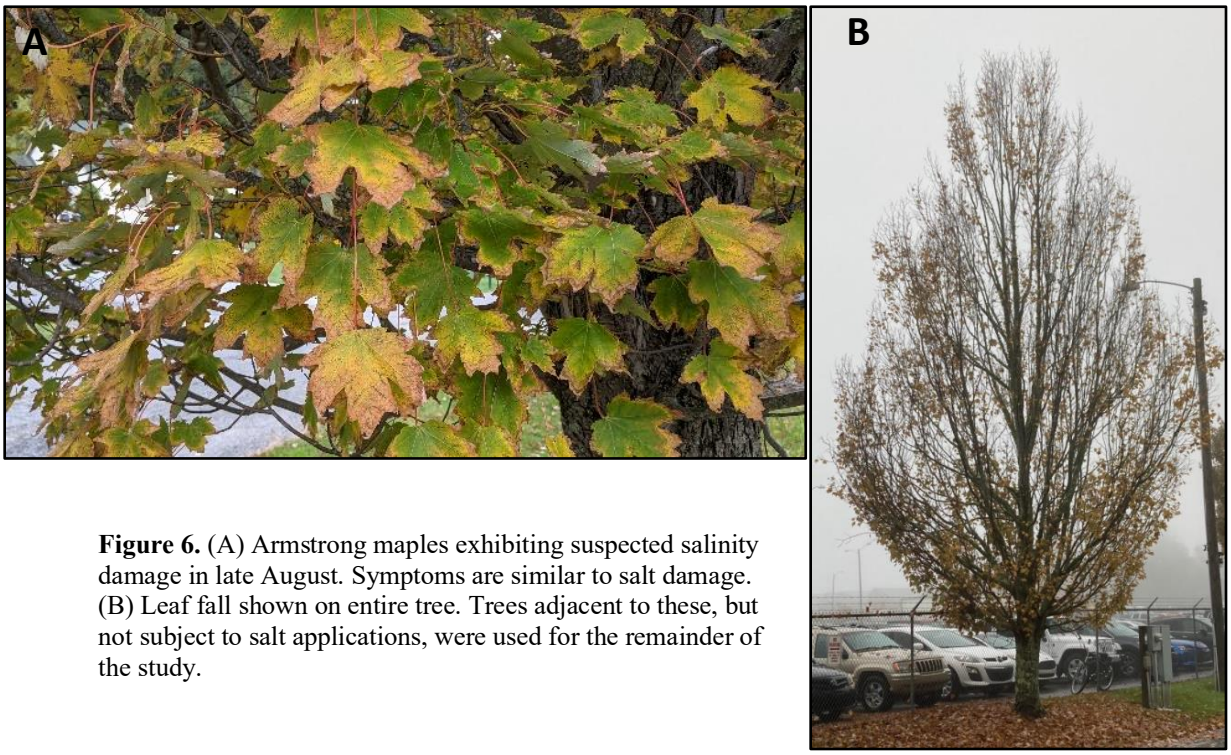

Figure 6. (A) Armstrong maples exhibiting suspected salinity damage in late August. Symptoms are similar to salt damage. (B) Leaf fall shown on entire tree. Trees adjacent to these, but not subject to salt applications, were used for the remainder of the study.

Anatomy and Morphology

Stomatal Characteristics

Stomatal density was assessed to determine if there might be anatomical differences between the different canopy locations. Sections were imprinted on dental sealant (low density polysiloxane) to assess stomatal density and size. Once the dental sealant hardened (2 mins) the molds were stored in envelopes for future analysis, at which time positive impressions were made by coating with clear nail polish and then mounting on slides for viewing, using a compound microscope at 200x magnification (Figure 7). I counted three fields on the abaxial surface for each leaf for stomatal densities using an Olympus IX-81 light microscope (Mickelbart 2019).

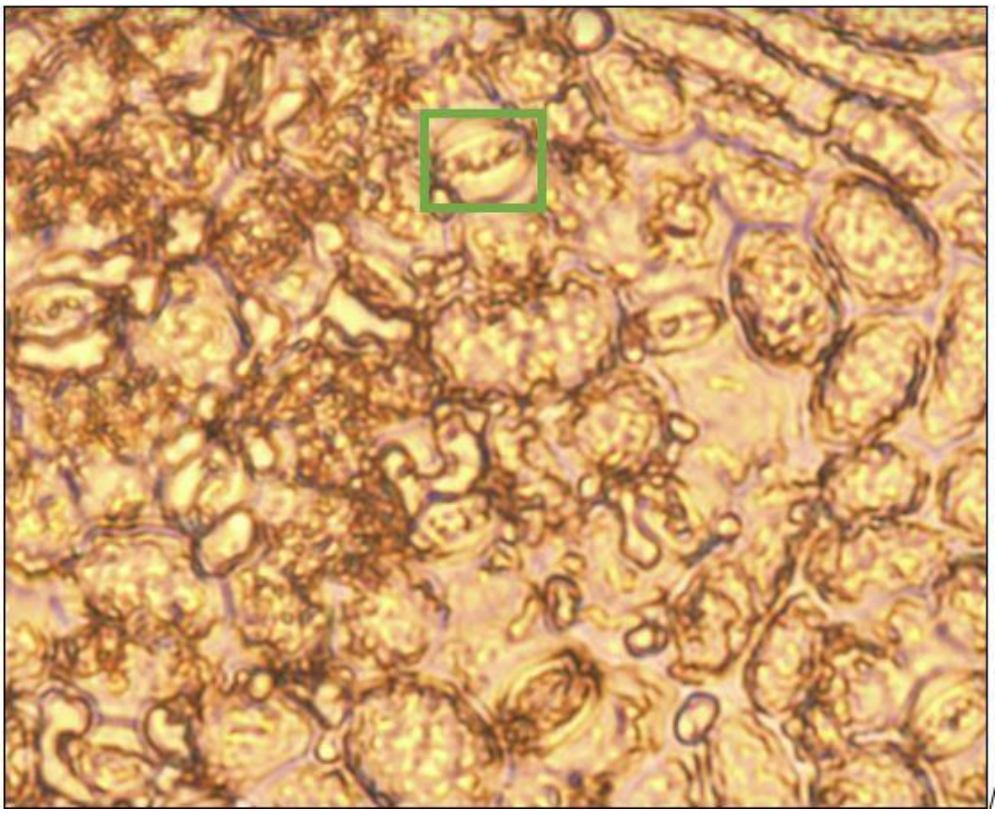

Figure 7. Stomatal impression on the abaxial surface of an upper Freeman maple leaf. Magnified at 200x. A stomate is outlined in green.

Leaf Areas and Weights

I collected intact leaves from the upper and lower canopies of both maple varieties at each sampling time over the season and measured their total area using Blackspot, a shareware program that uses a Canon high resolution scanner (Varma and Osuri 2013). The accuracy of this program is 99% or better, using known standards (Krogmeier 2021). Afterwards, I placed them in a drying oven for at least 24 hours at ~65°C, after which I obtained their dry weight. I calculated LMA as the ratio of leaf dry weight to area (g/cm^2).

Physiological Measurements

Gas Exchange

A Li-Cor 6800 portable gas exchange system (Li-Cor, Inc., Lincoln, NE) outfitted with the 6 cm^2 cuvette and LED lighting, was used at approximately monthly intervals from May to August to measure gas exchange of upper and lower leaves for both maple varieties. I used a bucket truck rented from the ASU Physical Plant, except for a few weeks in mid-summer when the truck malfunctioned and had to be repaired. Measurements were restricted to between the hours of 9 am and 12 pm on days with full or partial sunlight and low wind speeds.

I conducted light response curves in late May/early June on one leaf per canopy location per tree at a height of 8-9 m and another one at 2-3 m. PAR (photosynthetically active radiation) levels used for building the light response curves were applied as follows: 1500, 1250, 900, 600, 450, 250, 100, 50, and 0 μmol m^{-2} s^{-1}. I set the cuvette temperature to the closest temperature to ambient at the time of measurement, relative humidity to 50%, and the cuvette CO_2 to 415 ppm. I gave the leaf time to equilibrate at 1250 μmol m^{-2} s^{-1} before starting the light curve. I conducted light curves on two trees per day, for two days in a row, resulting in four trees total per site. The following week, I repeated this at the other location. From these curves I extracted the following parameters: DR, the rate of respiration in the dark; LCP, the light compensation point, which is the PAR at which A_{net} (net photosynthesis) is zero; AQE, the apparent quantum efficiency, a measure of the linear increase in A_{net} per unit increase in PAR received over the light-limited portion of the curve; LSP, the light saturation point, which is the PAR at which A_{net} reaches its maximum value (A_{max}); and A_{2000} which is A when PAR is 2000 μmol m^{-2} s^{-1}.

Light response curves were fit, where possible, in SigmaPlot v. 14.5 (Systat Software Inc., Chicago, IL) using a three-parameter exponential rise to maximum curve:

$$A_{net} = DR + a(1 - e^{(-b*PAR)}) \quad (1)$$

where:

A_{net} is net photosynthetic rate, DR is the estimated dark respiration at zero PAR, a and b are parameter estimates, and PAR is the light intensity. Some of the curves showed unusual depressions at high PAR and were not able to be modeled using the equation above. For those curves, I extracted A at the PAR where it was maximal, and at maximum PAR (A_{2000}).

On the third day of each week, I conducted rapid A-C_i curves on the same four trees at each of the locations. For these curves, the vapor pressure deficit of the leaf was set to 1.5 kPa, and the light level at 1250 μmol m^{-2} s^{-1}. In between trees 1 and 2 and between trees 3 and 4, I ran the program with an empty cuvette to obtain the leak correction for this protocol. Leaves were equilibrated at saturating light at a CO_2 concentration of 415 ppm. Once stable, the CO_2 was reduced to 10 ppm and when the sample chamber reached that level, I started the automatic A-C_i program. With this technique, the CO_2 concentrations were ramped up at a rate of 100 ppm/min until they reach a maximal concentration of 1010 ppm. Post-measurement analysis consisted of correcting the values for any leaks and then determining the rate of A when internal CO_2 concentration (C_i) was 350 ppm.

In August, once leaves started to change color, I modified the measurements in order to collect gas exchange data more frequently, due to time and budget constraints. I made gas exchange measurements at a PAR of 1250 μmol m^{-2} s^{-1} and in darkness. I continued doing the rapid A-C_i curves as before.

Water Potential Measurements

I measured leaf water potentials (ψ_{leaf}) of upper and lower canopy leaves using a Scholander Pressure Chamber (PMS, Inc., Corvallis, OR) on the same days that I measured gas exchange. I detached the leaves when I was in the bucket, immediately placed them in a plastic bag, removed excess air, and then dropped them down to an assistant who placed them in the pressure chamber. A total of 3-4 leaves from both the upper and lower locations were measured per tree before going to the next sample tree, which minimized the time differences between the measurements for upper and lower leaves.

Leaf Pigment Measurements

Each time I measured gas exchange, I also collected three leaves from each canopy location for each of the five replicate trees per site. I placed these leaves in a cooler and took them back to the lab, where I used a hole puncher to collect three samples (0.28 cm^2 each) from each leaf. Samples were placed directly into plastic vials containing N, N-dimethylformamide (DMF) for chlorophyll extraction and then refrigerated in the dark at 5ºC for at least 48 hours. After extraction, sample absorbances were measured in quartz cuvettes, using a Shimadzu Model 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan). After zeroing at 720 nm, samples were measured at wavelengths of 470, 647, and 664 nm and chlorophyll concentrations were determined using the equations from Porra (2002). Anthocyanins were extracted using a methanol:6 M HCL:water solution (23:7:70 v:v:v) for two days in the refrigerator at 5ºC and absorbances read at 530 and 653 nm. I used equations from Gould et al. (2002) to determine anthocyanin levels.

Leaf Nitrogen Analyses

Six leaves from each tree were collected (three from the upper canopy and three from the lower canopy) once during the middle of the growing season (August 4th for both varieties) and again during late senescence (September 28th for upper FM leaves and October 27th for lower leaves; October 27th for upper and lower AM leaves) to measure their nitrogen content. I also measured leaf areas and weights as described earlier. Senescent leaves were those that easily detached with a slight tug. I dried the leaves to constant weight at 60°C and then sent them for analysis to the nutrient testing lab at the University of Georgia Extension Services. Nitrogen amounts in summer (N_{summer}) and fall (N_{fall}) were expressed as a percent of leaf dry weight, and nitrogen retranslocation efficiency calculated according to Wang et al. (2020) as:

$$Percent\ Retranslocation = \left(1 - \left(\frac{N_{fall}}{N_{summer}}\right) * CF\right) * 100 \quad (2)$$

where *CF* is a correction factor for the change in mass from summer to fall and is equal to LMA_{fall}/LMA_{summer} (Vergutz et al. 2012).

Statistical Analysis

I performed statistical analyses using Microsoft Excel (Microsoft Corporation, Redmond, WA), and SigmaPlot V. 14.5. The experimental design consisted of three main factors: maple variety (FM and AM), date in the season, and canopy location (upper and lower). Canopy locations within a tree were not independent, so I used a repeated measures two-way analysis of variance, which can serve the same purpose as a paired *t*-test for variables that are not independent. Significance for all analyses was assumed at $p \leq 0.05$. When data failed to meet the assumptions for parametric analysis, I used non-parametric alternatives.

Results

Microclimate - Wind Speed

There were no significant differences in temperature or relative humidity between upper and lower locations at either site (data not shown). However, mean wind speeds averaged over 24 hrs at the upper location at both sites were higher by 0.11 ± 0.02 m/s for FM and 0.3 ± 0.05 m/s for AM and this persisted across all the months of the study (Figure 8; July $p < .000$, August $p = .003$, September $p = .001$, and October $p = .035$). Due to a lower weather station malfunction at the AM site, the dataset is not as complete, but for data collected, differences were significant for all months (July $p = .000$, August $p = .001$, and September $p = .011$).

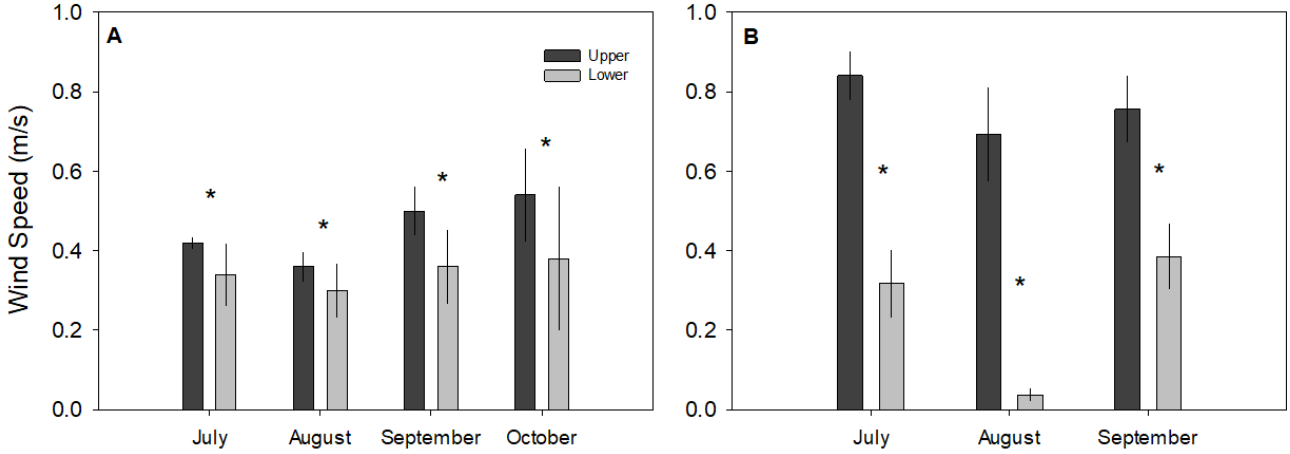

Figure 8. Mean wind speeds at (A) Freeman site (July: n= 29, August: n = 27, September: n = 27, October n: = 20) and (B) Armstrong site (July: n = 16, August: n = 7, September n: = 7). Bars are mean ± se. Asterisks indicate significant differences between upper and lower locations.

Microclimate - Solar Radiation

I compared total solar energy input (W/m^2) per month at each canopy location and found that it averaged 1350 ± 543 W/m^2 higher at the upper than the lower canopy location for FM (Figure 9). I also compared monthly sums for morning (sunlight hours before noon) and afternoon (sunlight hours after noon). At the FM site, solar energy input was higher in the afternoon than morning ($p = .001$) and at both times of the day the upper canopy location ($p =$

.004) received greater amounts. The differences between upper and lower leaves in both the morning and the afternoon were more distinct in July, August, and September, and smaller in October, when day lengths were shorter. Due to a weather station malfunction at the AM site, I was not able to do as full an analysis there, but on three sunny days in mid-summer I found a similar patter with regard to morning vs evening solar energy inputs, but I was not able to reach firm conclusions about whether upper or lower leaves received different inputs (Figure 9).

I also examined the solar energy on an hourly basis over the course of two full days at each site (Figure 10). On occasion, the upper canopy received more sunlight than the lower at the FM site, especially in the morning, while differences were less distinct at the AM site.

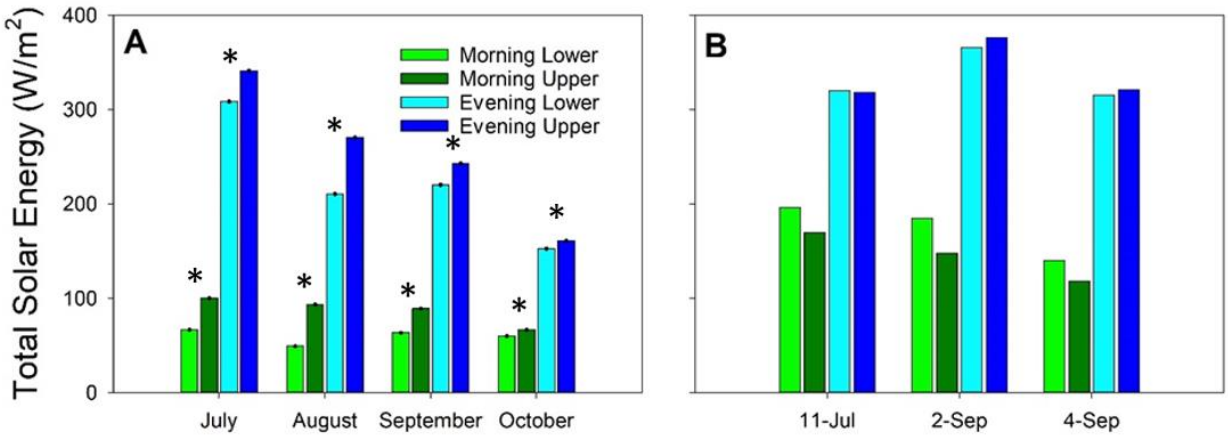

Figure 9. Figure A shows the average total solar energy per month to upper and lower stations at the FM site. Values are the daily sums averaged over the month ± se (July: n = 27, August: n = 27, September: n= 27, October: n = 17). Figure B shows the daily total on three days at the AM site where the lower station had good data, but comparisons should not be made due to a lack of data, i.e., no statistics possible.

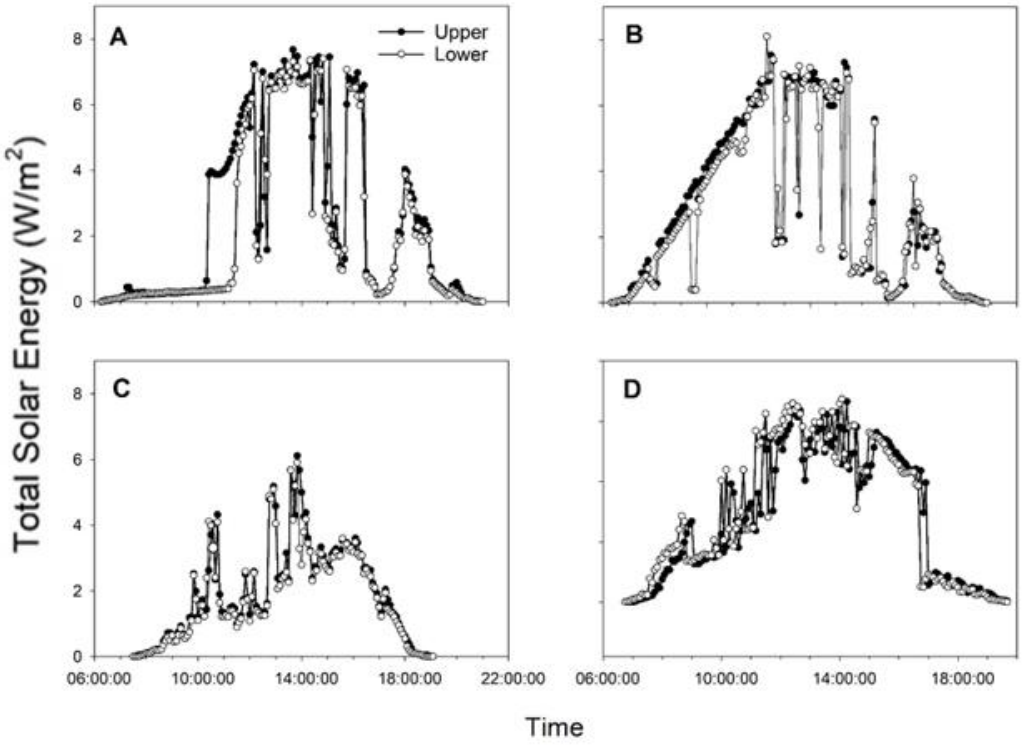

Figure 10. Mean hourly solar energy inputs over the course of a day at the FM site for (A) July 21st (B) October 3rd, and the AM site for (C) July 21st, and (D) for September 4th. Sudden drops result from the sensor being blocked by either clouds or an adjacent tree over the course of the day.

Phenology

The Freeman maples were chosen for study because they have the unusual coloring-up pattern in which they turn red at the top of the tree before the bottom, unlike the Armstrong variety (Figure 1), which turns red nearly simultaneously over the entire canopy. To determine if differences in the timing of reddening were associated with differences in leaf phenology, I followed leaves from budbreak, to leaf out to senescence.

Budbreak and Leaf-out

Buds on FM began expanding in the lower canopy first, around March 13th, but by the end of March (Julian Day ~90), the upper leaves had caught up developmentally to the lower leaves and both groups continued to develop nearly simultaneously (Figure 11). Lower leaves

had significant ($p < 0.001$) but slightly advanced budbreak timing compared to lower leaves for all dates except for days 90 and 105. By April 27th, the lower buds had fully opened and formed leaves, while the upper leaves fully opened a few days later (Figure 11). I do not have similar data for the Armstrong maples because this variety was chosen for study after it had already leafed out.

There was no statistical difference between upper and lower leaves for the date at which they achieved full leaf size (day 116 for lower, day 118 for upper). However, leaf survivorship declined sooner for upper leaves such that leaf lifespans were ~18 days shorter than for lower leaves (Figure 12; $p = 0.007$). FM upper leaves were 50% gone by day 281 and 75% gone by day 288, whereas for the lower leaves, these milestones were achieved by day 300 and day 304, respectively, or 12-19 days later. For AM, leaf survivorship extended to later in the fall than for FM, and, in distinct contrast to FM, upper and lower leaves senesced at nearly the same time. Differences in survivorship between canopy locations, for example, did not become apparent until day 292 ($p < 0.001$; Figure 12). AM upper leaves were 50% gone by day 296 and 75% gone on day 299, and for lower leaves by day 303 and day 305, respectively, about one week later.

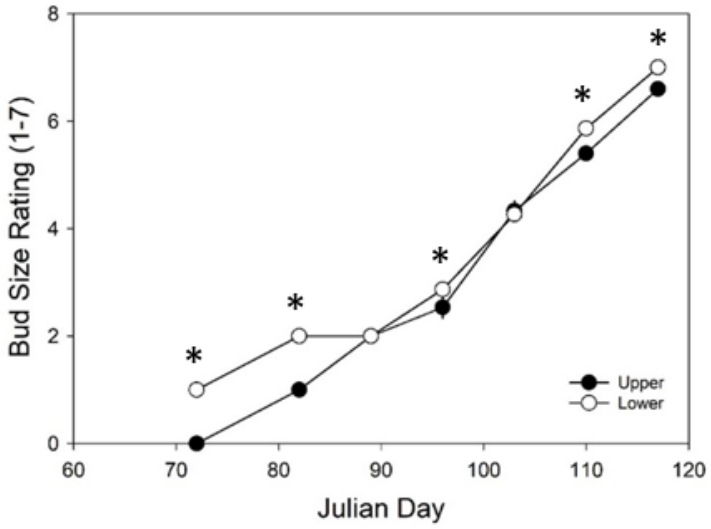

Figure 11. Bud development using a rating scale of 1-7, with 1 = dormant and 7 = leafed out, according to the protocol of Skinner and Parker (1994). Numbers are mean rating per tree ± se, n = 5. Asterisks indicate significantly different values on a particular day. Julian Day 60 is March 1st and Julian Day 120 is May 1st.

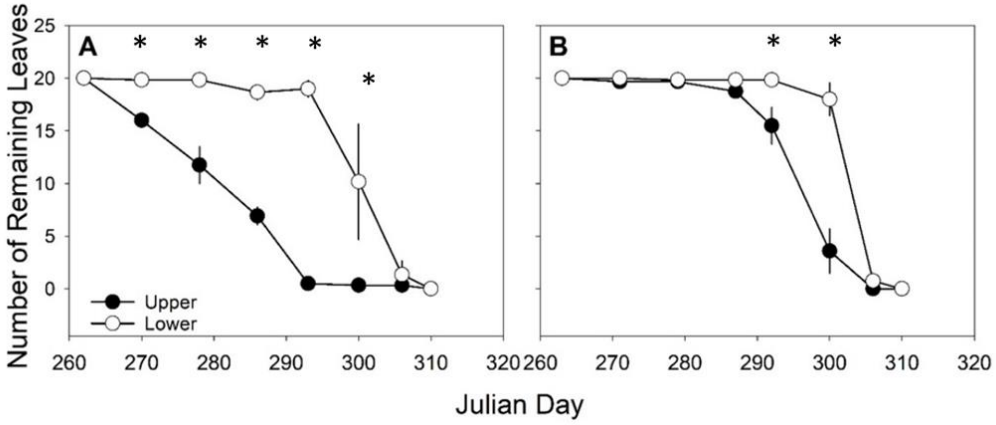

Figure 12. Leaf survivorship for (A) FM and (B) AM. Branches are considered from the terminal leaf pair to the 10th leaf pair. Symbols are mean ± se, n = 4. Julian Day 260 is September 17th and Julian Day 300 is October 27th.

Color Change Phenology

Figure 13 shows the representative color change gradient in Freeman maple leaves. The seasonal progression of color changes in the canopy of FM are shown in the upper part of Figure 14 while the lower panel of pictures shows this for AM.

Figure 13. Representative progressive color changes from green to red in leaves from the upper canopy of FM.

Interestingly, red coloration was first noted in the upper canopy leaves of FM as early as August 4th, before any cold temperatures were noted (e.g., the minimum July temperature was 10.3 ºC). However, in September the minimum temperature dropped as low as 3.8 ºC and in October to 3.1 ºC. In contrast with the upper leaves, lower leaves did not start turning red until about October 8th, over two months later ($p < .001$). Greenness was reduced to 20% ~55 days earlier in upper vs lower leaves (Figure 15), and by October 20th, the majority of all leaves on FM were red.

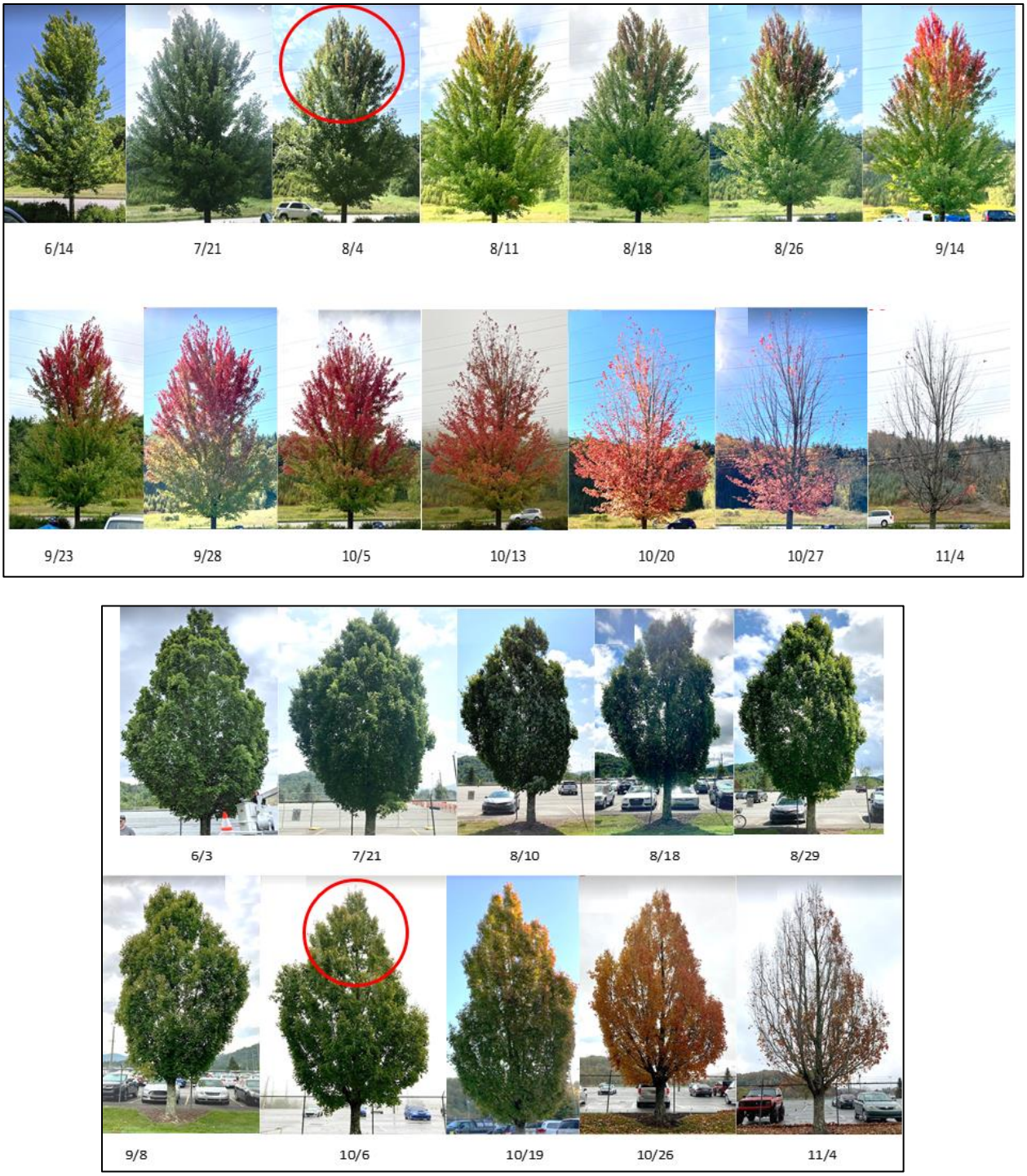

Figure 14. Representative progression of color change and leaf senescence in Freeman maples (top) and Armstrong maples (bottom) over the 2021 growing season. Red circles indicate first signs of red coloration, which was on August 4th for FM and October 16th for AM.

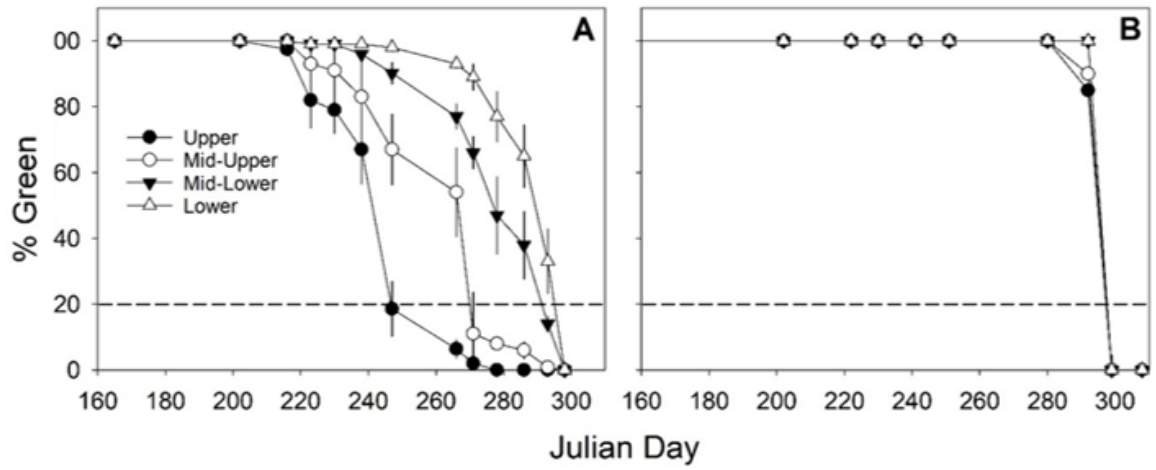

Figure 15. Percent greenness of canopy sectors for (A) FM and (B) AM trees. The dotted lines indicate when 20% of a sector still appeared green. Julian Day 140 is May 20th and Julian Day 300 is October 27th. Symbols are mean ± se, n = 5.

Color change occurred much later in the season for AM and was not visually apparent until October 6th when a few leaves at the top appeared red, but no substantial coloration was noted until October 19th, ~64 days later than for FM (Figures 14, 15). Upper leaves started to show declines in greenness by October 19th, but within just one week the lower leaves had caught up and very shortly thereafter all the leaves began to change color. By November 4th all of the leaves had senesced and fallen off, and statistically, there was no significant difference due to canopy location (p = 0.426).

Anatomy/Morphology

Stomatal Densities

There were no statistical differences in stomatal density between upper and lower leaves within either maple variety (FM, p = 0.106; AM, p = 0.241; Figure 16). However, when the densities for just the upper leaves were compared across varieties, they were substantially higher in FM (p = .021), but not different for lower leaves (p = .123).

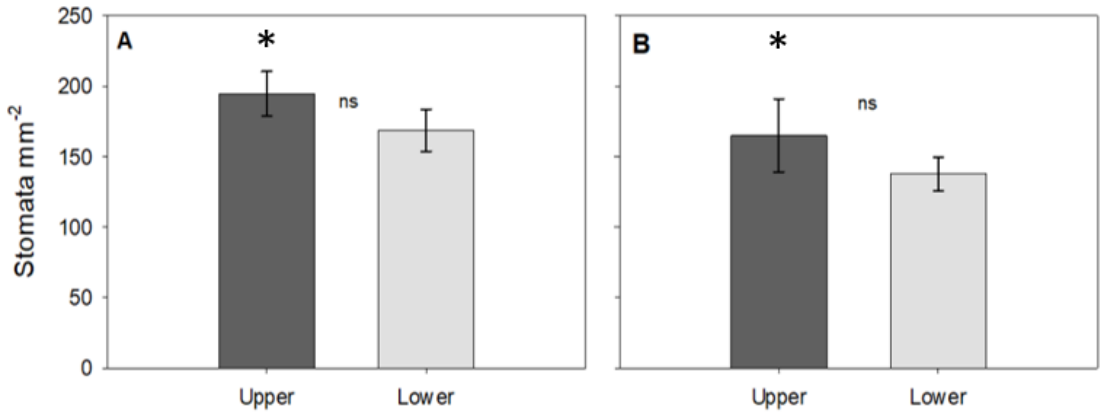

Figure 16. Stomatal densities for the abaxial surface of leaves from upper and lower canopies in (A) FM and (B) AM. Numbers are mean ± se, n = 5. ns means "not significant". Bars within a leaf class (upper or lower) with asterisk indicate difference across varieties at $p \leq .05$.

Leaf Area

Lower leaves of FM reached their full size around day 211, whereas upper leaves required an additional 19 days, as determined from fitting the Gompertz function to the data (Figure 17). There was no significant difference ($p = 0.561$) in the final leaf size between canopy locations although there were occasional differences during leaf expansion when upper and lower leaf sizes did differ on certain days 127 ($p = 0.043$) and 280 ($p = 0.009$). The difference on day 280 was most likely the result of random sampling error, while on day 127 it probably stemmed from the fact that lower leaves opened up and expanded first.

There were no significant time trends for leaf area for AM, most likely because we did not sample them early enough in the season (see Methods section for details), nonetheless, leaf development occurred earlier in this variety because leaves were essentially full size by the first sampling date (Julian Day 151 vs 219 and 230 for lower and upper FM leaves, respectively). However, in contrast to the pattern for FM, where upper and lower leaves were the same size, in AM, upper leaves were 37% smaller than lower leaves ($p = 0.005$).

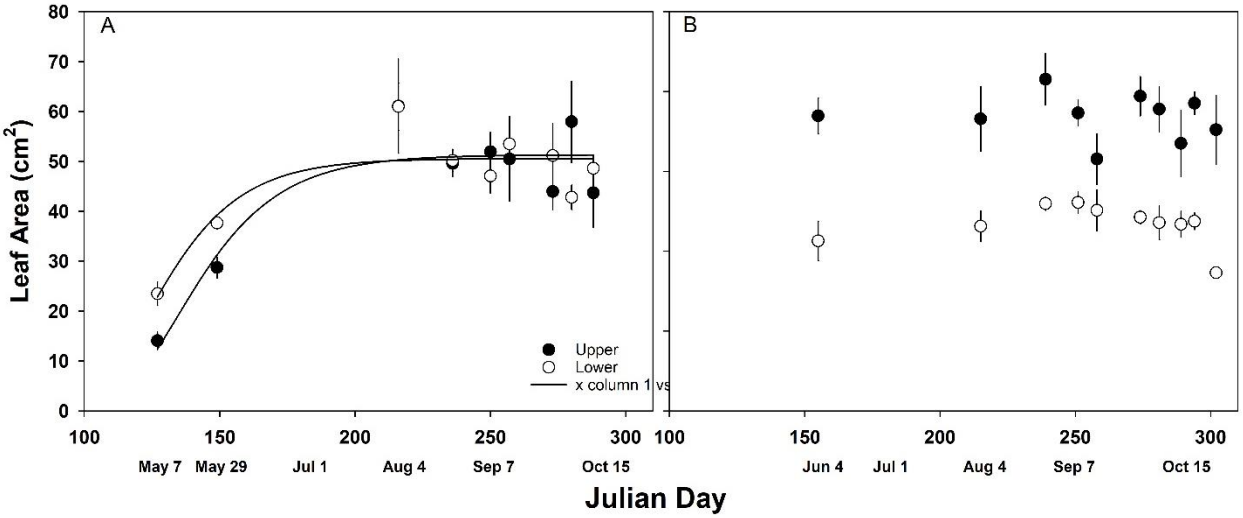

Figure 17. Development of leaf area over the season for (A) FM and (B) AM trees. Symbols are mean ± se, n=4-5. Julian Day 100 is April 10th and Julian Day 300 is October 27th.

Leaf Mass per Area

Leaf mass per area (LMA) was significantly higher in the upper leaves of both varieties ($p < .001$; Figure 18). Across varieties, LMA was higher for FM than AM for the upper ($p < .001$) but not the lower leaves.

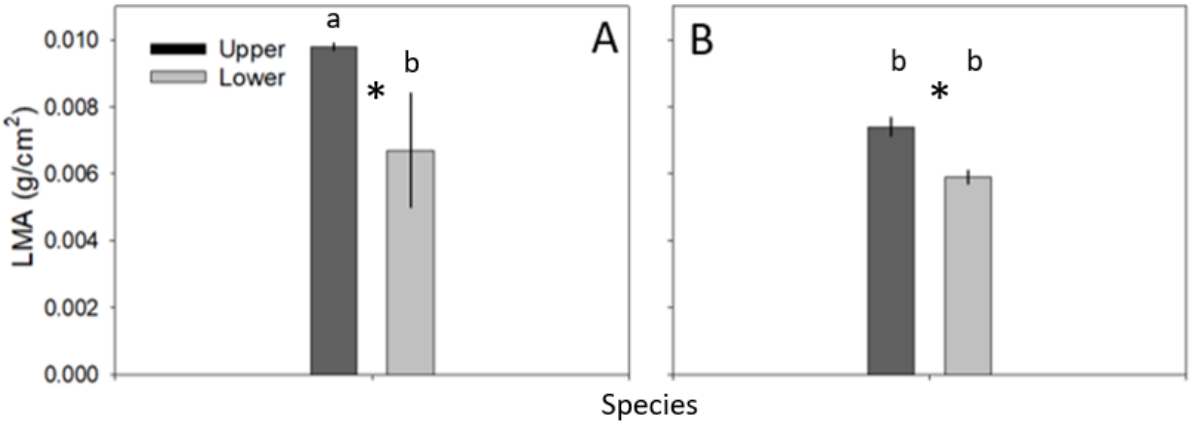

Figure 18. Leaf mass per area for (A) FM and (B) AM trees sampled on Aug 4th. Numbers are mean ± se, n = 5. Asterisks indicates significance between canopy locations within a variety while lowercase letters indicate significant differences between locations across varieties.

Pigment Contents Throughout Growing Season

Chlorophyll

Total chlorophyll amounts of FM leaves varied throughout the season for both canopy locations (Figure 19). In May, upper leaves averaged 5.05 ± 0.12 μg/cm^2, which was similar to that for lower leaves (4.7 ± 0.24 μg/cm^2), but for the rest of the measurements, chlorophyll amounts were significantly higher in the lower leaves ($p < 0.002$). Chlorophyll amounts peaked in August with the lower leaves averaging 8.4 ± 0.4 μg/cm^2 and upper leaves averaging 6.5 ± 0.49 μg/cm^2, after which they started to decline. By mid-October, chlorophyll amounts in upper leaves had decreased substantially (1.0 ± 0.37 μg/cm^2) while in lower leaves they remained much higher (4.8 ± 0.76 μg/cm^2; Figure 19).

Total chlorophyll amounts in AM leaves showed no significant difference ($p = 0.090$) between canopy locations throughout the season, but there was a significant time effect ($p = 0.001$). Chlorophyll peaked in August with upper leaves averaging of 8.8 ± 0.4 μg/cm^2 while in lower leaves it averaged 9.7 ± 0.4 μg/cm^2. Chlorophyll amounts in upper leaves started to decline in mid-October, whereas lower leaves showed little change until the end of October (Figure 19).

Anthocyanins

Anthocyanins appeared earliest ($p <0.001$) and increased to a greater extent ($p= 0.008$), in upper leaves of FM, compared to lower leaves. This difference persisted until the leaves fell off in mid/late October, when even by the end of their lifespan, lower leaves never accumulated as many anthocyanins as upper leaves (Figure 19). When anthocyanin absorbances were recalculated on a mass basis, to account for the differences in LMA between upper and lower leaves in FM, the results were qualitatively similar, suggesting that it was cellular concentrations

of anthocyanins that were responsible for difference and not just due to greater cell numbers per unit area.

Anthocyanins did not appear until mid/late October in AM leaves, and even then, the intensity of color was much less than in FM, and the color was different also, ranging from a rust-red to orange. There was no significant difference in anthocyanin content between canopy locations ($p = 0.887$), but it did change significantly over time ($p < 0.001$; Figure 19).

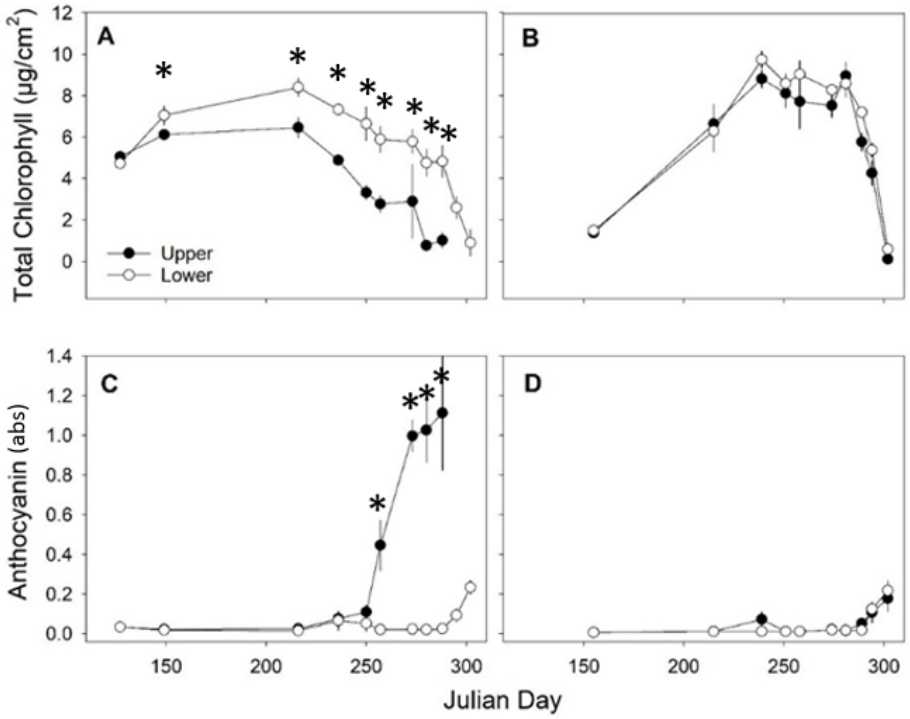

Figure 19. Total chlorophyll in (A) FM and (B) AM leaves over the 2021 growing season, and anthocyanin absorbance for (C) FM and (D) AM leaves. Symbols are mean ± se, n = 4. Julian Day 150 is May 30th and Julian Day 300 is November 14th. Asterisks indicate significant differences between canopy locations.

Gas Exchange

Light Response Curves

Light response curves were constructed for each species in late May and early June (Figure 20, Table 1). For the FM, there was no significant difference between upper and lower

leaves for any parameter except LSP, which was higher in the upper leaves (p = .020), and A_{2000}, which was also greater in the upper leaves (p = .029). For AM, dark respiration rates were significantly lower in upper leaves (p = 0.001).

In the FM, the upper leaves never achieved light saturation, while lower leaves appeared more sensitive and saturated at a PAR of 900 µmol m^{-2} s^{-1}. Additionally, lower leaves appeared to be inhibited when PAR exceeded this level (Figure 20A), and this was why there were differences in LSP and A_{2000} as noted above.

Leaves for AM were similarly inhibited at high PAR, but in this case, rates peaked at a lower PAR of 500 µmol m^{-2} s^{-1} and both upper and lower leaves showed similar patterns in this regard (Figure 20B). Across varieties, there were no differences in any of the parameters except for the LSP of upper leaves, which was higher in FM than AM (Table 1).

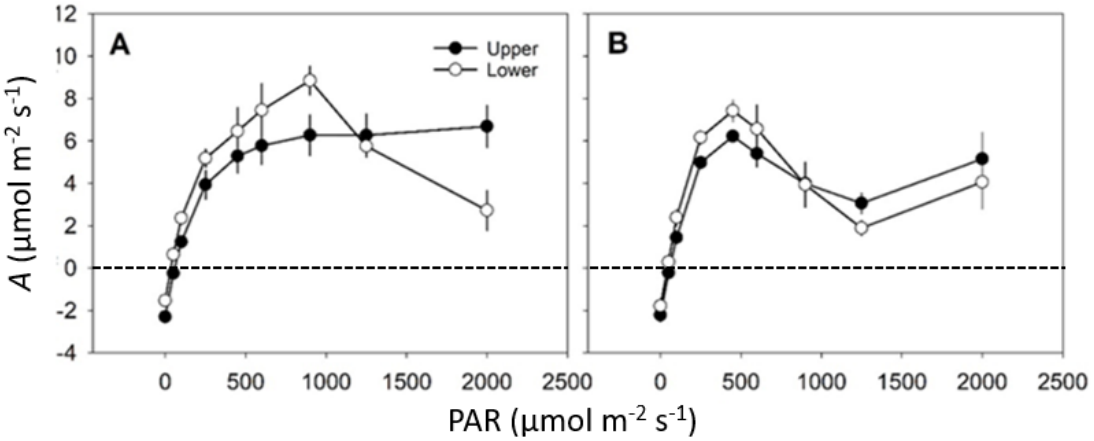

Figure 20. Light response curves for (A) FM and (B) AM. Measurements made in late May/early June. Symbols are means ± se; n=4. Dashed line shows light compensation point when A = 0. Line for upper FM leaves fitted using an exponential rise to maximum equation (see equation 1 in text) while all the others are simply point fits.

Table 1. Light response parameters for FM on May 27th and for AM on June 2nd. DR is dark respiration rate, LCP is light compensation point, AQE is apparent quantum efficiency, A_{max} is photosynthesis at saturating PAR, LSP is the PAR where A_{max} is achieved, and A_{2000} is photosynthesis when PAR = 2000 µmol m^{-2} s^{-1}. Bolded p values indicate differences between upper and lower leaves within a maple variety. The italicized values for LSP indicate a difference between upper leaves across the maple varieties. Symbols are mean ± se, n = 3 in FM and 4 in AM.

Parameters	Freeman Upper	Freeman Lower	*p* value	Armstrong Upper	Armstrong Lower	*p* value
DR ($\mu mol\ m^{-2}\ s^{-1}$)	-2.3 ± 0.05	-1.5 ± 0.26	*p* = .166	**-2.2 ± 0.32**	**-1.8 ± 0.34**	**p = 0.001**
LCP ($\mu mol\ m^{-2}\ s^{-1}$)	63 ± 4	37 ± 5	*p* = .088	59 ± 7	43 ± 8	p = 0.125
AQE ($\mu mol\ CO_2$/ μmol photons)	0.035 ± 0.003	0.039 ± 0.001	*p* = .500	0.037 ± 0.001	0.042 ± 0.001	*p* = .062
A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	6.8 ± 0.92	8.9 ± 0.67	*p* = .669	7.0 ± 0.61	7.5 ± 0.51	*p* = .601
LSP ($\mu mol\ m^{-2}\ s^{-1}$)	***1750 ± 217***	**817 ± 188**	**p = .020**	*838 ± 388*	838 ± 388	*p* = 1.00
A_{2000} ($\mu mol\ m^{-2}\ s^{-1}$)	**6.68 ± 1.14**	**2.72 ± 1.09**	**p = .029**	5.15 ± 1.25	4.05 ± 1.25	P = .071

Water Potentials

Water potentials measurements were taken each time gas exchange measurements were made to rule out water stress as a potential cause for differences in gas exchange between canopy locations (Figure 21). Since the upper leaves were on average 6 m higher up the tree than the lower leaves, the gravitational component of water potential would decrease values in the upper canopy by ~0.06 MPa. Thus, only differences greater than this would indicate additional water stress imposed by physiological processes in upper leaves. Over the growing season there were no instances where water potentials in FM differed significantly between upper and lower leaves.

However, for AM, there were two instances where upper leaves had significantly lower values than upper leaves (days 251 and 272, *p* = .019 and *p* = .029, respectively). In each case, upper leaves had water potentials that averaged 0.09 MPa more negative than lower leaves (Figure 21).

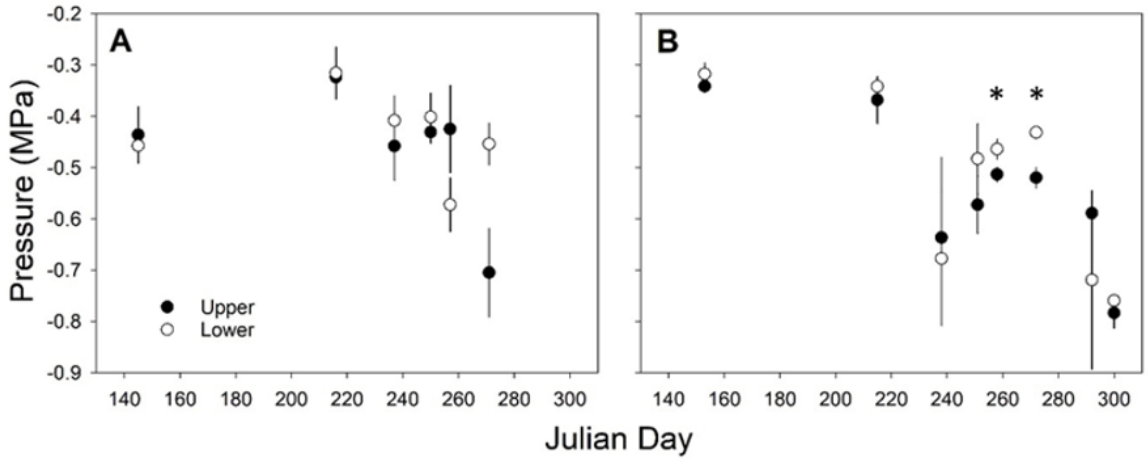

Figure 21. Water potential readings on (A) FM and (B) AM over the season. Symbols are mean ± se, n=4. Julian day 120 is April 30th and 300 is October 27th. Asterisks show significant differences between canopy locations at $p \leq .05$.

Gas Exchange Measurements

Rates of photosynthesis (A) at a standard PAR of 1250 µmol m^{-2} s^{-1} for FM varied significantly between upper and lower leaves during all measurement days except for August 4th when they were similar ($p = .909$). For all other measurements, rates were significantly higher in the lower leaves ($p < 0.001$). Over time, rates decreased significantly ($p < .001$) in both upper and lower leaves, although upper leaves decreased beginning in August on Day 216, and then rates remained nearly constant until they senesced, whereas upper leaves started declining much later after day 251 (Figure 22).

In AM, there was no significant difference in photosynthetic rates between locations, but there was a significant trend over time ($p < 0.001$; Figure 22). As anthocyanin levels increased and chlorophyll decreased, photosynthetic rates decreased, although the decreases were not notable until around day 270, when they dropped off sharply. Dark respiration rates did not differ between canopy locations for either FM or AM, nor with time except for AM, where rates on Day 300 were significantly higher than several earlier dates (data not shown).

Stomatal Conductance

Stomatal conductance (g_s) did not differ between canopy locations ($p = 0.110$) or with time ($p = 0.578$) for FM (Figure 22), although there was a tendency for g_s to peak in mid-summer. For AM, g_s did not differ statistically between canopy locations ($p = 0.318$), but did over time ($p < 0.001$). As chlorophyll was depleted and leaves began to senesce, g_s declined.

Water Use Efficiency

Water use efficiency (WUE), defined as the ratio of A/g_s, was significantly higher in lower than upper leaves for FM in early August, ($p < 0.001$), but not for any other times during the season (Figure 22). WUE of lower leaves steadily declined over time, due mainly to concomitant decreases in A, while for upper leaves it tended to peak in mid-summer, and followed patterns for g_s more so than for A, even though statistically, canopy location was not significant. For AM, there was no canopy location difference in WUE, and WUE decreased later in the season, again following declines in both A and g_s. Early in the season up to late summer (around Day 280), WUE was slightly higher in AM than FM leaves, more as a result of higher rates of A than from differences in g_s.

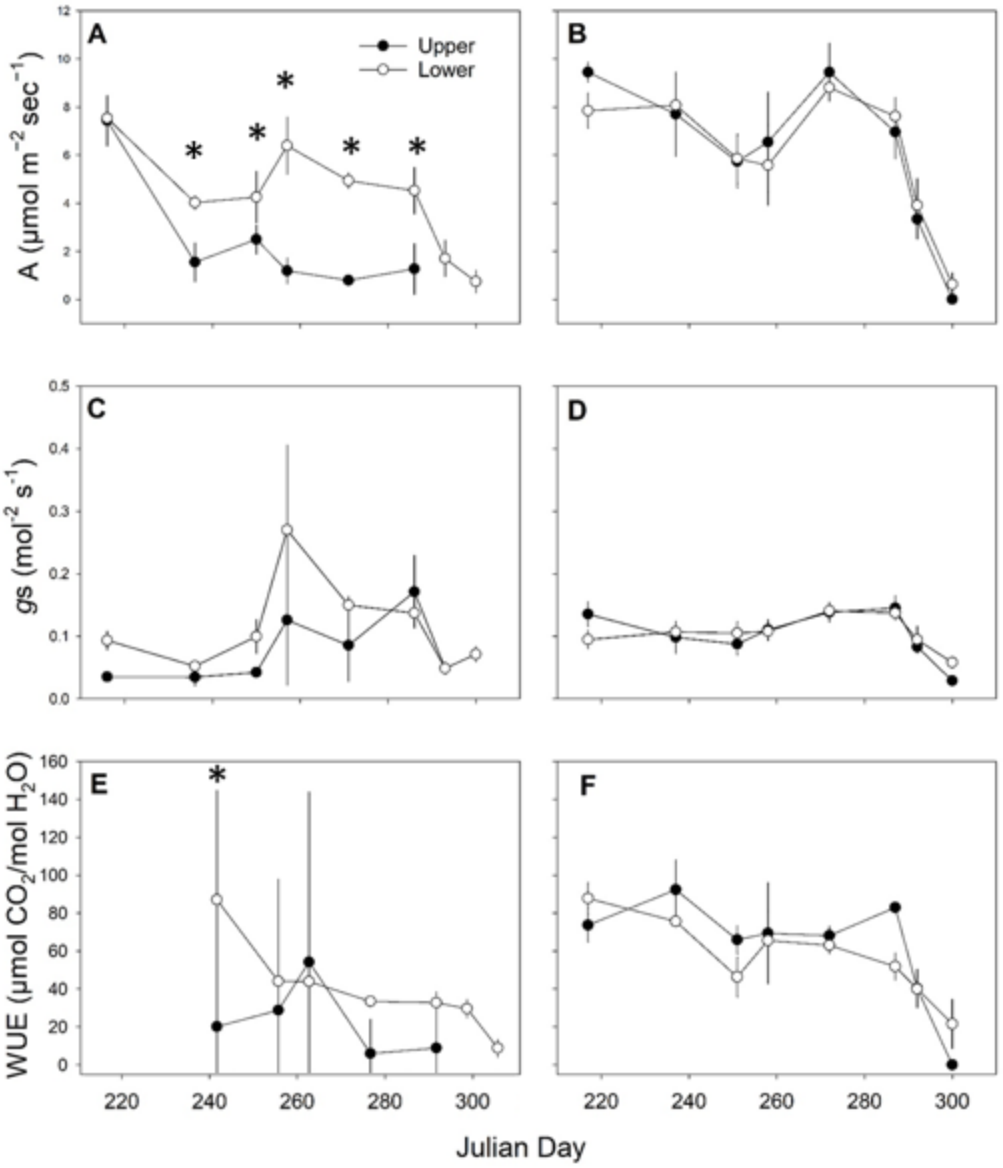

Figure 22. Gas exchange parameters at a PAR = 1250 µmol m^{-2} s^{-1} for FM (panels A, C, E) and for AM (panels B, D, F). Julian Day 220 is August 8th and Julian Day 300 is October 27th. Symbols are mean ± se, n = 4. Asterisks indicate significant differences between canopy locations within a variety. See text for details about cuvette conditions. All measurements completed prior to 1 pm on all days.

Rapid A-C_i *Curves*

I compared rates of photosynthesis at a standard internal CO_2 (C_i) of 350 ppm, which corresponded to an ambient CO_2 (C_a) of approximately 560 ppm. Neither variety had a significant difference in *A* between the upper and lower leaves (p = .407 for FM, p = .124 for

AM; Figure 23). However, there was a significant decrease from June to August and through to September in FM (p = .025). There were no significant differences between any of the dates in AM (p = .311) and this resulted in a significant difference between the varieties for measurements in August, but not in June or September (p = .001; Figure 23).

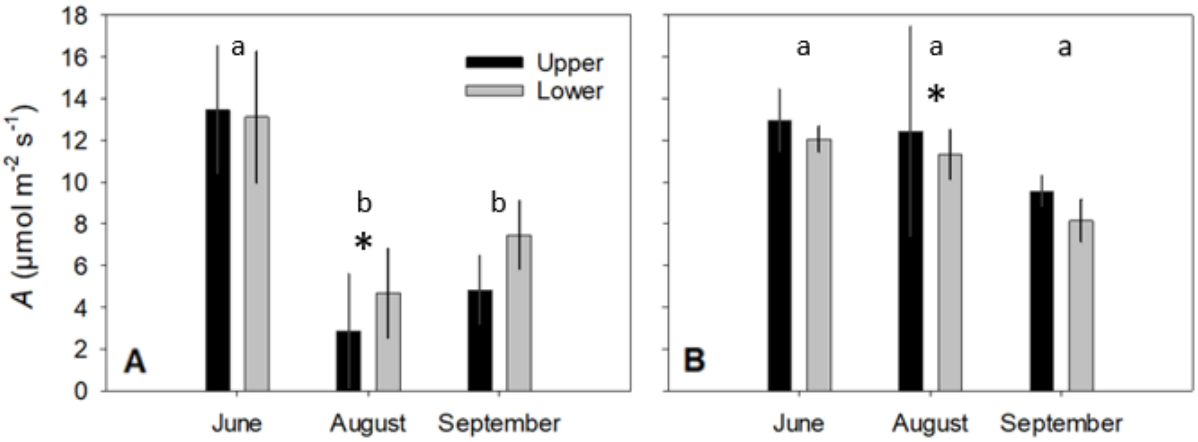

Figure 23. Photosynthetic rates at a C_i = 350 ppm CO_2 for (A) FM (B) AM throughout the 2021 growing season. PAR was 1250 µmol m^{-2} s^{-1} and temperatures were set closest to ambient for each day. Measurements completed prior to 1 pm on all days. There were no significant differences for canopy location for either variety, and therefore, months within a variety not sharing the same letter are significantly different. Asterisks indicate significant difference in August means (averaged over both upper and lower locations) across the two varieties. In all cases significance assumed if $p \leq .05$.

Nitrogen Levels

Nitrogen contents for lower leaves of FM, collected on August 4th, were significantly higher than in upper leaves (1.6 ± 0.21 % vs 0.9 ± 0.13 %; p = .008). By the fall, as leaves were nearing full senescence, contents were much lower (0.3 ± 0.01% vs 0.4 ± .01%; p = .002). In contrast to FM, there was no difference in nitrogen contents between upper and lower leaves for AM in either summer or fall (p = .981 and p = .951, respectively; Table 2). Retranslocation efficiencies did not differ between upper and lower leaves for either maple variety, but were higher in AM than FM trees (Table 2).

Table 2. Nitrogen contents for leaves collected August 4th and in fall, right before they dropped (September 28th for upper FM leaves, October 27th for lower FM leaves and upper and lower AM leaves). See text for how retranslocation efficiency is calculated. Values are mean ± se, n = 4. Significance assumed when $p \leq .05$ and indicated by bolding. RE = Retranslocation Efficiency.

	FM			AM		
Location	**Summer (N %)**	**Fall (N %)**	**RE (%)**	**Summer (N %)**	**Fall N %)**	**RE (%)**
Upper	0.9 ± 0.13	0.3 ± 0.00	53.7 ± 6.66	2.1 ± 0.12	0.5 ± 0.02	67.9 ± 2.71
Lower	1.6 ± 0.21	0.4 ± 0.01	50.4 ± 3.92	2.1 ± 0.23	0.5 ± 0.01	60.5 ± 2.34
***p* value**	**.008**	**.002**	.660	.981	.951	.656

Discussion

In my study of two urban red maple varieties, I found differences in the physiology and morphology of leaves between the upper and lower canopy locations, as well as between varieties. The most prominent canopy location differences occurred in the leaves of FM and included phenology, LMA, gas exchange rates, pigmentation timing and amounts, and nitrogen content. In contrast, location differences were restricted to just leaf size and LMA for AM with none for gas exchange, pigmentation or nitrogen content. Given that both varieties were exposed to similar weather and growing conditions, this suggests that differences between upper and lower leaves are physiologically based and varietal specific.

The Influence of Microclimate on Leaf Morphology and Lifespan

The microclimate data, obtained from the weather stations positioned at the heights of the upper and lower canopies, showed no significant differences at either site between locations for temperature or VPD. However, both sites were windier and had higher solar radiation inputs at the upper canopy location, and both parameters are known to affect leaf growth, morphology, and physiology in a large number of species (Boardman 1977, Grace and Russell 1977, Niklas 1992, Niklas 1996).

The Role of Wind

Higher wind speeds in the upper canopy result in smaller boundary layers at the leaf and tree canopy levels (Jarvis and McNaughton 1986), which in turn enhance evapotranspiration (Campbell and Norman 1998, Daudet et al., 1999). Furthermore, high wind speeds can cause mechanical damage due to flapping or the abrasion of leaves against one another or with branches (Van Gardingen and Grace 1991, Telewski 1995), although it must be said that I observed little or no visible damage in either variety. Abrasion of the cuticle, which would not be visible to the eye, would enhance non-stomatal water loss (Grace 1988, Van Gardingen et al. 1991, Bueno et al. 2022), while flapping and twisting of the petiole could cause mechanical disruption to the vascular connections (Grace 1988), which in turn would decrease water transport and increase water stress (Sack and Holbrook 2006). Height by itself, because of the gravitational component of water potential, would also cause greater tension in the xylem and lead to smaller, more dense leaves (Marshall and Monserud 2003). The resultant stress would reduce turgor pressures and limit the capacity for cellular expansion, resulting in smaller, denser leaves, with a concomitant higher LMA (Grace and Russell 1982). The fact that upper leaves of both varieties were smaller and had higher LMA is consistent with this possibility, although I did not observe significant differences in water stress between upper and lower leaves, nor did I specifically investigate damage to the conducting system in petioles.

On the other hand, leaves in some species may adjust their morphology and physiology in such ways that the effects of wind are minimized. For example, in *Berberis microphylla*, a shrub that grows in arid climates subject to constant winds, leaves on the windward side of the crown reduce water loss by closing their stomata, which reduces transpirational water losses, but they also produce a thicker cuticle, which lowers non-stomatal water losses. In contrast, the

conspecific shrub *Colliguaja integerrima* adopts a different strategy to minimize stress on the windward side of its crown by adjusting its hydraulic conductivity upward to cope with greater stomatal water losses. In each case, the physiological and morphological adjustments minimize differences in water stress between the windward and leeward sides of the crown (Iogna et al. 2013). However, such compensatory measures may not have occurred for my trees. For example, neither variety showed differences in stomatal density between upper and lower leaves, and there were few significant differences in water potential.

Leaves lower down in the canopy would experience reduced wind speeds and have lower evapotranspiration rates, which when coupled with lower amounts of mechanical injury, could result in a prolonged lifespan (Wu et al. 2021). However, in AM, the difference in the timing of leaf senescence between the upper and lower leaves was only about just two weeks, whereas it was nearly two months for FM. This suggests that wind may not be the factor determining the earlier leaf senescence in upper FM leaves; that the sensitivity of upper leaves to wind is greater in FM than AM; or finally, that there are inherent physiological differences between upper and lower leaves in FM but not AM. Experiments where trees are subject to controlled wind studies would be needed to determine which of the above hypotheses are correct.

The Role of Light

The other environmental parameter that differed significantly between the upper and lower canopy was solar radiation input. At the FM site, light levels were consistently higher in the upper than lower canopy, and radiation inputs were greater in the afternoon hours than morning hours. As the season progressed, and the days became shorter, total radiation inputs decreased and the difference between upper and lower canopy locations became smaller. On an hourly basis, I did not see major differences between the upper and lower canopies, but when

they did differ, it was usually in the direction of the lower canopy receiving less radiation than the upper canopy (Figure 10). Some of this difference may have resulted from shading of the lower sensor by nearby trees due to the position of the sun, but the duration of this shading would have been small compared to the length of time the sun was above the horizon. Furthermore, total daily differences were relatively small compared to the daily total input, but were consistent with the known correlation of high light with high LMA (Boardman 1977, Neufeld and Young 2014), as was found in upper leaves of both varieties. High light (Salisbury 1927, Iogna et al. 2013, Lehmann and Or 2015, Papanatsiou et al. 2017), and sometimes low light (Lei 1998) can induce greater stomatal densities, but neither species showed a difference between their upper and lower leaves, which would suggest a minimal role for light in this regard.

The most important determinant of leaf size in red maples is whether the leaves are positioned on long or short shoots, with larger leaves on the long shoots (Niklas and Cobb 2010). However, I restricted my sampling to only leaves on short shoots, at the exterior of the south-facing crown, so differences in light would be even less of a factor. That some parameters changed in FM, but not AM, suggests that the physiology of each variety plays a large role in determining these patterns.

Role of Light in Anthocyanin Production in the Fall

It is thought that leaves synthesize anthocyanins in the fall to protect their photosynthetic apparatus from the combination of high light and cooler temperatures (Hoch et al. 2001, Feild et al. 2001, Lee and Gould 2007, Hughes et al. 2007, Hughes et al. 2022). If the upper canopy leaves are receiving more sunlight than lower canopy leaves, they may be producing anthocyanins sooner to protect against this stress. However, it is unclear what the proximate cue

is for the genes to turn on that result in anthocyanin production, whether it be photoperiod, minimum temperatures, low nitrogen (Carpenter et al. 2014), or a combination of all of these (Chalker-Scott 1999, Naing and Kim 2021). But it is known that various environmental stresses cause the accumulation of reactive oxygen species (ROS), and that these can serve as signaling molecules to upregulate genes in the anthocyanin pathway (Naing and Kim 2021). If this results from the combination of high light and cold, anthocyanins may benefit the leaf by reducing photoinhibition (Hughes et al. 2005, 2007), and they may act as ROS scavengers to further reduce cellular damage (Gould et al. 2002, Hughes et al. 2005, Landi et al. 2014, Xu et al. 2017), often resulting in preservation of higher photosynthetic rates (Zhu et al. 2018), and allowing a longer time to retranslocate nutrients back into the stems from the leaves (Field et al. 2001, Hoch et al. 2001, Hoch et al. 2003).

There is further evidence that differences in radiation inputs between the upper and lower canopies are not the major contributor to the differences in the timing of anthocyanin production, at least in FM. The FM were oriented singly in an E-W line adjacent to a road, and this alignment would mean that the daily movement of the sun through the sky would result in only short periods of self-shading near midday. For other portions of the day, their crowns would be fully and equally exposed from top to bottom, at least within a similar compass sector. For AM, where they were aligned along a NE-SW line, self-shading would be limited to late in the day, at which time the trees would not be as physiologically active. In both cases, self-shading events for either maple variety would be of short duration, and occur at times in the day when temperatures are higher or near their peak. Thus, both upper and lower leaves would be fully exposed to high light early in the morning when temperatures are the lowest, and hence

differential light interception is unlikely to be the sole cause of any physiological differences between upper and lower leaves.

Temperature by itself does not seem to be the proximate cue for early anthocyanin production in the upper leaves of FM, since minimum temperatures at night were above 10ºC, and much higher during the day, when reddening was first noticed in early August. I cannot rule out sensitivity to the shortening of the photoperiod, even in early August, and genes in the anthocyanin pathway can be upregulated by short photoperiods (Gu et al. 2018, Seaton et al. 2018). Their function under short photoperiods is not fully understood, but may involve detoxification of ROS, which in some cases can become more prevalent under short days (Pétriacq et al. 2017).

Instead, these trees may be exhibiting a '*bet-hedging*' strategy (Wallace and Dunn 1980), whereby they synthesize anthocyanins early in the season to avoid damage from any unusual cold events that might occur in late summer or early fall. This also co-occurs with the decline in chlorophyll, which may make these leaves more sensitive to photo-oxidative stress. As Anderson and Ryser (2015) point out, solar radiation can be intense in late summer, whereas later in the season, when temperatures start to drop, solar radiation levels decrease due to changes in the sun's position in the sky and from increased cloudiness, which scatters and diminishes wavelengths that anthocyanins absorb, thereby decreasing their effectiveness at preventing photo-oxidative damage to leaves.

Upper and lower leaves, however, would be exposed to nearly the same amount of direct radiation, if located in the same compass sector of the crown, since the difference in timing between when the upper and lower canopies would receive direct radiation as the sun comes over the horizon (a difference in height of ~6 m) would be less than a second (The Curious Team

2015). If differences in radiation are not the reason for the earlier and greater accumulation of anthocyanins in upper leaves, then other factors must be at play, and the most likely would be the differences in LMA and nitrogen amounts. Hughes et al. (2007) found that young leaves of several tree species produced anthocyanins in the spring when there were fewer airspaces between cells, and the photosynthetic apparatus was still developing. These conditions resulted in limited CO_2 diffusion within the leaf, and greater susceptibility to photoinhibition as a result. It is possible that upper leaves of FM, which have a higher LMA, also have a reduced capacity for internal CO_2 diffusion that could result in greater susceptibility to photoinhibition compared to lower leaves, thus providing them with an impetus to produce anthocyanins both earlier and to a greater extent. It is known that trees in eastern North America experience high radiation and large temperature fluctuations in the fall (Anderson and Ryser 2015, Renner and Zohner 2019) which would support this hypothesis. Coupled with this possibility is that the lower nitrogen amounts in upper FM leaves, which were detectable in early August, also probably stimulate anthocyanin accumulation (Schaberg et al. 2003, Carpenter et al. 2014, Liang and He 2018).

If upper leaves of FM are more sensitive than lower leaves to high light-low temperature conditions, then waiting until temperatures drop in the fall to begin producing anthocyanins would be too late to prevent leaf damage, and this would favor the '*bet-hedging*' strategy alluded to above (Wallace and Dunn 1980). It also suggests that protecting leaves to retranslocate nutrients has priority over carbon uptake, or otherwise they would delay anthocyanin production.

Although upper leaves had lower leaf N, this does not support the hypothesis that low N leaves have a higher requirement for retranslocation (Nasholm et al. 1998; Keskitalo et al. 2005), as there were no differences in either maple variety with regard to the retranslocation efficiency between upper and lower leaves. This is consistent with the findings of Pena-Novas and Archetti

(2021) who did not find differences in retranslocation efficiency between trees that turn red and those that do not. They hypothesized that if anthocyanins are produced to enhance retranslocation under nutrient stress, then those that have lower foliar nitrogen should produce anthocyanins and those with higher leaf nitrogen less so. In a study of trees growing in a botanical garden, they found no difference in retranslocation efficiencies between trees that turn red and those that turn yellow (Pena-Novas and Archetti 2021). However, these findings have recently been challenged by Hughes et al. (2022), who argue that earlier synthesis of anthocyanins is linked to low leaf nitrogen and accumulation of high amounts of total non-structural carbohydrates (TNCs).

Other tree species exhibit similar patterns of early senescence and greater anthocyanin production in their upper canopy leaves, such as pin oak (*Quercus prinus, personal observations*) and sugar maple in North America (Moy et al. 2015) and several tree species in Japan (Koike et al. 2001, Schmitzer et al. 2009), including ash (*Fraxinus mandshurica* var. *japonica*), basswood (*Tilia japonica*) and several Japanese maples (*A. mono and A. palmatum*). This suggests that there may be generalizable physiological differences between leaves in the upper and lower canopy that which result in their earlier senescence. Koike et al. (1990) reported that late successional trees that turn red do so first from the outside of the canopy and that this is a function of differences in the light environment between outer and inner canopy leaves. However, in my study, all of the leaves in the upper and lower canopy were located on the exterior of the crown, and in similar compass sectors, so a gradient in light is probably not solely responsible for the difference in timing of anthocyanin production and leaf senescence. It is consistent with observations of other red maples (Neufeld, personal observations) where leaves turn red first on the east side of the canopy. Those leaves would be subject to high light early in

the morning when fall temperatures are the coldest and thus most in need of protection against photoinhibition.

Of course, this brings up the question of the reduced difference in timing of anthocyanin production between upper and lower leaves of AM. Perhaps upper leaves of AM are less sensitive to such injury, and therefore they don't turn color much before lower leaves. Upper and lower AM leaves were similar in their N content, and higher N is associated with greater tolerance to photoinhibitory conditions (Liang and He 2018). AM also produce fewer anthocyanins and are more orange colored than the deep red leaves of FM. Leaves of some tree species, particularly those that remain yellow in autumn, and which do not synthesize anthocyanins or do so only minimally, may rely primarily on non-photochemical quenching (NPQ) of excess energy using xanthophyll cycle intermediates rather than rely on photoprotection by anthocyanins. However, as pointed out by Hughes et al. (2022), some species might achieve photoprotection via a strategy by which they combine the protective effects of anthocyanins with those of carotenoids (Moy et al. 2015), hence the orange-red coloration of the leaves of AM.

Phenology Patterns and Intra-Canopy Variation in Gas Exchange

Budbreak and leaf expansion in lower leaves of FM occurred earlier in the season, and they persisted longer into the fall than the upper leaves, resulting in leaf lifespans that were up to 32 days longer. This early leaf out and maturation of lower leaves means they were probably more photosynthetically competent earlier in the season, giving them an advantage in terms of carbon gain over upper leaves. In understory seedlings, more light capture led to better growth and longer survival in leaves (Harrington 1989; Augspurger 2008). This is somewhat contradictory to the findings of Zani et al. (2020), who postulated that season-long productivity

of leaves results in a shorter, not longer, leaf lifespan, due to sink limitations. That is, an earlier leaf out would be correlated with a greater abundance of assimilates, leading to feedback inhibition and an earlier senescence in the fall, as has been documented in several tree species (Fu et al. 2014). However, more recent studies, using large datasets and MODIS satellite observations of phenology across a wide range of ecosystems, show that seasonal productivity does not appear to correlate with end of season timing (Lu and Keenan 2022), which is more in agreement with my results, where lower leaves retained their chlorophyll, maintained *A* later into the season, and persisted longer into the fall. Photosynthetic capacity of upper leaves began declining in early August almost as soon as anthocyanins were detected, and was linked with reductions in chlorophyll and lower nitrogen, indicative of less investment in the biochemistry of photosynthesis, such as reduced amounts of RUBISCO (Rabinowitch and Govindjee 1965, Kitaoka and Koike 2004). Generally speaking, the higher the chlorophyll level, the greater the photosynthetic capacity and plant productivity (Richardson et al. 2002, Croft et al. 2016).

Most noticeable was that upper leaves, which developed anthocyanins much earlier and to a greater extent, also began senescing much earlier than lower leaves, as also found by Anderson and Ryser in Canada (2015). When the phenological results are combined with the lower *A*, it leads me to conclude that upper leaves probably contribute, on a per leaf basis, much less to the carbon economy of the tree than lower leaves over the course of a season. And as most tree crowns are generally wider toward their base, and probably have more leaves, it is logical to conclude that total carbon assimilation within the crown probably decreases with height, although variation in shading and production of long- and short-shoot leaves could complicate this pattern (Niklas and Cobb 2010).

Nitrogen Resorption Patterns and Efficiencies

Resorption rates ranged between 54-68%, and were higher in AM than FM, but did not differ between upper and lower leaves. These rates are well within those found for most temperate tree species (Vergutz et al. 2012), and nearly identical to those found for red maple in PA by Pena-Novas and Archetti (2021). For FM, this would mean that retranslocation rates would be higher when expressed as a function of seasonal carbon gain, which was substantially lower in upper leaves, and it may be for this reason that upper leaves place a higher priority on photoprotection earlier in the season and to a greater extent (Hughes et al. 2022). Lower nitrogen in upper leaves of FM does not seem to be a result of premature nitrogen withdrawal though, because leaf chlorophyll amounts, which are highly correlated with leaf nitrogen (Evans 1989), were at their peak when the leaves were sampled for nutrient analysis (see Figure 18). Although Pena-Novas and Archetti (2021) argue that the lack of a difference between yellow and red turning species does not support the hypothesis that anthocyanins enhance retranslocation efficiencies, but rather, aid in the resorption of chlorophyll, it should be noted that a major source of nitrogen in the leaf is contained in the chlorophyll and associated protein complexes (Evans 1989), so it is hard to disprove the hypothesis that they do not aid in nutrient retention.

Light Response Curves for Young Leaves Early in the Summer

AM leaves reached full size almost two months earlier (by the end of May) than FM leaves and this would suggest they were more mature than FM leaves at equivalent times during the season. When light response curves were performed on both species in late May and early June, there was evidence of high light inhibition in both varieties (see Figure 19). For AM, both upper and lower leaves exhibited this inhibition while for FM, it was primarily the lower leaves

that showed a similar pattern. In contrast, upper leaves in FM had typical light response curves that leveled off at or near full sunlight (PAR = 2000 μmol m^{-2} s^{-1}).

Red maples are known to be intermediate in shade tolerance (Fowler 1965, Abrams 1998), but able to adjust their leaf anatomy and physiology in response to high light (Wallace and Dunn 1998, Jurik 1986, Jurik et al. 1988), which may be one reason why this species is particularly widespread (Abrams 1998). Among the authors cited here, all of whom worked with wild varieties of red maple, none reported instances of photoinhibition at high PAR in their light response curves, whether done early or late in the season, so it is possible that this is something unique to these varieties, or to trees growing in urban locations. The depression in *A* in AM leaves at high PAR suggests that while having achieved full size, they may not have been *physiologically* mature at the time these curves were done, and were thus prone to high light injury. FM leaves, in contrast, were still increasing in size (their estimated time to full size didn't occur until mid- to late-August), and so it's logical to conclude that this pattern could have resulted because these leaves were still developing and were sensitive to high light. Upper leaves of FM may have achieved photosynthetic maturity sooner and developed greater tolerance to high light even though they too were still increasing in size, since it was mostly lower leaves that showed this unusual light response pattern. This demonstrates that photosynthetic competency and carbon gain are highly dependent not only on the location of leaves with respect to light within the canopy (Wallace and Dunn 1980, Jurik 1986, Jurik et al.1988, Koike 1990), but to the vertical position along the trunk from the upper to lower canopy (my results). That red maple leaves might be sensitive to high light (Kitaoka and Koike 2004) is suggested by the findings of Wallace and Dunn (1980) who noted that after logging, red maples that were formerly in the shaded understory re-oriented their leaves up to 37^{o} from horizontal, which would reduce light

absorption and help avoid photoinhibitory conditions. I did not measure leaf angles for my trees, but those in the Li-6800 cuvette would have been oriented perpendicular to the incoming light during the light response curves and unable to avoid high light injury and hence might have showed photoinhibition if sensitive. Unfortunately, I was not able to conduct additional light response curves later in June or in July because the bucket truck was out of commission and I could not access the leaves. Further studies of light responses as leaves mature throughout the season would help elucidate how these maple varieties adjust to tolerate differing levels of PAR.

Seasonal Patterns of Gas Exchange

As soon as upper FM leaves began showing signs of reddening (early August), their photosynthetic rates also began to decline (Figure 21). This coincided with decreases in chlorophyll and photosynthetic capacity as assessed via A-Ci curves (Figure 22) and suggests that these leaves had begun senescencing as early as late summer. Prior to the declines in chlorophyll, there were no significant difference in A_{350} (photosynthesis when C_i = 350 ppm) between canopy locations for either species. However, in the FM, A_{350} started declining in August, particularly in the upper leaves, which coincides with the decline in chlorophyll (and presumably RUBISCO, which can make up as much as 27% of the nitrogen content of a leaf (Evans 1989), and this occurred much earlier than in leaves of AM. Because A_{350} minimizes stomatal limitations due to the larger diffusion gradient between the ambient air and the sub-stomatal spaces, it suggests that any differences between upper and lower leaves, or in the timing between FM and AM, result from reduced biochemical activity, presumably due to degradation of the electronic transport reactions, as well as reductions in the amount and activity of RUBISCO (Evans 1989).

Lower leaves did show a decline in *A* from June into August, but then they maintained relatively constant rates all the way up to mid-October, after which rates declined rapidly with the onset of cold weather, similar to patterns reported by others for deciduous trees (Kitaoka and Koike 2004). Stomatal conductances, on the other hand, were more variable, and even tended to rise slightly as upper leaves senesced, while there was a peak in early August for lower leaves, followed by progressive declines into October. Water use efficiencies (WUE) were very high in early August, more than double the values later on, a result of much higher rates of *A* in early August. Afterwards, *A* and g_s both declined for upper leaves, but rates of *A* were maintained above those of upper leaves until late in the season. The variability in g_s was primarily responsible for the resultant highly variable WUE. There was no evidence throughout the 2021 growing season for any severe water stress (water potentials never decreased below -0.8 MPa), so the physiological consequences of these differences in WUE are probably small.

AM had higher concentrations of chlorophyll and nitrogen than both the upper and lower canopies of FM, along with generally higher *A* (Figure 21), which is consistent with the fact that *A* and leaf nitrogen are highly correlated in most plants (Mooney and Gulmon 1979, Field 1983, Mei and Thimann 1984, Evans 1989, Daughtry et al. 2000). AM maintained high rates of *A* until around October 7th, after which both chlorophyll and *A* showed similar and rapid declines, accompanied by a small amount of anthocyanin production just before the leaves fell off. These results are similar to those found in sugar and red maples (Anderson and Ryser 2015, Moy et al., 2015) where increases in anthocyanins coincided with rapid declines in total chlorophyll.

Speculation on Why Nitrogen Distribution Patterns Differ Between FM and AM Trees

There are a number of factors that could be responsible for the difference in nitrogen allocation between the FM and the AM, including transport properties from root to shoot,

hormone distribution, and sink locations and sizes. Cytokinins comprise a class of hormones that is involved in many plant processes including cell division, signaling, nutrient allocation, senescence, and apical dominance (Binns 1994; Keiber 2002; Wingler et al., 1998). Cytokinins are also involved in nitrogen uptake (Horgan and Wareing 1980; Simpson et al. 1982 ; Trèková and Kamínek 1999). A study looking at seasonal variations in cytokinin in upper and lower sugar maple canopies found that there was increased accumulation in the upper canopy in mid-season, with less in the lower canopy, suggesting that cytokinin distribution is regulated differently in the upper and lower canopy. Seasonal variations in cytokinin may be related to microclimatic differences in the leaves, e.g., temperature, sunlight, humidity (Held et al., 2005).

Many plants accumulate anthocyanins when they are treated with cytokinins (Klein and Hagen 1961; Deikman and Hammer 1995, Ji et al. 2015) and it is possible that cytokinin concentrations are greater in upper FM leaves, and that they contribute to the earlier and greater anthocyanin accumulation. Additionally, other hormones, including gibberellins, jasmonic acid and abscisic acid, have been shown to modulate anthocyanin production (Loreti et al. 2008), and could be at higher concentrations in upper leaves of FM. There are few studies linking the timing and extent of anthocyanin production to hormone levels in trees and this could be the subject of future investigations.

Xylem Structure and Potential Influences on Anthocyanin Production

Water flows up plants due to the tension created in the xylem as a result of evaporation from leaves (Dixon and Joly, 1895). Differences in lumen diameters, pit pore apertures, abundances of pits and density of xylem elements, all could translate to differences in water transport with differ with height between FM and AM. For example, xylem conduits grade from narrower to wider from tip to base in trees, and this also scales with resistance to flow through

those pits (Olson et al. 2021). Similar gradients are also known to exist for the phloem (Clerx et al. 2020), which is responsible for transporting primarily sugars and hormones. If hydraulic flows differ between upper and lower leaves, and between FM and AM, they might lead to changes in anatomy and morphology that are particular to each location and variety. It would be interesting to compare xylem and phloem anatomy between these two varieties.

Climate change implications

Climate change has affected tree phenology considerably, especially over the past few decades (Piao et al. 2019). Plant phenology, including flowering, leaf development, and autumn leaf coloration, are all dependent on weather, especially in temperate areas where trees have a dormant period in the winter (Yang 2009). In May of 2021, global atmospheric carbon dioxide levels peaked at a monthly average of 419 ppm, which are the highest levels since scientists started taking measurements in 1958 (NOAA 2021), not to mention over the past 400,000 years (Petit et al. 1999). This increase in atmospheric greenhouse gas concentrations is forecast to cause higher temperatures and increasingly stochastic weather events such as intense convective storms, interspersed with longer periods of drought (IPCC 2007; Coble et al. 2017). A study by Wu et al. (2020) suggested that autumn senescence will be delayed in trees at high elevations when there is a decline in wind. Less wind leads to a reduction in evapotranspiration, so in late autumn when leaves would be senescing, they would be under less water stress, and hold on to their leaves longer. Also, with less wind petioles are less likely to detach from the stem, and leaves will persist on the trees for longer periods of time.

Trees may fix higher amounts of carbon in temperate areas if temperatures increase because of longer leaf retention (Dragoni et al. 2011). However, there is some controversy about this. One paper concludes that high CO_2 may hasten the onset of leaf senescence in the fall

because sink limitations in trees are reached earlier (Zani et al. 2020, Norby 2021, Zani et al. 2021). There is also evidence that in years with higher rainfall, autumn colors are less vibrant because of reduced photosynthesis and possibly higher nitrogen uptake (Kyne and Diver 2012). Here in the Southern Appalachian Mountains, the latest climate predictions for slight increases in precipitation (~10%), but greater stochasticity of precipitation, and moderate warming (Kunkel 2020). Whether upper and lower leaves of the maple varieties I studied will react to these changes similarly is unknown but worthy of study.

Conclusions

The results of my study indicate that climatic differences between canopy locations were minimal except for wind and light, which were higher in the upper than lower canopy. While these may have influenced leaf physiology and morphology to some degree, it appears that other factors, primarily physiological, played greater roles in affecting the phenology of leaf survivorship, pigmentation, and gas exchange. Since the trees were growing in similar conditions, this suggests that there are varietal specific physiological mechanisms at play, particularly regarding nitrogen allocation, which was lower in FM than AM, and lower in upper versus lower leaves of FM. These differences, coupled with losses in chlorophyll, most likely contributed to the early production, and shorter survivorship, of upper leaves of FM compared to lower leaves, and to the later senescence of leaves of AM. Future studies could include comparing cytokinin and other signaling hormones, TNCs, and xylem and phloem anatomy between these two maple varieties and canopy locations to see if they differ. These results will help with future ecophysiological modeling studies, and further the study of urban forestry and tree physiology.

References

Adir N, Zer H, Shochat S (2003) Photoinhibition – a historical perspective. Photosynth Res 76:343.

Agati G, Guidi L, Landi M, Tattini M (2021) Anthocyanins in photoprotection: knowing the actors in play to solve this complex ecophysiological issue. New Phytol 232:2228-2235.

Anderson R, Ryser P (2015) Early autumn senescence in red maple (*Acer rubrum* L.) is associated with high leaf anthocyanin content. Plants 4:505–522.

Aphalo PJ, Jarvis PG (1993) The boundary layer and the apparent responses of stomatal conductance to wind speed and to the mole fractions of CO_2 and water vapour in the air. Plant Cell Environ 16:771-783.

Archetti M (2000) The origin of autumn colours by coevolution. J of Theor Biol 205:625-630.

Archetti M, Brown SP (2004) The coevolution theory of autumn colours. Proc R Soc Lond 271:1219-1223.

Archetti M, Doring TF, Hagen SB, Hughes NM, Leather SR, Lee DW, Lev-Yadun S, Manetas Y, Ougham HJ, Schaberg PG, Thomas H (2009) Unravelling the evolution of autumn colours: an interdisciplinary approach. Trends in Ecol and Evol 24:166-173.

Armson D, Stringer P, Ennos AR (2013) The effect of street trees and amenity grass on urban surface water runoff in Manchester, UK. Urb For and Urb Green 12:282-286.

Augspurger CK (2008) Early spring leaf out enhances growth and survival of saplings in a temperate deciduous forest. Oecologia 156:261-286.

Bachtell KR (1989) A fortunate blend: Freeman maple, a hybrid of red and silver maples, shows promise for tough urban sites. Am Nursery 169:40-43.

Balakumar T, Vincent VHB, Paliwal K (1993) On the interaction of UV-B radiation (280-315 nm) with water stress in crop plants. Physiol Planta 87:217–222.

Bassow SL, Bazzaz, FA (1997) Intra- and inter-specific variation in canopy photosynthesis in a mixed deciduous forest. Oecologia 109:507-515.

Beadle C, Sands R (2004) Physiology and Silviculture, in: Encyclopedia of Forest Sciences. Tree Physiol 4:1568–1577.

Binns AN (1994) Cytokinin accumulation and action: biochemical, genetic, and molecular approaches. Ann Rev Plant Physiol Plant Molec Biol 45:173-196.

Boardman NK (1977) Comparative photosynthesis of sun and shade plants. Ann Rev of Plant Physiol 28:355-377.

Brant AN, Chen HYH (2015) Patterns and mechanisms of nutrient resorption in plants. Crit Rev in Plant Sci 34:471–486.

Bueno A, Alonso-Forn D, Peguero-Pina JJ, de Souza AX, Ferrio JP, Sancho-Knapik D, Gil-Pelegrin E (2022) Minimum leaf conductance (g_{min}) is higher in the treeline of *Pinus uncinata* Ram. In the Pyrenees: Michaelis' hypothesis revisited. Frontiers in Plant Science 24: doi.org/10.3389/fpls.2021.786933.

Campbell GS, JM Norman (1998) An Introduction to Environmental Biophysics. Springer, NY, NY.

Carpenter KL, Keidel TS, Pihl MC, Hughes NM (2014) Support for a photoprotective function of winter leaf reddening in nitrogen-deficient individuals of *Lonicera japonica*. Molecules 19:17810–17828.

Chalker-Scott L (1999) Environmental significance of anthocyanins in plant stress responses. Photochem Photobiol 70:1–9.

Chapin FS III, Bloom AJ, Field CB, Waring RH (1987) Plant responses to multiple environmental factors. BioSci 38:49-57.

Chen Y, Huang Z, Tang K (2021) Invisible red in young leaves: Anthocyanin likely plays a defensive role in some other way beyond visual warning. Flora 280:151833.

Choinski JS Jr, Ralph P, Eamus D (2003) Changes in photosynthesis during leaf expansion in *Corymbia gummifera*. Aust J of Bot 51:111-118.

Clerx LE, Rockwell FE, Savage JA, Holbrook NM (2020) Ontogenetic scaling of phloem sieve tube anatomy and hydraulic resistance with tree height in *Quercus rubra*. Am J of Bot 107:852-863.

Cobbina J, Miller MH (1987) Purpling in maize hybrids as influenced by temperature and soil phosphorus. Agronomy J 75:576–582.

Coble AP, Cavaleri MA (2014) Light drives vertical gradients of leaf morphology in a sugar maple (*Acer saccharum*) forest. Tree Physiol 34:146–158.

Coble AP, Vadeboncoeur MA, Berry ZC, Jennings KA, Mcintire CD, Campbell JL, Rustad LE, Templer PH, Asbjornsen H (2017) Are Northeastern U.S. forests vulnerable to extreme drought? Ecolog Proces 6(34):1-13.

Critchfield WB (1960) Shoot growth and heterophylly in *Acer*. J of Arnold Arbor 52:240-266.

Croft H, Chen JM, Luo X, Bartlett P, Chen B, Staebler RM (2016) Leaf chlorophyll content as a proxy for leaf photosynthetic capacity. Glob Change Biol 23:3513-3524.

Crous KY, Ellsworth DS (2004) Canopy position affects photosynthetic adjustments to long-term elevated CO2 concentration (FACE) in aging needles in a mature Pinus taeda forest. Tree Physiol 24:961–70.

Crowder B (2015) Trees and Shrubs of North America. National Geographic Society. Washington, D.C. 15-16.

Daudet FA, Le Roux X, Sinoquet H, Adam B (1999) Wind speed and leaf boundary layer conductance variation within tree crown: Consequence on leaf-to-atmosphere coupling and tree functions. Agric Forest Meteorol 97:171-185.

Daughtry CST, Walthall CL, Kim MS, de Colstoun EB, McMurtrey JE (2000) Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote sensing Environ 74:229-239.

DeForest JL, McCarthy BC (2011) Diminished soil quality in an old-growth, mixed mesophytic forest following chronic acid deposition. Northeast Natural 18:177–184.

Deikman J, Hammer PE (1995) Induction of anthocyanin accumulation by cytokinins in *Arabidopsis thaliana*. Plant Physiol 108:47-57.

Dickson RE, Shive JB (1982) CO_2 fixation, translocation, and carbon metabolism in rapidly expanding leaves of *Populus deltoides*. Annals of Bot 50:37–47.

Dixon HH, Joly J (1895) On the ascent of sap. Philo Trans Roy Soc 186:563–576.

Dragoni D, Schmid HP, Wayson CA, Potter H, Grimmond CSB (2011) Evidence of increased net ecosystem productivity associated with a longer vegetated season in a deciduous forest in south-central Indiana, USA. Glob Change Biol 17:886–897.

Drake PL, Froend RH, Franks PJ (2013) Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. J of Exp Bot 64:495–505.

Eensalu E, Kupper P, Sellin A, Rahi M, Sober A, Kull O (2008) Do stomata operate at the same relative opening range along a canopy profile of *Betula pendula*? Func Plant Bio 35:103-110.

Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78:9-19.

Feild C (1983) Allocating leaf nitrogen for the maximization of carbon gain: Leaf age as a control on the allocation program. Oecologia 56:341-347.

Feild TS, Lee DW, Holbrook NM (2001) Why leaves turn red in autumn. The role of anthocyanins in senescing leaves of red-osier dogwood. Plant Physiol 127:566-574.

Ferreyra MLF, Serra P, Casati P (2021) Recent advances on the roles of flavonoids as plant protective molecules after UV and high light exposure. Physiol Planta 173:736-749.

Freeman, OM (1941) A red maple, silver maple hybrid. J of Hereditary 32:11-14.

Fu YSH, Campioli M, Vitasse Y, De Boeck HJ, Van den Berge J, AbdElgawad H, Asard H, Piao S, Deckmyn G, Janssens IA (2014) Variation in leaf flushing date influences autumnal senescence and next year's flushing date in two temperate tree species. Proceed of the Nat Acad of Science 111:7355-7360.

Geiger R, Aron RH, Todhunter P (2003) The climate near the ground. Rowman & Littlefield Publishers, Inc Lanham, MD USA.

Gill SE, Handley JF, Ennos AR, Pauleit S (2007) Adapting cities for climate change: the role of the green infrastructure. Built Env 33:115-133.

Gould KS, McKelvie J, Marham KR (2002) Do anthocyanins function as antioxidants in leaves? Imaging of H_2O_2 in red and green leaves after mechanical injury. Plant, Cell Environ 25:1261-1269.

Grace J (1988) Plant response to wind. Agric Ecosys Environ 22:71-88.

Grace J, Russell G (1977) The effect of wind on grasses. III. Influence of continuous drought or wind on anatomy and water relations in *Festuca arundinacea*. J of Exp Bot 28:268-278.

Grace J, Russell G (1982) The effect of wind and a reduced supply of water on the growth and water relations of *Festuca arundinacea* Schreb. Annals of Bot 49:217-225.

Gu Z, Chen H, Yang R, Ran M (2018) Identification of DFR as a promoter of anthocyanin accumulation in poinsettia (*Euphorbia pucherrima*, willd. ex Klotzsch) bracts under short-day conditions. Scientia Horticulturae 236:158-165.

Harborne JB, Williams CA (2000) Advances in flavonoid research since 1992; Phytochem 55:481–504.

Harrington TB (1989) Stand development and individual tree morphology and physiology of young Douglas-fir (*Pseudotsuga menziesii)* in association with tanoak (*Lithocarpus densiflorus*). Dissertation Oregon State Univ., Corvallis, OR.

Held, MA, Quesnelle PE, Emery RJN (2005) Seasonal changes of cytokinins in upper and lower leaves of a sugar maple crown. Bio Planta 49:455-458.

Hirose T, Werger MJA (1987) Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. Oecologia 72:520-526.

Hoch WA, Zeldin EL, McCown BH (2001) Physiological significance of anthocyanins during autumnal leaf senescence. Tree Physiol 21:1-8.

Hortensteiner S (2006) Chlorophyll degradation during senescence. Ann Rev Plant Bio 57:55-77.

Hughes NM, George CO, Gumpman CB, Neufeld HS (2022) Coevolution and photoprotection as complementary hypotheses for autumn leaf reddening: a nutrient centered perspective. New Phytol 233:22-29.

Hughes NM, Morley CB, Smith WK (2007) Coordination of anthocyanin decline and photosynthetic maturation in juvenile leaves of three deciduous tree species. New Phytol 175:675–685.

Hughes NM, Neufeld HS, Burkey KO (2005) Functional role of anthocyanins in high-light winter leaves of the evergreen herb *Galax urceolata.* New Phytol 168:575-587.

Hutnik RJ, Yawney HW (1961) Silvical characteristics of red maple (*Acer rubrum*). Station Paper NE. Upper Darby, PA: U.S. Dept Ag, FS, Northeastern Forest Exp Station 142(18):1-18.

Hsiao TC (1973) Plant Responses to Water Stress. Ann Rev Plant Physiol 24:519–570.

Hutchison BA, Matt DR, McMillen RT, Gross LJ, Tajchman SJ, Norman JM (1986) The architecture of a deciduous forest canopy in eastern Tennessee, U.S.A. J of Ecol 74:635-676

Iogna PA, Bucci SJ, Scholz FG, Goldstein G (2013) Homeostasis in leaf water potentials on leeward and windward sides of desert shrub crowns: water loss control vs. high hydraulic efficiency. Oecologia 173:675-687.

Jarvis PG, McNaughton KJ (1986) Stomatal control of transpiration: scaling up from leaf to region. Advance in Ecol Res 15:1-49.

Ji XH, Wang YT, Zhang R (2015) Effect of auxin, cytokinin and nitrogen on anthocyanin biosynthesis in callus cultures of red-fleshed apple (*Malus sieversii* f. *niedzwetzkyana*). Plant Cell Tis Org Cult 120:325–337.

Jurik TW (1986) Patterns of leaf photosynthetic capacity in successional northern hardwood tree species. Am J of Bot 73:131-138.

Jurik TW, Weber JA, Gates JM (1988) Effects of temperature and light on photosynthesis of dominant species of a northern hardwood forest. Bot Gaz 149:203-208.

Karageorgou P, Manetas Y (2006) The importance of being red when young: anthocyanins and the protection of young leaves of *Quercus coccifera* from insect herbivory and excess light. Tree Physiol 26:613-621.

Keskitalo J, Berquist G, Garestrom P, Jansson S (2005) A cellular timetable of autumn senescence. Plant Physiol 139:1635-1648.

Kjelgren RK, Clark JR (1992) Microclimates and tree growth in three urban spaces. J of Env Hort 10:139-145.

Kitaoka S, Koike T (2004) Invasion of broad-leaf tree species into a larch plantation: seasonal light environment, photosynthesis and nitrogen allocation. Physiologia Planta 121(4):604-611.

Klein AO, Hagen CW Jr (1961) Anthocyanin production in detached petals of *lmpatiens balsamina* L. Plant Physiol 36:1-9.

Koike T (1990) Autumn coloring, photosynthetic performance and leaf development of deciduous broad-leaved trees in relation to forest succession. Tree Physiol 7:21–32.

Koike T, Kitao M, Maruyama Y, Mori S, Lei TT (2001) Leaf morphology and photosynthetic adjustments among deciduous broad-leaved trees within the vertical canopy profile. Tree Physiol 21:951–958.

Krautler B, Jaun B, Bortlik K-H, Schellenberg M, Matile P (1991) On the enigma of chlorophyll degradation: the constitution of a secoporphinoid catabolite. Angew Chem Int Ed 30:1315– 1318.

Krogmeier KR (2021) Impacts of polyploidy and subsequent natural selection on the ecophysiology of *Solidago altissima*. Masters Thesis, Appalachian State University. 81 pgs.

Kunkel KE (2020) North Carolina Climate Science Report. North Carolina Institute for Climate Studies, 233 pp.

Kyne A, Diver K (2012) Climate change and autumn colors in New England's forests. NE Geo 4:34-53.

Landi M, Guidi L, Pardossi A, Tattini M, Gould KS (2014) Photoprotection by foliar anthocyanins mitigates effects of boron toxicity in sweet basil (*Ocimum basilicum*). Planta 240:941-953.

Lee DW, Gould KS (2007) Anthocyanins in leaves and other vegetative organs: An introduction. Adv Bot Res 37:1-16.

Lee DW, Lowry JB, Stone BC (1979) Abaxial anthocyanin layer in leaves of tropical rain forest plants: enhancer of light capture in deep shade. Biotropica 11:70-77.

Lehmann P, Or D (2015) Effects of stomata clustering on leaf gas exchange. New Phytol 207:1015-1025.

Lei T (1998) Diverse responses of maple saplings to forest light regimes. Annals Bot 82:9–19.

Liang J, He J (2018) Protective role of anthocyanins in plants under low nitrogen stress. Biochem and Biophys Res Comm 498:946-953.

Lev-Yadun S, Yamazaki K, Holopainen JK, Sinkkonen A (2012) Spring versus autumn leaf colours: Evidence for different selective agents and evolution in various species and floras. Flora- Morph, Dist, Funct Ecol of Plants 207:80-85.

Long SP, Humphries SM (1994) Photoinhibition of photosynthesis in nature. Ann Rev Plant Phys Plant Molec Biol 45:633-662.

Loreti E, Povero G, Novi G, Solfanelli C, Alpi A Perata P (2008) Gibberellins, jasmonate and abscisic acid modulate the sucrose-induced expression of anthocyanin biosynthetic genes in Arabidopsis. New Phytol 179:1004–1016.

Lu X, Keenan TF (2022) No evidence for a negative effect of growing season photosynthesis on leaf senescence timing. Global Change Biol 28:3083-3093.

Matile PH, Flach BMP, Eller BM (1992) Autumn leaves of *Ginkgo biloba* L.: optical properties, pigments and optical brighteners. Botanica Acta 105:13–17.

Marshall JD and Monserud RA (2003) Foliage height influences specific leaf area of three conifer species. Can J of Res 33:164-170.

McNamara S, Zuzek K, Rose N, Pellett H, Hokanson SC (2005) 'Firefall' Freeman Maple. HortSci 40:269-271.

Mei H-S, Thimann KV (1984) The relation between nitrogen deficiency and leaf senescence. Physiol Planta 62:157-161.

Miret JA, MunnéBosch S (2015) Redox signaling and stress tolerance in plants: a focus on vitamin A. Ann Rev N.Y. Acad Sci 1340:29–38.

Mooney HA, Gulmon SL (1979) Environmental and evolutionary constraints on photosynthetic characteristics of higher plants. Top Plant Pop Bio 1979:316-337.

Moy A, Sherry L, Verhoeven A (2015) Different strategies for photoprotection during autumn senescence in maple and oak. Physiol Planta 155:205-216.

Naing AH, Kim CK (2021) Abiotic stress-induced anthocyanins in plants: Their role in tolerance to abiotic stresses. Physiol Planta 172:1711-1723.

Nasholm T, Ekblad A, Nordin A, Giesler R, Hogberg M, Hogberg P (1998) Boreal forest plants take up organic nitrogen. Nature 392:914-916.

Neufeld HS and Young DR (2014) Ecophysiology of the herbaceous layer in temperate deciduous forests. In "The Herbaceous Layer in Forests of Eastern North America", ed. F.S. Gilliam. Oxford University Press, NY. pgs. 35-95.

Nielsen SL, Simonsen AM (2011) Photosynthesis and photoinhibition in two differently coloured varieties of *Oxalis triangularis*- the effect of anthocyanin content. Photosynthetica 49:346-352.

Niklas K (1996) Differences between *Acer saccharum* leaves from open and wind-protected sites. Annals of Bot 78:61-66.

Niklas K (1992) Plant Biomechanics: An Engineering Approach to Plant Form and Function. University of Chicago Press, Chicago, USA.

Niklas KJ, Cobb ED (2010) Ontogenenetic changes in the numbers of short-vs. long-shoots account for decreasing specific leaf area in *Acer rubrum* (Aceraceae) as trees increase in size. Am J of Bot 97:27-37.

NOAA (2018) Carbon dioxide peaks near 420 parts per million at Mauna Loa observatory. https://research.noaa.gov/article/ArtMID/587/ArticleID/2764/Coronavirus-response-barely-slows-rising-carbon-

Nobel PS, Zaragoza LJ, Smith WK (1975) Relation between mesophyll surface area, photosynthetic rate, and illumination level during development for leaves of *Plectranthus parviflorus henckel*. Plant Physiol 55:1067–1070.

Norby RJ (2021) Comment on “Increased growing-season productivity drives earlier autumn leaf senescence in temperate trees”. Sci 371:1438.

Nozzolillo C, Isabelle P, Das G (1990) Seasonal changes in the phenolic constituents of jack pine seedlings (*Pinus banksiana*) in relation to the purpling phenomenon. Can J of Bot 68:2010–2017.

Olson ME, Anfodillo T, Gleason SM, McCulloh KA (2021) Tip-to-base xylem conduit widening as an adaptation: causes, consequences, and empirical priorities. New Phytol 229:1877-1893.

Öoquist, G, Chow WS, Anderson JM (1992) Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. Planta 186:450-460.

Osmond CB, Bjorkman O, Anderson DJ. (1980) Physiological processes in plant ecology. Toward a Synthesis with *Atriplex*. Springer 468:1-11

Papanatsiou M, Amtmann A, Blatt MR (2017) Stomatal clustering in *Begonia* associates with the kinetics of leaf gaseous exchange and influences water use efficiency. J of Exp Bot 68:2309-2315.

Pate JS (1973) Uptake, assimilation and transport of nitrogen compounds by plants. Soil Biol Biochem 5:109-119.

Paź-Dyderska, S, Dyderski MK, Nowak K, Jagodziński AM (2020). On the sunny side of the crown – Quantification of intra-canopy SLA variation among 179 Taxa. Forest Eco Mgmt 472:118254.

Peltoniemi MS, Duursma RA, Medlyn BE (2012) Co-optimal distribution of leaf nitrogen and hydraulic conductance in plant canopies. Tree Physiol 32:510-519.

Pena-Novas I, Archetti M (2021) Nitrogen resorption efficiency in autumn leaves correlated with chlorophyll resorption, not with anthocyanin production. doi.org/10.1101/2021.05.12.443938

Perica S (2006) Seasonal fluctuation and intracanopy variation in leaf nitrogen level in olive. J of Plant Nutr 24:779-787.

Petit JR and many others (1999) Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. Nature 399:429-436.

Pétriacq P, and many others (2017) Photoperiod affects the phenotype of mitochondrial complex I mutants. Plant Physiol 173:434-455.

Piao S, Liu Q, Chen A, Janssens IA, Fu Y, Dai J, Liu L, Lian X, Shen M, Zhu X (2019) Plant phenology and global climate change: current progresses and challenges. Glob Chang Biol 25:1922-1940.

Poorter H, Niinements U, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol 182:565-588.

Porra RJ (2002) The checquered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photo Res 73:149-156.

Rabinowitch EI, Govindjee (1965) The role of chlorophyll in photosynthesis. Sci Am 213:74-83.

Radyukina NL, Mikheeva LE, Karbysheva EA (2019) Low molecular weight antioxidants in cyanobacteria and plant cells. Biol Bull Rev 9:520-531.

Rahman MA, Armson D, Ennos AR (2015) A comparison of the growth and cooling effectiveness of five commonly planted urban tree species. Urb Ecosyst 18:371-389.

Rahman MA, Moser A, Rotzer T, Pauleit S (2019) Comparing the transpirational and shading effects of two contrasting urban tree species. Urban Eco 22:683-697.

Reeves I, Emery RJN (2007) Seasonal patterns of cytokinins and microclimate and the mediation of gas exchange among canopy layers of mature *Acer saccharum* trees. Tree Physiol 27:1635-1645.

Reeves I, Farrow SC, Emery RJN (2007) Patterns of cytokinins and leaf gas exchange among canopy layers of a mature sugar maple (Acer saccharum) stand. Trees 21:181-189.

Renner SS, Zohner CM (2019) The occurrence of red and yellow autumn leaves explained by regional differences in insolation and temperature. New Phytol 224:1464-1471.

Richardson AD, Duigan SP, Berlyn GP (2002) An evaluation of non-invasive methods to estimate foliar chlorophyll content. New Phytol 153: 185-194.

Sack L, Holbrook NM (2006) Leaf hydraulics. Ann Rev of Plant Biol 57:361-381.

Salisbury EJ (1927) On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. Phil Trans of the Royal Soc 216B:1-65.

Schaberg PG, van den Berg AK, Murakami PF, Shane JB, Donnelly JR (2003) Factors influencing red expression in autumn foliage of sugar maple trees. Tree Physiol 23:325–333.

Schaefer HM, Rolshausen G (2005) Plants on red alert: do insects pay attention? BioEssay 28:65-71.

Schmitzer V, Stampar F, Veberic R, Osterc G (2009) Phase change modified anthocyanin synthesis in *Acer palmatum* Thunb. (Japanese maple) cultivars. Acta Physiol Planta 31:415-418.

Seaton DD, Toledo-Ortiz G, Ganpudi A, Kubota A, Imaizumi T, Halliday KJ (2018) Dawn and photoperiod sensing by phytochrome A. Proc of the Nat Acad of Science 115:10523-10528.

Sendall, KM, Vourlitis GL, Lobo, FA (2009) Seasonal variation in the maximum rate of leaf gas exchange of canopy and understory tree species in an Amazonian semi-deciduous forest. Braz J of Plant Physiol 21:65-74.

Sibley JL, Eakes DJ, Gilliam CH, Keever GJ Dozier WA Jr (1995) Growth and fall color of red maple selections in the southeastern United States. J of Environ Hort 13:51-53

Skinner M, Parker B (1994) Field guide for monitoring sugar maple bud development. The University of Vermont Entomology Research Laboratory.

Tanaka Y, Sugano SS, Shimada T, Hara-Nishimura I (2013) Enhancement of leaf photosynthetic capacity through increased stomatal density in *Arabidopsis*. New Phytol 98:757– 764.

Telewski FW (1995) Wind-induced physiological and developmental responses in trees. Wind crand Tree. Cambridge Univ Press: 237-263.

Terashima I, Evans JR (1988) Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. Plant and Cell Physiol 29:143-155.

Tischner R (2000) Nitrate uptake and reduction in higher and lower plants. Plant, Cell, and Environ 23:1005-1024.

Trèková M, Kamínek M (1999) Effect of an exogenous cytokinin on nitrate uptake and nitrogen assimilation in wheat plants. Biol of Plants 42:86-88.

USDA, NRCS (2012) The PLANTS Database (http://plants.usda.gov, 20 May 2012). National Plant Data Team, Greensboro, NC 27401--4901 USA.

Van Gardingen PA, Grace J (1991) Plants and wind. Advan Bot Res 18:189-253.

Varma V, Osuri, AM (2013) Black Spot: a platform for automated and rapid estimation of leaf area from scanned images. Plant Ecol 214:1529-1534.

Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecol Monographs 82:205-220.

Villar R, Ruiz-Roblet J, Ubera JL, Poorter H (2013) Exploring variation in leaf mass per area (LMA) from leaf to cell: An anatomical analysis of 26 woody species. Am J of Bot 100:1969-1980.

Wallace LL, Dunn E (1980) Comparative photosynthesis of three gap phase successional tree species. Oecologia 45:331-340.

Wang X, Chen Y, Liu F, Zhao R, Quan X, Wang C (2020) Nutrient resorption estimation compromised by leaf mass loss and area shrinkage: variations and solutions. For Ecol and Mgmt 472:118232.

Wilson BF (1966) Development of the shoot system of *Acer rubrum* L. Harvard Forest Paper no. 14:1-21.

Wingler A, Von Schaewen A, Leegood RC, Lea PJ, Quick WP (1998) Regulation of leaf senescence by cytokinin, sugars, and light. Plant Physiol 116:329-335.

Wu C, Wang J, Phillippe C, Penuelas J, Zhang X, Sonnentag O, Tian, F, Wang X, Wang H, Liu R, Fu, YH, Ge Q (2020) Widespread decline in winds delayed autumn foliar senescence over high latitudes. PNAS 118(16): e2015821118.

Xu Z, Mahmood K, Rothstein SJ (2017) ROS induces anthocyanin production via late biosynthetic genes and anthocyanin deficiency confers the hypersensitivity to ROS-generating stresses in *Arabidopsis*. Plant and Cell Physiol 58: 1364–1377.

Yang J (2009) Assessing the impact of climate change on urban tree species selection: a case study in Philadelphia. J of Forestry 107(7):364-372.

Zani D, Crowther TW, Mo L, Renner SS, Zohner CM (2020) Increased growing-season productivity drives earlier autumn leaf senescence in temperate trees. Science 370:1066-1071.

Zhu H, Zhang TJ, Zheng J, Huang XD, Yu ZC, Peng CL (2018) Anthocyanins function as a light attenuator to compensate for insufficient photoprotection mediated by nonphotochemical quenching in young leaves of *Acmena acuminatissima* in winter. Photosynthetica 56:445–454.

Vita

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