GENETIC VARIATION OF FUNCTIONAL TRAITS RELATED TO DROUGHT TOLERANCE IN YELLOW BIRCH SEEDLINGS

By
Ashley Sarah Maloney

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ABSTRACT

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Key Words: yellow birch, *Betula alleghaniensis*, common garden, provenance, adaptive variation, leaf morphology, drought tolerance

Understanding patterns of variation in drought-related traits of hardwood trees is crucial for conserving and managing North American temperate forests under climate change. In this study, I examined provenance variation of yellow birch (*Betula alleghaniensis* Britton) in traits related to drought resistance. Yellow birch is a widespread and economically important eastern North American hardwood species. A common garden approach was used to compare height, diameter, biomass, leaf morphology, and stable carbon isotopes among ten seed sources originating from across Canada and Northern US states. Analysis of variance (ANOVA) of seedling height and diameter did not reveal significant variation in either trait, while ANOVA of a subsample (n=40) revealed significant variation in height and leaf characters (average horizontal width, horizontal width, maximum perpendicular width, perpendicular width 1, and perpendicular width 2). Simple linear regressions revealed significant correlations between variation in leaf morphological traits and climate at seed origin. Temperature-related climate variables were more strongly correlated with leaf traits than precipitation-related climate variables. Height was slightly correlated with the climate variable summer precipitation (PPT_SM) (R^2=0.344, p<0.1). The multiple linear regression model including degree days above 5°C (DD5), mean annual temperature (MAT), and annual heat moisture index (AHM), explained a total of 71% of the variation among provenances in leaf perpendicular width 2. Principal component analysis (PCA) and canonical correlation analysis (CANCOR) were used to describe relationships among and between tree and climate variables. PC1 strongly correlated with all leaf characters, whereas PC2 strongly correlated with height. Cumulatively, PC1 and PC2 explained 90.7% of the variation among measured tree variables. For the CANCOR, canonical variable 1 (CV1) explained 67.23% of the variance among the two data sets. All leaf characters had a strong correlation with CV1, whereas height had a weak positive correlation with CV1. Climate variable DD5 had the strongest positive correlation with CV1, followed by MAT and AHM. Climate variable PPT_SM had a strong negative correlation with CV1. The results indicate that variation in leaf traits among yellow birch populations is associated with adaption to local climate. Conversely, variation in height growth was uncorrelated with climate at seed origin. Temperature and heat-moisture indices were strong predictors of leaf width in both univariate and multivariate analyses, as leaf width decreased with warmer and drier climates. High levels of within-provenance variation were present, which appears to be a common characteristic of this species. In addition, other site variables not included in this study, such as soil pH, soil moisture, and light availability, may have contributed to unexplained variation among populations.

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CHAPTER I

PROJECT INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Understanding patterns of variation in drought-related traits of prominent species such as yellow birch (*Betula alleghaniensis* Britton) is crucial for conserving and managing North American hardwood forests under climate change. Increases in frequency and severity of extreme climatic events are expected to negatively affect forest health and productivity (Allen et al., 2010). Stressors such as drought are directly involved in trends of overall forest decline and loss of ecosystem function, with increased tree mortality over recent decades having been linked to elevated drought stress (Bréda & Badeau, 2008; Dale et al., 2001; Jones et al., 1993; O'Brien et al., 2017; Zierl, 2004). Drought and temperature-stressed trees have been shown to be more susceptible to mortality due to insect attacks and wildfires (Allen et al., 2010) and temperate forests with slow growing and smaller trees tend to have higher mortality rates due to drought (O'Brien et al., 2017). Environmental conditions such topography and site water content are strong indicators of drought-induced mortality in temperate forest regions. Trees are at an increased risk to mortality caused by drought when exposed to long periods of warmer temperatures (McDowell et al., 2008). Eventual mortality of stressed trees may either be physiological (carbon starvation or hydraulic failure) or indirect (takeover by pests or pathogens).

Trees have mechanisms to either avoid or resist drought through a variety of responses. Isohydry (drought avoidance) causes the stomata to close, reducing transpiration after a certain water potential threshold is reached (McDowell et al., 2008). This mechanism prevents xylem cavitation by avoiding extremely low water potential levels but may eventually lead to carbon starvation due to the lack of photosynthesis occurring while respiration continues or severe heat stress. Anisohydry (drought tolerance) keeps stomata slightly open, allowing transpiration to continue at relatively high rates. This response allows the plant to continually uptake carbon with open stomata, but may lead to higher risk of cavitation, ultimately causing tree mortality or increased chance of a lack of carbon in future events (Allen et al., 2010; McDowell et al., 2008).

Changes in leaf morphology allow plants to efficiently acclimate to changes in environmental conditions by either increasing or decreasing the rates of photosynthesis and transpiration (Xu et al., 2009). Therefore, variation in leaf morphology can be a good indicator of a plant's uptake, efficiency, and resource usage (Xu et al., 2009). In paper birch (*Betula papyrifera* Marshall), significant variation in leaf morphology has been shown to correlate strongly with climate at seed origin (Pyakurel & Wang, 2013). Variation of leaf morphology among yellow birch populations has been significantly correlated to site conditions such as latitude, longitude, elevation, and soil characteristics (Dancik & Barnes, 1975). Plants in dry environments tend to have lower specific leaf area (SLA, the ratio of leaf area to leaf dry mass, cm^2 g^{-1}), and relative growth rates (Wright & Westoby, 1999) as thicker leaves with low SLA have been shown to be more resilient to wilting in dry and hot climates (Warren et al., 2005). Therefore, in drought conditions it would be expected that SLA would decrease. However, a recent study on paper birch seedlings found a positive correlation between SLA and warm, dry climates at seed origin (Pyakurel & Wang, 2013). In contrast, significant variation in leaf traits of red ironbark (*Eucalyptus sideroxylon* Wools) populations was independent of mean annual rainfall at seed origin (Warren et al., 2005).

Stable carbon isotope analyses are commonly used to provide understanding of WUE within individual trees (Farquhar et al., 1989). In the process of photosynthesis, discrimination occurs against the two isotopes (^{12}C and ^{13}C) and can be measured using a ratio of mass per isotope (O'Leary, 1988). The discrimination ratio (Δ) of carbon isotopes can then be used to compare WUE, as those with more positive $\delta^{13}C$ values tend to be more water-efficient (Dawson et al., 2002). Furthermore, individuals with thicker leaves (lower SLA) tend to have higher Δ values (Zhang et al., 1993), indicating higher water-use efficiency (Lamont et al., 2002). Carbon isotope analysis has been used to investigate genetic variation of WUE in tree species such as Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco) (Zhang et al., 1993), cluster pine (*Pinus pinaster* Aiton) and sessile oak (*Quercus petraea* (Mattuschka) Lieblein) (Picon et al., 1996). Significant variation in Δ values among Douglas-fir populations was strongly correlated with altitude at seed origin (Zhang et al., 1993). In both cluster pine and sessile oak, increased drought intensity was associated with an increase in leaf $\delta^{13}C$ values (Picon et al., 1996). Within-site variation of $\delta^{13}C$ in leaf and wood tissue has been investigated in *Juniperus* and *Pinus* (Leavitt & Long, 1986), revealing variation of these values within-populations, individuals, and tissue types sampled from the same site or individual. The effects of soil nitrogen and nitrogen dioxide ($NO_{2)}$ on WUE among *Populus* seedlings were investigated using carbon isotope analysis, revealing that exposure to NO_2 was non-toxic and it caused an increase in $\delta_{13}C$ values, ultimately increasing long-term WUE (Siegwolf et al., 2001). Populations of American sea rocket (*Cakile edentula* (Bigelow) Hooker), growing in dry environments exhibited higher fitness associated with low

WUE in plants with smaller leaves, or conversely, high WUE in plants with larger leaves (Dudley, 1996).

Common garden studies are crucial for predicting the potential response of tree populations to climate change (Matyas, 1996). An individual plant’s phenotype is the result of its genotype plus the environment it is growing in (Frankham et al., 2013). Therefore, when different genotypes of the same species are grown in a controlled, uniform environment, phenotypic variation that is observed can be attributed to be the result of underlying genetic differences. While provenance studies have provided significant insight into patterns of local adaptation for widespread boreal conifers (e.g. (Joyce & Rehfeldt, 2013; Lu et al., 2016; Prunier et al., 2013; Rehfeldt et al., 1999; Thomson et al., 2010), such studies are currently lacking for many important eastern North American temperate trees (but see (Clausen & Garrett, 1968; Gaucher et al., 2005; Gunderson et al., 2012; Leites et al., 2019; Pyakurel & Wang, 2013)).

Yellow birch is a widespread and ecologically important temperate hardwood tree that ranges from the Canadian Atlantic Coast to southeastern Manitoba, across the Midwest states and south to the Appalachian Mountains (Erdmann, 1990). The species is an important component of several major temperate forest types (e.g. Hemlock-Yellow Birch, Sugar Maple-Beech-Yellow Birch, and Red Spruce-Yellow Birch) and is an important source of food and shelter for many small mammals, birds, and insects. Economically, yellow birch is used for hardwood flooring, cabinets, doors, and furniture (Juncus, 1998). As a widespread, wind-pollinated tree species, yellow birch is expected to harbour significant levels of genetic variation (Thomson et al. 2015). Indeed, provenance studies of yellow birch conducted in the 1960s and 1970s have revealed significant intraspecific variation in a variety of traits such as catkin characteristics

(Clausen, 1968), height growth and cessation (Clausen, 1967), leaf morphology (Dancik & Barnes, 1975; Sharik & Barnes, 1979), and flowering times (Clausen, 1980). A study of the response of yellow birch to varying soil moisture revealed significant differences in the architecture of branches among water-stressed and well-watered trees (Rasheed & Delagrange, 2016). Using LIDAR technology, it was shown that individuals under water stress significantly decreased the long axis branches and increased short axis branches. Furthermore, the seedlings under water stress decreased total biomass by 40% compared to those in well-watered conditions. Yellow birch is a moderately shade-tolerant species, so the ability to increase height growth and root biomass while growing under the canopy is a crucial trait for competing against other tree species (Beaudet & Messier, 1998). A study involving yellow birch found that root biomass and morphology were significantly correlated with tree size but weakly correlated with light availability (Cheng et al., 2005). While these studies provide valuable information about how yellow birch trees can respond to varying degrees of light and soil moisture, they do not reveal genetic variation that may be present across the species, as the seedlings originated from a single population or seed source.

Dancik and Barnes (1974) and Sharik and Barnes (1979) found significant intraspecific variation in yellow birch leaf traits but did not test populations in common garden so it is unclear whether the observed variation is due to genetic or environmental effects. Wearstler and Barnes (1977) tested yellow birch populations in common garden and found significant variation between provenances in height, seed weight, initial germination, and germination percentage. While significant correlations between trait values and geographic variables (i.e. latitude) were observed, correlations with climate at seed origin were not explicitly examined.

To the best of my knowledge, only one previous study has specifically investigated patterns of adaptive variation in relation to climate of functional traits in yellow birch. Clausen (1967) observed significant variation in height and growth cessation among yellow birch populations tested in common garden. The date of growth cessation was found to correlate significantly with growing season length, annual precipitation, and average July temperature, while height did not correlate significantly with any of the three climate variables tested. No common garden studies examining patterns of adaptive variation in leaf morphology and water-use efficiency currently exist for yellow birch. Therefore, whether yellow birch demonstrates local adaptation to climate in traits related to drought tolerance is currently not well-understood. Thus, the objective of my research was to investigate intraspecific variation in morphological and physiological traits in relation to climate of yellow birch seedlings originating from Canadian and northern United States populations. I focused on functional traits such as height, diameter, biomass, leaf morphology, and stable carbon isotopes, which have been shown to be indicators of drought tolerance in other plant species and are therefore of importance in understanding potential response of yellow birch populations to climate change. Understanding patterns of adaptive variation is crucial for understanding and predicting climate change effects on forest tree species and the conservation of genetic diversity (Leites et al., 2019). The results of this research will provide important information on the potential adaptive genetic variation that exists in yellow birch populations that have yet to be studied as well as insight for conserving this prominent species under climate change conditions.

The purpose of this thesis was twofold. The first objective (Chapter 2) was to investigate patterns of variation in functional traits of yellow birch provenances. Traits which showed significant variation among provenances were then related to climate variables at the seed origin using simple and multiple linear regression analyses. The second objective (Chapter 3) was to summarize the variation among traits and climate variables as well as the relationships between the two data sets, using principal component analysis and canonical correlation. Project goals were accomplished through the establishment of a greenhouse common-garden study at Lakehead University, involving 10 Canadian and Northern Great Lake States yellow birch provenances. Two-year-old seedlings were measured for functional traits such as height, diameter, leaf morphology, biomass, and stable carbon isotopes. Data describing climate at seed origin was obtained to investigate patterns of local adaptation among the yellow birch provenances. Due to the wide-spread distribution of yellow birch and its polyploid genome, I hypothesize that (i) high levels of genetic variation among provenances will be present, and (ii) significant variation in functional traits will strongly correlate with climate at seed origin, providing evidence for adaptive variation among yellow birch populations.

LITERATURE REVIEW

ENVIRONMENTAL VARIATION IN PLANT FUNCTIONAL TRAITS

Biomass Allocation

Functional traits specifically linked to drought stress (such as biomass allocation) have been shown to vary in response to differing soil moisture conditions (Aspelmeier & Leuschner, 2006; Rasheed & Delagrange, 2016; Zhang et al., 2004), nutrient conditions (Wang et al., 1998), and light conditions (Beaudet & Messier, 1998; Delagrange et al., 2004; Logan, 1965; Messier & Nikinmaa, 2000). To cope with dry conditions, trees can increase root biomass to increase water uptake, usually caused by a shift in hormones (i.e. abscisic acid) (Wilkonsin & Davies, 2010). Depending on the abundance of nutrients, water, or sunlight, individuals will change the concentration of nutrients to the roots, stem, branches, or leaves (Beaudet & Messier, 1998; Delagrange et al., 2004; Messier & Nikinmaa, 2000). Studies have found intraspecific variation in biomass allocation under varying environmental gradients for hardwood trees (Logan, 1965), including yellow birch (Rasheed & Delagrange, 2016), silver birch (*Betula pendula* Roth) (Aspelmeier & Leuschner, 2006) and paper birch (Wang et al., 1998).

Wang et al. (1998) investigated variation in biomass and relative growth rate among four geographically distinct paper birch populations when exposed to varying water and nitrogen treatments. They found less variation in measured traits among seedlings grown in the high water, high nitrogen treatment, or when resources were not limited. Among all populations, relative growth rate was positively correlated with foliage biomass. Furthermore, there were no significant differences in total biomass among populations when grown in the low water, high nitrogen treatment. Greater root:shoot ratios were found in low nitrogen treatments, but water availability did not significantly affect root:shoot ratios. Overall, nitrogen availability had a greater effect on seedling biomass than water availability, suggesting these populations were better adapted for drought conditions than for low soil nutrient composition.

In another study, leaf and root morphological responses to drought treatments were compared between four genotypes of silver birch originating from climates with varying amounts of annual rainfall (Aspelmeier & Leuschner, 2006). Well-watered plants were significantly different in total leaf area (LA) and specific leaf area (SLA), but root characteristics did not vary significantly among genotypes. Drought treatments resulted in an increase in SLA and decrease in LA among all genotypes, as well as a decrease in specific fine root surface area (SRA). Interestingly, the ratio of total root to leaf surfaces remained constant under drought stress in all genotypes, despite the increase of fine root:leaf mass ratio (FR:LM). This was caused by a decrease in diameter and number of fine root hairs under drought conditions. The results from this study revealed that morphological responses of silver birch to drought are under strong genetic control.

Significant variation of plant morphology under drought conditions has also been shown in populations of *Populus davidiana* (Dode) (Zhang et al., 2004). Seedlings originating from dry, wet, and in-between climates were subjected to varying soil moisture treatments, resulting in significantly different plant responses. For example, seedlings originating from the wet climate resulted in greatest height, total biomass, total leaf area, and SLA when grown in the drought treatment. Furthermore, populations originating from the dry climate were the most negatively affected by drought, with the lowest height, total biomass, total leaf area, and SLA measurements. The authors concluded these differences are caused by adaptations to the environment; the dry climate populations responded to drought by slow growth in order to maintain physiological functions under long periods of drought, whereas the wet climate populations take advantage of water availability and grow relatively quickly to increase their potential in the face of competition.

Leaf and Branch Morphology

Changes in leaf morphology are an efficient way for trees to acclimate to current environmental conditions, allowing for a change in rates of photosynthesis and transpiration (Xu et al., 2009). Tree survival and fitness may also be influenced by changes in leaf morphology (Westoby et al., 2004). Therefore, variation in leaf morphology is a good indicator of a plant's uptake, efficiency, and resource usage (Xu et al., 2009). Significant variation in leaf shape, size, and thickness has been shown to occur globally across numerous tree species (Beaudet & Messier, 1998; Pyakurel & Wang, 2013; Westoby et al., 2013; Wright et al., 2005, 2018). One important leaf character is specific leaf area (SLA), which is the ratio of leaf area to leaf dry mass (cm^2 g^{-1}). It is a critical factor in growth rate as a higher SLA in shaded environments increases the area for capturing light per unit of leaf biomass (Beaudet & Messier, 1998; Xu et al., 2009). Thicker leaves tend to have a lower SLA, whereas thinner leaves have a higher SLA. Trees with high SLA are generally adapted to cool and wet environments, whereas trees with a lower SLA tend to perform best in hot and dry environments (Xu et al., 2009). Significant variation of SLA among coastal and interior populations of Douglas fir has been negatively correlated with altitude at seed origin (Zhang et al., 1993).

Variation in leaf morphology of Southern beech (*Nothofagus cunninghammi* Hooker) was compared among seedlings grown at different sites with varying elevation to seedlings grown in a uniform glasshouse environment (Hovenden & Vander Schoor, 2004). Increasing altitude had a strong negative correlation with SLA among seedlings grown in the glasshouse, but conversely, a positive correlation among field-grown sites. Field grown leaves had an increase in stomatal density with increasing altitude of sites, but within the glasshouse, seedlings originating from higher elevations had decreased stomatal density. This study provided important insight to the differences of environment on leaf characteristics among Southern beech populations, with environmental conditions explaining a greater proportion of variation among populations compared to altitude of seed origin.

Coastal and inland species of the flowering plant *Mimulus* have been shown to vary significantly in response to drought treatments (Wu et al., 2010). In that study, coastal species which typically inhabit areas with high soil moisture had the lowest SLA values, but also thicker and wider leaf venation, whereas species originating from drier areas had relatively high SLA values. However, the contrasting SLA trends may be due to perennial plants reportedly having lower SLA compared to annual plants.

Rasheed & Delagrange (2016) used LIDAR technology to examine variation in branch architecture among yellow birch seedlings grown water-stressed and well-watered conditions. Individuals in the water-stressed treatment had significantly decreased long axis branches and increased short axis branches compared to individuals grown in the well-watered treatment. Low water treatments also significantly decreased the number of leaves, total leaf area, and average leaf length. This decrease in leaf biomass may be an adaptation for drought conditions, allowing the seedling to decrease water consumption without drastically changing larger structures or functions. However, there was no significant variation in root-to-shoot ratios among the seedlings. While the findings of this study are insightful, the data is limited due to the small sample size (n=30) and the seedlings originated from a single population. Past research on yellow birch has shown significant variation in leaf shape among populations, with site characteristics such as elevation and soil characteristics to be correlated with reported variation (Dancik & Barnes, 1975; Sharik & Barnes, 1979).

Water-use Efficiency

Water use efficiency (WUE) is an important measurement of carbon and water ratios occurring within and around a plant’s tissue and ecosystem (Donovan & Ehleringer, 1994; Farquhar et al., 1989; Kondo et al., 2004; O’Leary, 1988). WUE can be broken into three types: instantaneous, intrinsic, and integrated (Farquhar et al., 1989). Instantaneous WUE, W_t $(= A/E)$, refers to the ratio of photosynthetic carbon gain (assimilation) to water loss by transpiration, which occurs at the leaf level and is appropriate for short time scales (Farquhar et al., 1989). Integrated WUE is a more long-term measurement as it refers to the total carbon gain to the total water loss of a leaf, plant, or ecosystem and can be written as (Farquhar et al., 1989):

$$W_{\mathrm{p}} = \frac{\mathrm{A}\,(1 - \text{ø}_c)}{,\ \ (1 + \text{ø}_w)}$$

In this equation, ø_c refers to the fraction of assimilated carbon lost due to respiration and ø_w refers to the fraction of total “unproductive” water loss due to non-photosynthetic tissues or opened stomata at night. Lastly, intrinsic WUE, W_g (= A/g_s), is the ratio of net assimilation to stomatal conductance, occurring at the leaf and plant level (Osmond et al., 1980). Although each of these measurements of WUE are insightful, we are mostly concerned with integrated WUE, as it reflects the overall water use throughout the plant over long periods of time (Seibt et al., 2008). Plants with thicker leaves (low SLA) tend to have higher levels of carbon isotope discrimination (Δ) (Zhang et al., 1993). Furthermore, high leaf-level WUE is considered an important adaptive trait that may assist in minimizing water loss in drought conditions (Diefendorf et al., 2010; Ferguson et al., 2018; Seibt et al., 2008). For example, in *Arabidopsis,* significant variation within the species has been shown in functional traits when subjected to well-watered and low-water treatments (Ferguson et al., 2018). Traits such as stomatal conductance and carbon assimilation were positively correlated with one another, whereas traits such as WUE, transpiration, and stomatal conductance were negatively correlated with one another. However, no correlations occurred between measured physiological traits and plant growth traits.

A common way to measure WUE is through stable carbon isotope analysis, which has provided critical understanding of plants and their response to the environment (Dawson et al., 2002; Farquhar et al., 1989; Leavitt & Long, 1986). Carbon exists in two stable forms, ^{12}C and ^{13}C. During photosynthesis, plants discriminate against ^{13}C, which is the heavier isotope and forms slightly stronger bonds (Dawson et al., 2002; Farquhar et al., 1989; O'Leary, 1988). This discrimination (Δ) of carbon isotopes provides the foundation of isotope analysis in plant tissues (Dawson et al., 2002). Plants contain less ^{13}C than the atmosphere, due to the processes involved in CO_2 uptake and the discrimination against ^{13}C (Dawson et al., 2002). The diffusion of $^{13}CO_2$ is slower than $^{12}CO_2$ because of this difference in mass. The equation, $R= {}^{13}C/ {}^{12}C$ is the ratio these two isotopes exist in plant tissue, the atmosphere, wood, or other materials (Leavitt & Long, 1986). For isotope analysis, R values are converted to $\delta^{13}C$ values using the following equation:

$$d131 = \left[\frac{3\,(sample)}{3\,(sample)} - 1\right] x\ 1{,}000$$

(referred to as the PeeDee (PDP) limestone standard) and presented in "per mil" units (‰) (Dawson et al., 2002). During the process of photosynthesis, C3 plants fix CO_2 using the enzyme ribulose biphosphate carboxylase. This fixation leads to a difference in isotope abundance (O'Leary, 1988). A majority of plants (C3, C4, and CAM) have $\delta^{13}C$ values in the range of -25‰ to -35‰ (Craig, 1953, 1954). However, studies have shown a clear distinction of $\delta^{13}C$ values between C3 and C4 plants, with a mean of -28‰ for C3 plants and a mean of -14‰ for C4 plants (O'Leary, 1988).

Due to the strong relationship between plant photosynthesis and carbon isotopes, many studies have been performed globally and across plant species. Stable carbon isotope values were measured in a variety of plants (C_3 trees and C_4 grasses) across habitat gradients in South Africa (Codron et al. 2013). Unexpectedly, this study found no climate effects correlated with $\delta^{13}C$ values, but there were significant differences between plants in riverine habitats (low $\delta^{13}C$ values) compared to those in arid, dry, and open woodland habitats (high $\delta^{13}C$ values). Conversely, a meta-analysis found strong positive correlations between mean annual rainfall (MAP) and Δ_{leaf} values of woody plants (Diefendorf et al., 2010). Significant differences in fractionation existed between major biomes included in the meta-analysis, with highest Δ_{leaf} values found in tropical rainforests and lowest Δ_{leaf} values in xeric woodlands. Furthermore, Δ_{leaf} values were greater among cool-cold deciduous forests than Δ_{leaf} values in cool-cold evergreen forests.

Intraspecific variation of $\delta^{13}C$ has been reported among Douglas fir (*Pseudotsuga menziesii* (Mirbel) Franco), with significant differences occurring among populations originating from the Rocky Mountains when grown in a common garden. This study found significant correlations between geographic location of seed origin and $\delta^{13}C$ values, with populations from the Southern Rockies having the highest $\delta^{13}C$ and therefore, lowest WUE, compared to the Northern Rockies populations. These results are opposite of in situ trends previously reported for Douglas fir, which the authors concluded in situ trends of $\delta^{13}C$ may be more strongly affected by the environment than genetic variation.

Genetic variation of $\delta^{13}C$ has also been reported among ponderosa pine (*Pinus ponderosa* Douglas ex. Lawson) seedlings, with limited water availability significantly decreasing these values (Olivas-Garcia et al., 2000). Two-year-old seedlings grown from seed originating from Nebraska, Wyoming, New Mexico, and South Dakota were compared in a variety of physiological traits, including carbon isotope discrimination (Δ). This study found drought treatment, seed source, and family within seed source to strongly affect Δ, with lowest values occurring in seedlings from New Mexico and Nebraska.

Among hardwoods, genetic variation of $\delta^{13}C$ has been shown in species such as black poplar (*Populus nigra* Linnaeus) (Guet et al., 2015), *Poplar* hybrids (Dillen et al., 2008), and European beech (*Fagus sylvatica* Linnaeus) (Robson et al., 2012) when grown in a common garden. The results from these studies are similar in that intraspecific variation is strongly correlated to the geographic location of seed source. Furthermore, variation in $\delta^{13}C$ values tend to be more prominent when plants are exposed to drought stress compared to well-watered treatments.

COMMON GARDEN RESEARCH

Common garden studies with sampling from multiple populations across a species range are crucial to understand the adaptive nature of functional traits that may vary among tree populations (Rehfeldt et al., 2018). Traditionally, common garden studies were used to identify optimal seed sources for reforestation projects (Matyas, 1996) but more recently, common garden research has largely been used to predict population responses to climate change (Lu et al., 2016). An individual plant's phenotype is the result of its genotype plus the environment it is growing in (Frankham et al., 2013). Therefore, when different genotypes of the same species are grown in a controlled, uniform environment, phenotypic variation that is observed can be attributed to be the result of underlying genetic differences.

Functional traits such as height, diameter, biomass, and leaf morphology have been shown to vary significantly among provenances in hardwood species such as yellow birch (Clausen, 1967; Wearstler et al, 1977), paper birch (Pyakurel & Wang, 2013), trembling aspen (Thompson, 2014), and white birch (Oke, 2009) when grown in a uniform environment. Provenance data has also been used to detect local adaptation for conifers including white spruce (Lesser, 2005; Lu et al., 2016), black spruce (Parker, Riddell, & Lesser, 2004), jack pine (Thomson & Parker, 2008), and white pine (Joyce & Rehfeldt, 2013). Understanding the variation in functional traits within a species can provide insight as to how specific populations may respond to future climate change conditions.

Data from common garden studies can also be used to investigate variation in patterns of local adaptation among species. For example, provenance data for five North American hardwood species including yellow birch, red maple, black walnut, black cherry *(Prunus serotine* Ehrhart*)*, and northern red oak, revealed contrasting pattens suggesting that evolutionary trade-offs favouring either cold tolerance or increased growth had occurred (Leites et al., 2019). Different climatic variables were associated with varying strength of growth responses in each species. For height, mean temperature of the coldest month (MTCM) had the strongest effect with red maple, mean annual temperature (MAT) had the strongest effect with black walnut, and both mean temperature of the warmest month (MTWM) and mean maximum temperature of the warmest month (MMTWM) had the strongest effect with northern red oak. All climate variables had strong correlations with height of black cherry, whereas no climate variables were significantly correlated with height of yellow birch.

ADAPTIVE VARIATION TO CLIMATE IN NORTHERN HARDWOOD TREES

Temperate hardwood and boreal forest trees typically have growth traits strongly shaped by environmental gradients (Aitken & Bemmels, 2016; Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008; Clausen, 1975, 1980; Leites et al., 2019;

Matyas, 1996; Vitasse et al., 2009). These traits may include height, leaf phenology and morphology, flowering times, and growth cessation. Among conifers, photoperiod has been shown to correlate with bud burst and bud set in Norway spruce (*Picea abies* (Linnaeus) Karsten) (Matyas, 1996), Douglas fir (Campbell and Sugano 1975) and cold-tolerance of lodgepole pine (Rehfeldt et al., 1999). In both conifers and hardwoods, trees originating from warmer climates tend to have higher growth rates in mild environments and lower growth rates in colder environments, leading to a strong correlation between length of growing season and phenological traits such as bud burst, bud set, and period of leaf and shoot development (Howe et al., 2003; Leites et al., 2019).

Northern hardwood and boreal forest tree species have shown strong to moderate response of growth and phenology in relation to environmental gradients (Aitken et al., 2008). For widespread tree species, it has been suggested that temperature is the driving force behind genetic differentiation among a species, whereas both temperature and precipitation are the drivers of the spatial distribution of the species (Moles et al., 2014; Rehfeldt et al., 2018). Several studies have compared yellow birch to other temperate hardwood species such as sugar maple, American beech, or northern red oak (Beaudet & Messier, 1998; Delagrange, 2011; Delagrange et al., 2004; Gaucher et al., 2005; Leites et al., 2019; Logan, 1965; Messier & Nikinmaa, 2000). For example, one study tested latitudinal gradients and frost tolerance of three temperate hardwood species (Calmé et al., 1994). Yellow birch, sugar maple, and northern red oak seedlings were subjected to freezing trials by being overwintered outdoors. After observing primary root tissue, total percent root damage of each individual was calculated. Bud measurements, such as bud set and bud burst, and mitotic frequency were recorded. Northern red oak was least tolerant of freezing damage in terms of stem and root health and function, with only 7%

survival, followed by 50% survival of sugar maple. Overall, yellow birch was found to be the most frost-tolerant species, with 100% of the seedlings surviving the winter. Northern red oak root tissue had a freezing tolerance of -23ºC, whereas the roots of sugar maple could withstand -33ºC. In contrast, yellow birch roots remained alive in -33ºC, which was the lowest recorded temperature of the test. All three species have similar distributions in the northern range, but northern red oak extends farther south than the other two species. A study comparing yellow birch and sugar maple seedlings revealed significant variation of traits such as leaf mass area, leaf nitrogen content, photosynthesis rates, and leaf nitrogen partitioning in differing light environments and during three growing season stages (Delagrange, 2011). Both species showed high plasticity of leaf morphology in varying light conditions, which appeared to be the most efficient way for both species to respond to low light conditions while maintaining physiological functions.

BETULA ALLEGHANIENSIS (BRITTON)

Species Range and Importance

Yellow birch is an ecologically important tree ranging from the Atlantic Coast to southeastern Manitoba, across the Midwest states and south to the Appalachian Mountains (Clausen, 1973) (Figure 1). The species optimally grows in mesic forest types with 50% full sun exposure and moist, well-drained soils, such as these major Eastern North American forest types: Hemlock-Yellow Birch, Sugar Maple-Beech-Yellow Birch, and Red Spruce-Yellow Birch (Erdmann, 1990). The average annual temperature across its range is reported at 7°C, with extremes from -40° C to 38° C, while the growing season ranges from 60 to 150 days (Clausen, 1973). Annual precipitation ranges from 1270 mm on the eastern coastal range to around 640 mm in the western limits (Erdmann, 1990).

Economically, yellow birch is extremely valuable and is predominantly used for hardwood flooring, furniture, cabinets, doors, and other wood products (Juncus, 1998). The common name, yellow birch, comes from the distinct caramel-yellow bark, which peels in thin, short strips and gives off a wintergreen scent when crushed (Erdmann, 1990). The species is monoecious, with male and female catkins that mature in September through October, and disperse by wind throughout the winter months (Clausen, 1973). Yellow birch is considered an early to mid-successional species with intermediate shade tolerance, commonly outcompeted by paper birch and sugar maple during the seedling stage, as the species is relatively light and soil sensitive. Yellow birch require openings in the canopy and disturbances to soil beds for successful establishment (Erdmann, 1990).

Individual yellow birch trees can live up to 300 years and provide many benefits to the forest ecosystems they inhabit (Erdmann, 1990). A variety of birds, squirrels, and chipmunks eat the wind-dispersed seeds throughout the winter, while white-tailed deer, snowshoe hare, and moose graze on seedlings for nutrients. Yellow bellied sapsuckers rely on food supplies of birch sap in the spring and feed on insects and larvae that are burrowed in the trees (Erdmann, 1990). Recent research discovered a significant number of unique lichen species that are supported by yellow birch trees on the Avalon Peninsula of Newfoundland (Wigle et al., 2021). A variety of beetles, bugs, caterpillars, and larvae burrow in yellow birch trees, lay eggs, and feed on the leaves throughout the summer (Erdmann, 1990). Many fungi are detrimental to yellow birch, causing disease and dieback within entire stands.

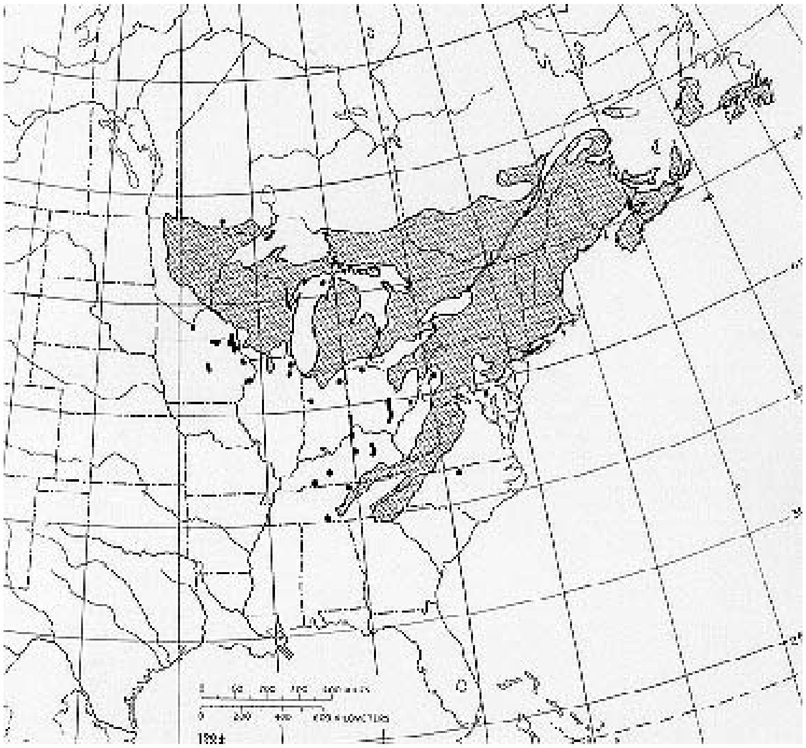

Figure 1. Native range of yellow birch (Erdmann 1990).

Intraspecific Genetic Variation

Most provenance research of yellow birch took place during the late 1900's (Beaudet & Messier, 1998; Calmé et al., 1994; Clausen, 1967, 1968, 1972, 1974, 1975, 1980; Dancik & Barnes, 1975; Wearstler et al., 1977). Those studies revealed variation among and within populations in seedling height growth and cessation (Clausen, 1967), early growth and survival (Clausen, 1974), flowering times (Clausen, 1980), and catkin characteristics (Clausen, 1968). Clausen (1967) investigated phenotypic variation amongst yellow birch seedlings collected from 55 localities across the range when grown in a common garden. The greenhouse design was randomized in blocks, with 5-10 individuals representing each seed source. After the first and second growing seasons, the height of 20 seedlings from each block was measured and recorded. Significant variation of height growth was present among seedlings from different provenances. However, height growth of 2-year-old seedlings was not significantly correlated to any of the tested geographic or climate variables. In contrast, the timing of growth cessation differed significantly among provenances, with the eastern Newfoundland population ceasing growth one week earlier than the average, and the southern Kentucky population remaining active over one week later than the average. These results described an inverse correlation with growth cessation and the latitudinal gradient, most likely due to photoperiod cues (Clausen, 1967).

Clausen (1968) investigated fruit and catkin characteristics using the same seed sources from the previous study. Catkins were collected from 17-25 trees in each population, and five catkins from each tree were randomly measured. Characteristics measured were lengths of rachises, dimensions of bracts and nutlets, as well as length and width ratios. Significant variation of all characteristics was demonstrated, although there was no apparent clinal pattern of variation among populations. Clausen (1974) reported on the survival and height growth among these seed sources after five years growing in the field at 11 sites across the U.S. and Canada. Seed origin was strongly correlated to seedling survival, as more northern provenances had higher survival rates than southern provenances when grown in the most extreme climates (i.e. Wisconsin and northern Michigan plantations). However, variation among provenances was not as prevalent at the other plantation sites with milder climates. Despite significant variation in height growth among provenances, it was independent from seed origin in all plantations. This study revealed differences in performance across provenances when seedlings were grown in varying climates, indicating the importance of seed source selection depending on the location.

To better-understand the possible causes of phenotypic variation in yellow birch that had been shown by Clausen's studies, Dancik and Barnes (1975) investigated the potential correlation between site characteristics and variation in leaf morphology. Populations in Michigan, Wisconsin, and Minnesota were divided into 18 districts within six major regions categorized by their physiographic characteristics. Leaves from each individual tree were collected, pressed, and measured for a variety of phenotypic characters. Soil characteristics (such as acidity and drainage), elevation, and coordinates were also recorded for each collection location. Significant variation in leaf characters was observed, and multiple regression analyses revealed that variation was explained by site factors such as drainage and pH. Moreover, significant variation in leaf characters occurred between populations located near Lake Superior and those that were more inland or continental. The authors concluded that the variation in morphological traits were not due to genetic variation, but to variations in phenotypic responses to site variables. However, a study of yellow birch populations in Michigan found that variation in seedling height was significantly correlated to latitude of seed origin, suggesting the impacts of temperature and photoperiod on seedling development (Wearstler et al., 1977).

Sharik and Barnes (1979) found significant variation among yellow birch populations growing in the Appalachian Mountains. Of the 47 tree characteristics observed, 42 were significantly different among the 30 populations of yellow birch. Measured traits included leaf characters (i.e. blade length, blade width, tooth serration, venation, and bract length), fruit (i.e. samara length and width), bark, and pollen.

Phenotypic variation between populations was shown to be related to provenance latitude and elevation. For example, increasing latitude was associated with increased blade length and width, as well as wider leaf venation, coarser leaf serration, and narrower samara bodies. Conversely, increasing elevation was associated with opposite trends, which may have leveled out significant variation among provenances. While significant differences in traits occurred among populations, high within-population variation was observed. The lack of consistency in clinal patterns and weak correlations with site variables (*r* values <= 0.72) led the authors to conclude their study was unsuccessful in revealing strong trends in adaptive variation among yellow birch populations. As such, further studies including a greater range of populations and sample size were recommended.

CHAPTER II

VARIATION IN DROUGHT RELATED TRAITS AMONG YELLOW BIRCH POPULATIONS AND EFFECT OF CLIMATE AT SEED ORIGIN

INTRODUCTION

Increases in frequency and severity of extreme climatic events is expected to negatively affect forest health and productivity due to climate change (Allen et al., 2010) and increased tree mortality over recent decades has been linked to elevated drought stress (O'Brien et al., 2017). Eventual mortality of stressed trees may either be physiological (carbon starvation or hydraulic failure) or indirect (takeover by pests or pathogens). Understanding of variation in functional traits within tree species can provide insight as to how specific populations may respond to stress due to climate change.

Common garden (aka 'provenance') studies are effective ways to reveal genetic variation within a species (Matyas, 1996). When grown under a controlled, uniform environment, observed phenotypic variation can be inferred due to underlying genetic differences, rather than plastic responses to the environment. Functional traits such as height, diameter, biomass, and leaf morphology have been shown to vary significantly among provenances in hardwood species such as yellow birch (Clausen, 1967; Wearstler et al., 1977), paper birch (Pyakurel & Wang, 2013), trembling aspen (Thompson, 2014), and white birch (Oke, 2009) when grown in a uniform environment.

Yellow birch is an ecologically important North American hardwood tree that ranges from the Atlantic Coast to southeastern Manitoba, across the Midwest states and south to the Appalachian Mountains (Clausen, 1973). Although common garden studies have been conducted for yellow birch (Clausen, 1967; Clausen & Garrett, 1968; Dancik & Barnes, 1975; Sharik & Barnes, 1979) no studies to date have tested specifically whether phenotypic variation in leaf traits and water use efficiency is due to local adaptation to climate. Therefore, the objectives of this study were to (i) investigate the variation in functional traits such as height, diameter, leaf morphology, biomass, and stable carbon isotopes that may be present among Canadian and Great Lakes States yellow birch populations and (ii) examine whether trait variation is significantly correlated to variations in climate at seed origin.

MATERIALS & METHODS

SEED SOURCES AND EXPERIMENTAL DESIGN

Yellow birch seed was obtained from 10 provenances across Canada and Northern US states, including northwestern and eastern Ontario, New Brunswick, Newfoundland, Michigan, and Minnesota. Each location had at least five trees collected, while some locations' seeds were collected from up to 10 trees. Seed sources ranged from 45.94713º to 48.67692º latitude and -58.19208º to -93.89938º longitude (Figure 2). Northwestern Ontario seed sources (Squaretop and Greenwood) were collected on October 6 and October 10, 2019, respectively. Dominant trees were selected at least 50 meters apart to minimize the chance of sampling full or half-sibs (Thomson et al., 2015). The collected seeds were sent to the National Tree Seed Centre for further cleaning and processing. The remainder of the seed sources were provided by the National Tree Seed Centre in Fredericton, New Brunswick (Provenance 1-4) and J.W. Toumey Nursery in Watersmeet, Michigan (Provenance 7-10).

Table 1. Provenance number, location, and geographic coordinates.

Provenance #	Location	Province/State	Lat	Long
9	Chippewa NF	MN	47.58378	-93.89938
8	Superior NF	MN	47.89521	-91.01247
5	Greenwood	ON	48.39344	-90.75198
10	Ottawa NF	MI	46.3587	-89.81761
6	Squaretop Mt.	ON	48.28006	-89.39645
7	Hiawatha NF	MI	46.23304	-86.50807
4	Petawawa	ON	46.0000	-77.43333
1	Black Brook	NB	47.4500	-67.4500
3	Big Pond	NL	45.94713	-60.4426
2	Gallants	NL	48.67692	-58.19208

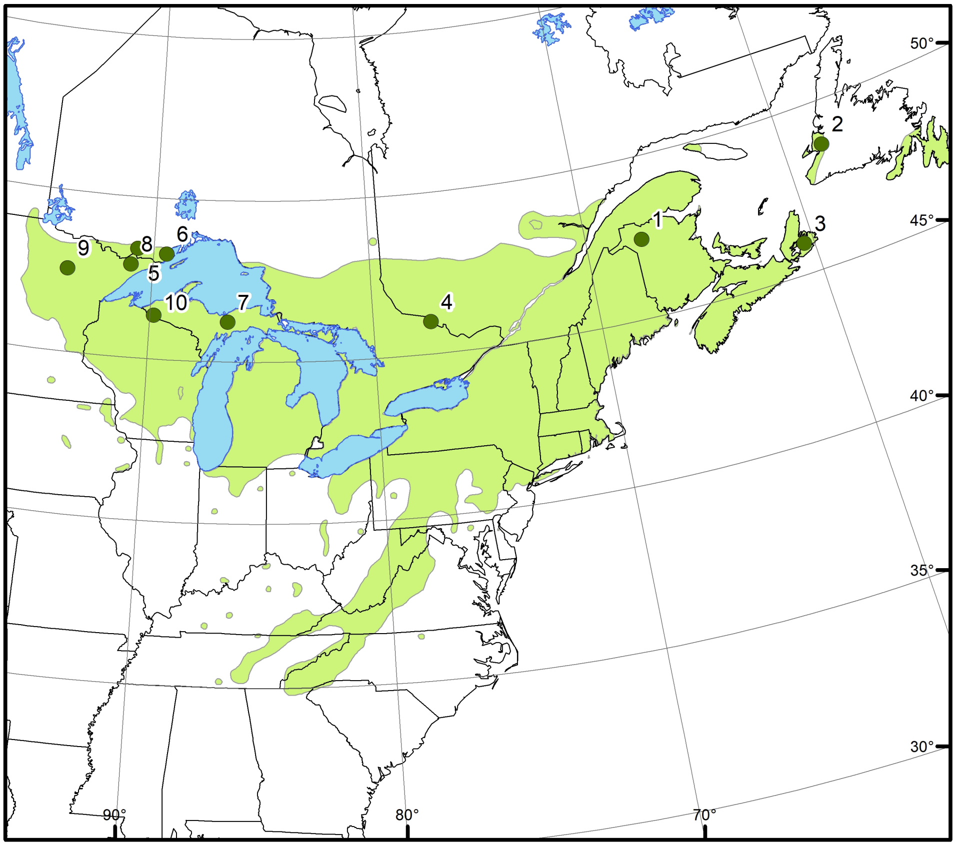

Figure 2. Provenance locations. Green dots indicate the location of seed origin while numbers indicate the number used to track each seed source in the greenhouse experiment. The green shading indicates the natural range of yellow birch.

Prior to sowing, the seeds underwent a week-long stratification process, where they were transferred back and forth from the freezer to the fridge at 24-hour intervals. Seeds were sown on May 15 and 16, 2020. The growing medium was a 60:40 peat and vermiculite mixture and at least two seeds were sown per cell in a 28/340 ml Styrofoam SuperBlock (Beaver Plastics Ltd.). The seedlings were grown in ambient conditions (min temp =17.91ºC, max temp = 24.67ºC, ave temp = 19.2ºC) for the duration of the experiment. After germination, the seedlings were thinned so that only one seedling remained per cell. The seedlings were fertilized with 15-15-18 Fertilizer (Plant Products, Soilless Feed) and were kept in the greenhouse throughout the winter. Once seedlings were dormant, the watering schedule was reduced to twice a week. On May 21, 2021, 200 seedlings were randomly chosen to be transplanted into 6.5x7 inch pots in a study design with four blocks each containing five replicates of each of the ten provenances. Once transplanted, the seedlings were fertilized with a slow release 16-14-18 granular fertilizer (Scott's Fertilizer, 2% added iron). The seedlings continued to be well-watered throughout the summer and were treated with a Neem oil solution for aphid control for the months of September and October 2021.

GROWTH AND BIOMASS MEASUREMENTS

Total seedling heights were measured for all 200 seedlings (20 seedlings per provenance) to the nearest half centimeter on September 15, 2021. Diameter of the 200 seedlings was also measured to the nearest millimeter at the base of the root collar using digital calipers. On October 9, 2021, 40 trees (four seedlings per provenance) were randomly selected for biomass, leaf morphology, and stable isotope measurements. A subsample of one seedling per provenance per block was used due to the destructive nature of these data collection methods. The top 3rd, 4th, and 5th mature leaves were collected, placed in plastic bags, and stored in the fridge prior to scanning for leaf morphology measurements. The remaining parts of the trees were prepared for biomass measurements by being separated into aboveground (leaves, branches, and stem) and belowground (roots) biomass (Gao et al., 2017). The seedlings were gently rinsed off to remove excess soil and debris prior to being dried in an oven at 60 degrees Celsius for 72 hours. Dry weights were taken of each component and recorded to the nearest gram using a precision balance.

LEAF MORPHOLOGY

Three scanned leaves from each seedling were analyzed using WinFOLIA software 2004a (Regent Instruments Inc. 2004). Advanced and basic morphology were chosen for the analysis, which included measurements of individual leaf area, horizontal width, perpendicular width, vertical length, perimeter, form coefficient, and aspect ratio. Other parameters included in the analysis were two lobe angles measured at two different heights of blade length (10% and 25% of height) and two blade width measurements taken at two different heights of blade length (50% and 90% of height). The blade length was measured from the blade/petiole junction to the topmost point of the leaf. These measurements were included as they provide a more complete picture of leaf shape, rather than overall size that could be measured by area or perimeter. Perpendicular width 1 was measured at the middle of the leaf (50% of height), whereas perpendicular width 2 was measured at the topmost portion of the leaf (90% of height). Maximum perpendicular width was measured as the maximum width perpendicular to blade length. An image including a visual representation of these parameters is included in Appendix VII. Total leaf area was calculated using the data output from WinFOLIA. Specific leaf area (SLA) was calculated as the ratio of total leaf area to leaf dry mass (Beaudet and Messier, 1998). Images of scanned leaves by provenance are included in Appendix VI.

CARBON AND NITROGEN STABLE ISOTOPE ANALYSIS

The same leaves that were scanned were also dried in the oven and weighed, then added to the total leaf weight data. The dried leaves were homogenized using mortar and pestle, weighed to approximately 30 milligrams, and shipped in glass vials to the Environment Isotope Lab at the University of Waterloo for $\delta^{13}C$ and $\delta^{15}N$ isotope analysis. The $\delta^{13}C$ data ($\delta^{13}C$ IRMEA / VPDB column) is the corrected delta value, reported in per mil (‰) units, against the primary reference scale of Vienna Pee Dee Belemnite (VPDB) (EIL, University of Waterloo). Further details of the methods used to conduct the stable-isotope analysis can be found in Appendix II.

CLIMATE DATA

Climate N.A. Software was used to generate climate variables for all seed sources (Wang et al., 2016). The coordinates for each provenance were entered into the software and 29 climate variables were selected based on annual and seasonal data. In the case of bulk seed provided for a provenance (Provenance 7, 8, & 9), a single centralized coordinate was used to obtain climate information. Climate data for years 1980-2010 was chosen for this analysis. Climate variables were chosen based on a review of the strength of their effect on tree species’ growth and function (Aitken & Bemmels, 2016; Leites et al., 2019; Moles et al., 2014) and are further listed with their definitions and units in Appendix III (Table 13). Climate variables expected to play key roles in driving variations of tree characteristics are listed in Table 2, along with the ranges by provenance. MAT differed from 3ºC in Provenances 1 and 6, 4.5ºC in Provenance 10, and 5.7ºC in Provenance 7 (Table 2). DD5 differed from 1438 in Provenance 6, 1485 in Provenance 1, 1766 in Provenance 10, and 1850 in Provenance 7. AHM ranged from 12.2 in Provenance 1 to 18.6 in Provenance 7. PPT_SM varied the most between Provenance 1 and 7, with an average of 321 mm and 239 mm of rainfall, respectfully.

Table 2. Key climate variable values represented by each provenance. See Appendix III for a complete list of climate variables with their associated definitions and units.

Provenance	MAT	MWMT	MAP	AHM	SHM	DD_0	DD5	DD18	EREF	TAVE_SP	TAVE_SM	PPT_SP	PPT_SM
1	3	17.6	1069	12.2	35	1348	1485	71	601	2.1	16.2	221	321
2	4.3	16.4	1295	11.1	30.8	817	1361	49	515	1.6	14.9	242	322
3	5.7	17.7	1510	10.4	33.1	632	1606	84	537	2.6	16	349	307
4	5.1	19.7	842	17.9	47.6	1105	1906	173	706	4.6	18.4	192	247
5	2.4	17.8	701	17.7	40.4	1574	1510	81	626	2.2	16.5	136	282
6	3	16.9	728	17.8	41.5	1325	1438	60	583	2.3	15.7	158	255
7	5.7	19	845	18.6	47.4	857	1850	145	696	4.4	17.8	184	239
8	2.8	18	731	17.5	39.5	1479	1548	89	633	2.6	16.7	150	291
9	4	19.4	684	20.5	43.2	1352	1806	157	650	4.1	18.2	153	288
10	4.5	18.8	833	17.4	40.5	1144	1766	131	671	4.1	17.7	189	282

STATISTICAL ANALYSIS

Data analysis was performed using SPSS statistics software (IBM SPSS, version 28). First, the data was checked for outliers and errors. Normal distribution of the data was checked by reviewing values of skewness and kurtosis using the Kolmogorov-Smirnov and Shapiro-Wilk tests, respectively. Factors such as SLA, horizontal width, maximum perpendicular width, perpendicular width 1, petiole length, petiole area, and root:shoot ratio were Log base 10 transformed to reach near-normal distribution. Horizontal width, maximum perpendicular width, and perpendicular width 1 values were reflected prior to transformation to obtain positive values. A two-way analysis of variance (ANOVA) was performed to determine whether significant variation occurred in height and diameter between provenances. All effects were considered random. The linear model used for the 200 seedlings was

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{ijk}$$

where i = 1 to 10 seed source, j = 1 to 4 blocks per test, k = 1 to 5 replicates per seed source per block, Y_{ijk} is the measured variable value of replication k of seed source i in block j, μ is the population mean, A_i is the random effect of seed source i, B_j is the random effect of block j, AB_{ij} is the random interaction effect of the ith seed source with the jth block, and ε_{ijk} is the random error effect of replication k of provenance i in block j (Thomson & Parker, 2008).

One-way ANOVAs were performed to examine whether significant differences in measured variables (i.e. height, diameter, leaf morphology, biomass, and stable carbon isotopes) occurred between provenances for the subsample of 40 seedlings. The linear model was modified due to the absence of within-block repetitions:

$$Y_{ij} = \mu + A_i + \varepsilon_{ij}$$

where $i = 1$ to total no. of seed sources, $j = 1$ to total no. of replicates for each seed source, Y_{ij} is the measured variable value of replication j of seed source i, μ is the population mean, A_i is the random effect of seed source i, and ε_{ij} is the random effect of replication j of provenance i (Thomson & Parker, 2008). Post-hoc Tukey HSD tests were performed to identify where the significant variation in measured traits occurred between provenances.

Simple linear regressions were performed to examine the relationship between provenance trait means and climate at seed origin. Highly correlated climate variables ($r>0.7$) were eliminated prior to the multiple regression analysis, resulting in four unrelated climate variables: MAT, DD5, AHM, and PPT_SM. Multiple stepwise univariate linear regressions were performed to identify the strongest groups of climate predictors and their correlations with provenance means. The criterion for entry and removal was based on the F-statistic set at 0.1 in order to produce significant models in SPSS.

RESULTS

ANALYSIS OF VARIANCE

The ANOVA for the full set of 200 seedlings did not result in significant variation among provenance mean height ($p=0.182$) or diameter ($p=0.215$) and can be found in Appendix V, Table A. 12. Block effects were significant for seedling diameter ($p=0.002$) but not height ($p=0.852$) (Appendix V, Table A. 12). The ANOVA for the subset of 40 randomly selected seedlings revealed significant differences among provenance means for six measured variables including height ($p=0.049$), average horizontal leaf width ($p=0.048$), horizontal leaf width ($p=0.028$), maximum perpendicular width ($p=0.048$), perpendicular width 1 ($p<0.001$), and perpendicular width 2 ($p=0.016$) (Table 3). Provenance 1 (Black Brook, NB) had the lowest height, measuring 38.5 cm, whereas Provenance 5 (Greenwood, ON) was the tallest at 73.9 cm (Table 3). A post-hoc Tukey HSD test showed significant variation in height between Provenances 1 and 5 (Appendix V, Table A. 9) which are geographically located in New Brunswick and Northern Ontario, respectively. These two provenances are near the far east and west extremes of the species range.

Average horizontal width varied between 0.89 cm for Provenance 7 (Hiawatha, MI) and 1.12 cm for Provenance 1 (mean = 1.039 cm +/- 0.018). Horizontal width varied between 0.06 cm from Provenance 1 and 0.16 cm from Provenance 7 (mean = 0.111 cm +/-0.009). Furthermore, maximum perpendicular width ranged from 0.06 cm (Provenance 1) to 0.16 (Provenance 7) (mean = 0.110 cm +/- 0.009). Perpendicular width 1 ranged from 0.05 cm (Provenance 1) to 0.17 (Provenance 7) (mean = 0.107 cm

+/- 0.029). Provenance 7 (Hiawatha, MI) had the narrowest perpendicular width 2 measurement, 0.17 cm, whereas Provenance 6 (Squaretop Mt., ON) had the widest, 0.34 cm (Table 3). Therefore, Provenance 1 and Provenance 7 had consistent significant differences in leaf morphology.

Table 3. One-way ANOVA results with mean, min and max values, significance, and standard error values. Mean values are all measured in units of cm. Horizontal width, maximum perpendicular width, and perpendicular width 1 were reflected and log-based 10 transformed.

Measured Variable	Mean	Min	Max	Sig	Std Error
Height	57.80	38.50	73.90	0.049	2.232
Ave Horizontal Width	1.04	0.90	1.12	0.028	0.018
Horizontal Width	0.11	0.07	0.17	0.048	0.009
Max Perp Width	0.11	0.07	0.17	0.048	0.009
Perp Width 1	0.11	0.05	0.18	<0.001	0.029
Perp Width 2	0.26	0.17	0.35	0.016	0.012

Post Hoc Tukey HSD tests determined significant variation in leaf morphology between specific populations. Provenance 1 differed significantly from Provenances 7 and 10 in mean perpendicular width 1 (p=0.004 and p=0.022, respectively) (Appendix V, Table A. 10). Furthermore, Provenance 6 differed significantly from Provenance 7 and 10 in mean perpendicular width 1 (p=0.007 and p=0.041, respectively). Perpendicular width 2 significantly differed between Provenance 1 and Provenance 7 (p=0.049) (Appendix V, Table A. 11). Provenance 1 originated from New Brunswick, Provenance 6 originated from Northwestern Ontario, and both Provenance 7 and 10 originated from Michigan (Table 1, Figure 2). Each of these provenances are close to the geographic extremes of the yellow birch range (Figure 2).

SIMPLE LINEAR REGRESSIONS

Mean provenance trait values were significantly correlated ($p<0.1$) with 12 climate variables including: mean summer precipitation (PPT_sm), mean warmest month temperature (MWMT), mean annual temperature (MAT), annual heat moisture index (AHM), summer heat moisture index (SHM), degree-days above 5ºC (DD5), degree-days above 18ºC (DD18), precipitation as snow (PAS), Hargreaves reference evaporation (EREF), Hargreaves climatic moisture deficit (CMD), spring mean temperature (TAVE_SP), and summer mean temperature (TAVE_SM), as well as the geographic variable longitude (LONG) (Table 4). Regression plots with p values ranging between 0.05 and 0.1 are presented in Appendix IV.

Six significant climate variables (MWMT, MAT, DD5, DD18, TAVE_SP, and TAVE_SM) were temperature-related, while two variables (PPT_SM and PAS) were precipitation-related and four variables (AHM, SHM, CMD, and EREF) were climate-moisture related (Table 4). The climate variable TAVE_SP significantly correlated ($p<0.05$) with every measured trait except height (Table 4). Although there were many correlations that were significant at both the 90% and 95% confidence levels, the correlations were relatively weak (Table 4). The highest R^2 value (0.676) was obtained for the regression between perpendicular width 2 and DD5 ($p=0.004$) (Table 4). The majority of the R^2 values ranged from 0.315 (perpendicular width 2 and MAT) to 0.497 (perpendicular width 2 and EREF) (Table 4).

Table 4. Simple linear regression results including measured variables and the corresponding R^2 values, significance, and climate variables. Significance levels indicated as $p<0.1$* and $p<0.05$**. See Appendix III, Table A. 2 for climate variable definitions.

Measured Variable	R2	Sig.	Correlated Climate Variable
Height	0.344	0.075*	PPT_SM
Ave Horizontal Width	0.395	0.051*	AHM
	0.325	0.086*	SHM
	0.397	0.051*	DD5
	0.385	0.055*	DD18
	0.406	0.047**	PAS
	0.444	0.035**	EREF
	0.407	0.047**	CMD
	0.455	0.032**	TAVE_SP
	0.426	0.041**	TAVE_SM
	0.354	0.069*	LONG
Horizontal Width	0.376	0.059*	AHM
	0.371	0.062*	DD5
	0.353	0.07*	DD18
	0.418	0.043**	PAS
	0.391	0.053*	EREF
	0.352	0.071*	CMD
	0.44	0.037**	TAVE_SP
	0.397	0.051*	TAVE_SM
Max Perp Width	0.327	0.084*	MWMT
	0.359	0.067*	AHM
	0.359	0.067*	DD5
	0.34	0.077*	DD18
	0.402	0.049**	PAS
	0.375	0.06*	EREF
	0.332	0.081*	CMD
	0.426	0.041**	TAVE_SP
	0.384	0.056*	TAVE_SM
	0.363	0.065*	LONG

Table 4 Ctd. Simple linear regression results continued.

Measured Variable	R2	Sig.	Correlated Climate Variable
Perp Width 1	0.37	0.062*	MWMT
	0.441	0.036**	DD5
	0.401	0.049**	DD18
	0.307	0.097*	PAS
	0.341	0.076*	EREF
	0.486	0.025**	TAVE_SP
	0.402	0.049**	TAVE_SM
Perp Width 2	0.315	0.091*	MAT
	0.608	0.008**	MWMT
	0.351	0.071*	SHM
	0.676	0.004**	DD5
	0.663	0.004**	DD18
	0.497	0.023**	EREF
	0.418	0.043**	CMD
	0.665	0.004**	TAVE_SP
	0.601	0.008**	TAVE_SM

No climate variables were significantly correlated with tree height at the 0.05 level. However, PPT_SM was significantly correlated with provenance height at $p<0.1$ ($R^2=0.344$; $p=0.075$) (Appendix IV, Figure A. 1). Average horizontal width had significant negative correlations with TAVE_SM ($R^2=0.426$, $p=0.041$), EREF ($R^2=0.444$, $p=0.035$), CMD ($R^2=0.407$, $p=0.047$), and TAVE_SP ($R^2=0.455$, $p=0.032$) as well as a positive correlation with PAS ($R^2=0.406$, $p=0.047$), (Figure 3). Other climate variables correlated at a 90% confidence level with average horizontal width such as MWMT, AHM, degree-days above 5ºC (DD5), and the geographic variable of longitude (Table 4; Appendix IV, Figure A. 2). Horizontal leaf width had a significant negative correlation with PAS ($R^2=0.418$, $p=0.043$) and a positive correlation with TAVE_SP ($R^2= 0.44$, $p=0.037$) at $p < 0.05$ (Figure 4). Climate variables AHM, DD5, CMD, and TAVE_SM were significantly correlated at $p < 0.1$ (Table 4; Appendix IV, Figures A. 3 & A. 4).

Provenance mean values of maximum perpendicular width had a significant negative correlation with PAS ($R^2=0.402$, $p=0.049$) and a positive correlation with TAVE_SP ($R^2=0.426$, $p=0.041$) (Figure 5). Other climate and geographic variables that correlated with maximum perpendicular width ($p<0.1$) included DD18, EREF, TAVE_SM, and LONG (Table 4; Appendix IV, Figures A. 5 & A. 6).

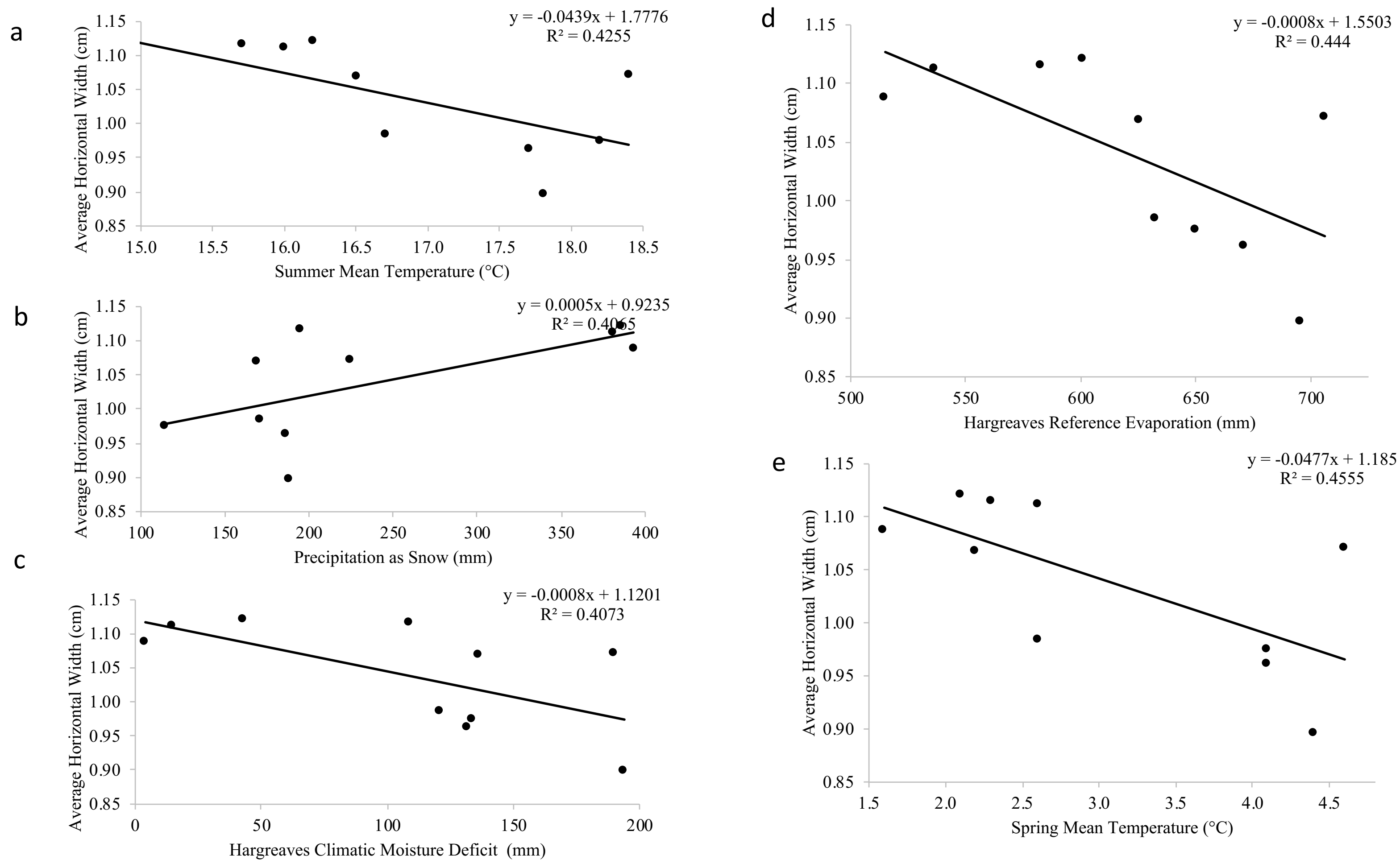

Figure 3. Simple linear regression analysis of average horizontal width and (a) summer mean temperature; (b) precipitation as snow; (c) Hargreaves reference evaporation; (d) climate moisture deficit; and (e) spring mean temperature.

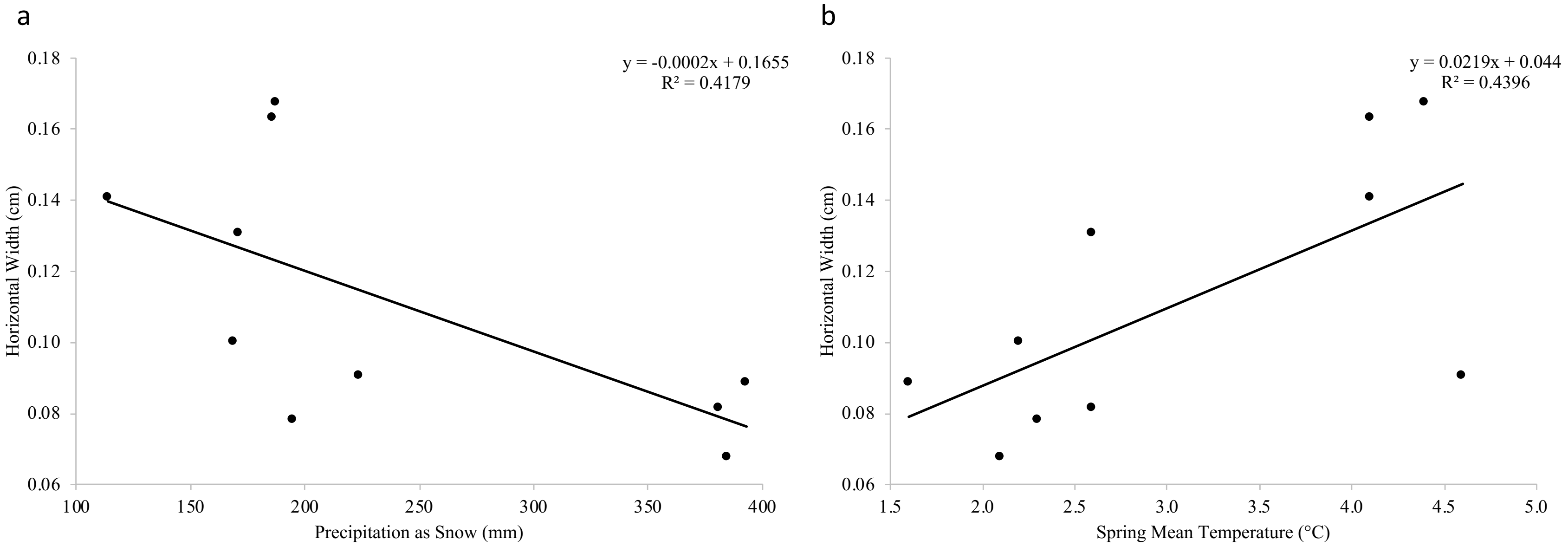

Figure 4. Simple linear regression analysis of horizontal width and (a) precipitation as snow and (b) spring mean temperature.

Provenance mean values of perpendicular width 1 resulted in significant positive correlations with climate variables DD5 (R^2=0.441, p=0.036), DD18 (R^2=0.401, p=0.049), TAVE_SP (R^2=0.486, p=0.025), and TAVE_SM (R^2=0.402, p=0.049) (Figure 6). Climate variables that correlated with perpendicular width 1 ($p<0.1$) included MWMT, EREF, and PAS (Table 4; Appendix IV, Figure A. 7).

Perpendicular width 2 had the strongest correlations with selected climate variables, resulting in seven climate variables negatively correlating at the 95% confidence level (Table 4). The significant climate variables include MWMT (R^2=0.608, p=0.008), DD5 (R^2=0.676, p=0.004), DD18 (R^2=0.663, p=0.004), EREF (R^2=0.497, p=0.023) (Figure 7), (CMD) (R^2=0.418, p=0.043), TAVE_SP (R^2=0.665, p=0.004), and TAVE_SM (R^2=0.601, p=0.008) (Figure 8). Furthermore, MAT and SHM correlated with perpendicular width 2 at $p<0.1$ (Table 4; Appendix IV, Figure A. 8).

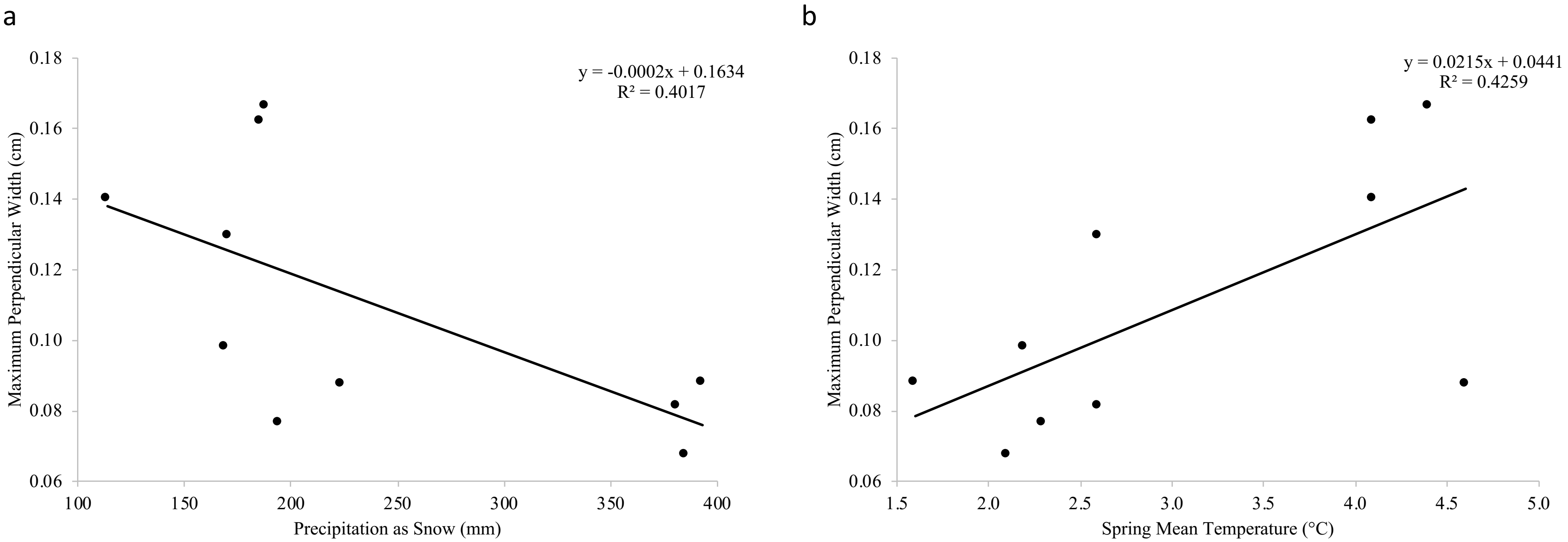

Figure 5. Simple linear regression analysis of maximum perpendicular width and (a) precipitation as snow and (b) spring mean temperature.

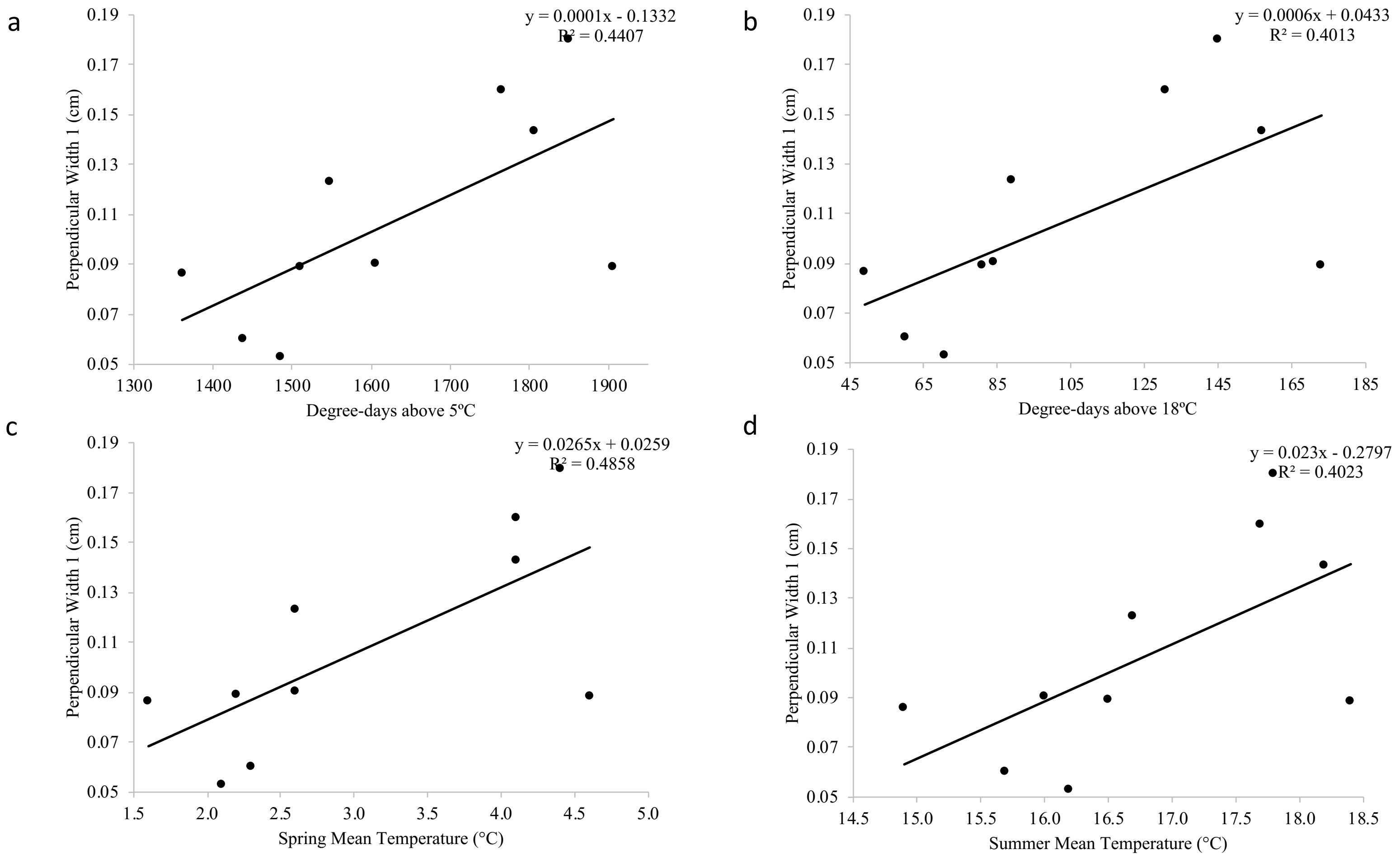

Figure 6. Simple linear regression analysis of perpendicular width 1 and (a) degree-days above 5ºC; (b) degree-days above 18ºC; (c) spring mean temperature; and (d) summer mean temperature.

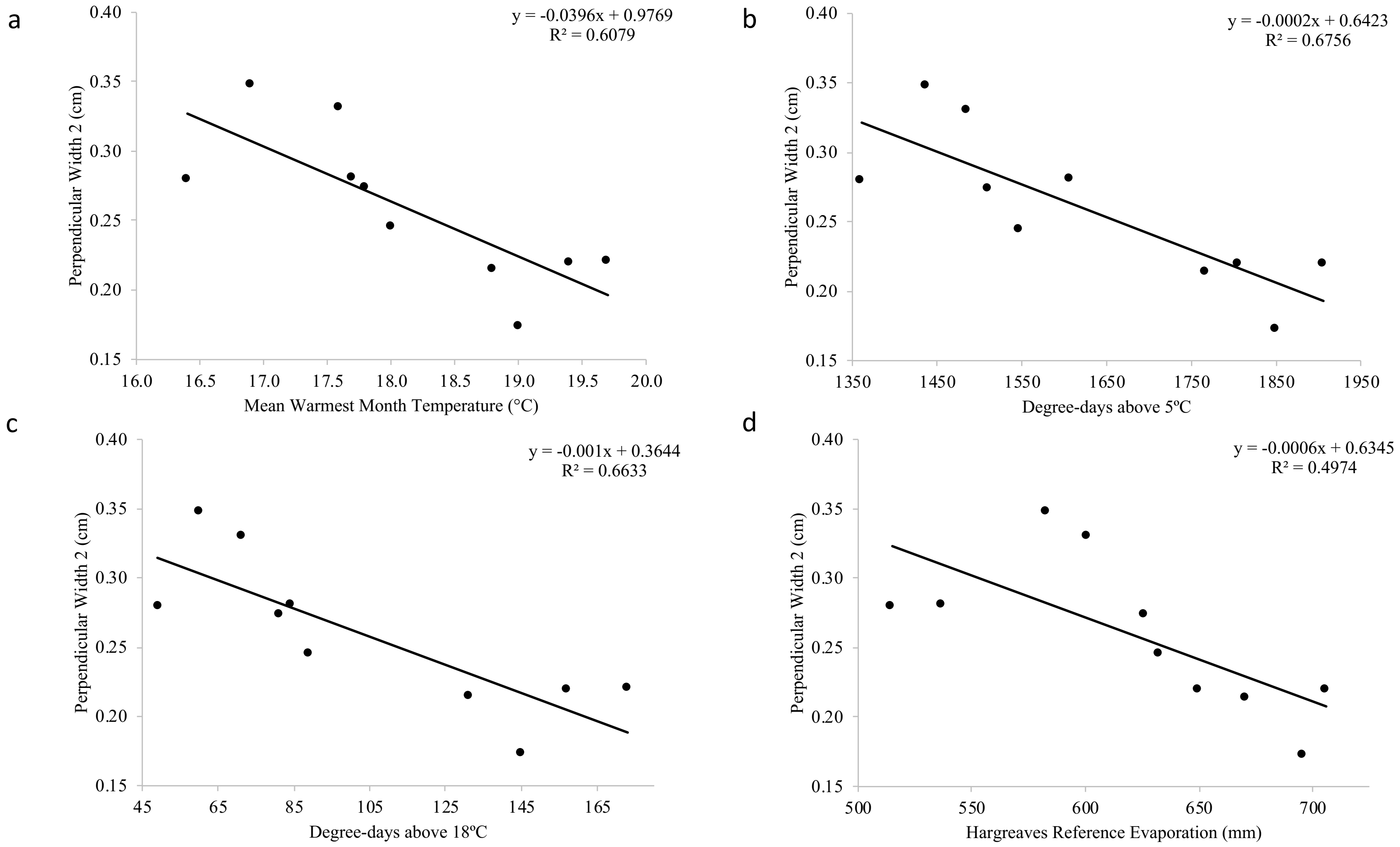

Figure 7. Simple linear regression analysis of perpendicular width 2 and (a) mean warmest month temperature; (b) degree-days above 5ºC; (c) degree-days above 18ºC; and (d) Hargreaves reference evaporation.

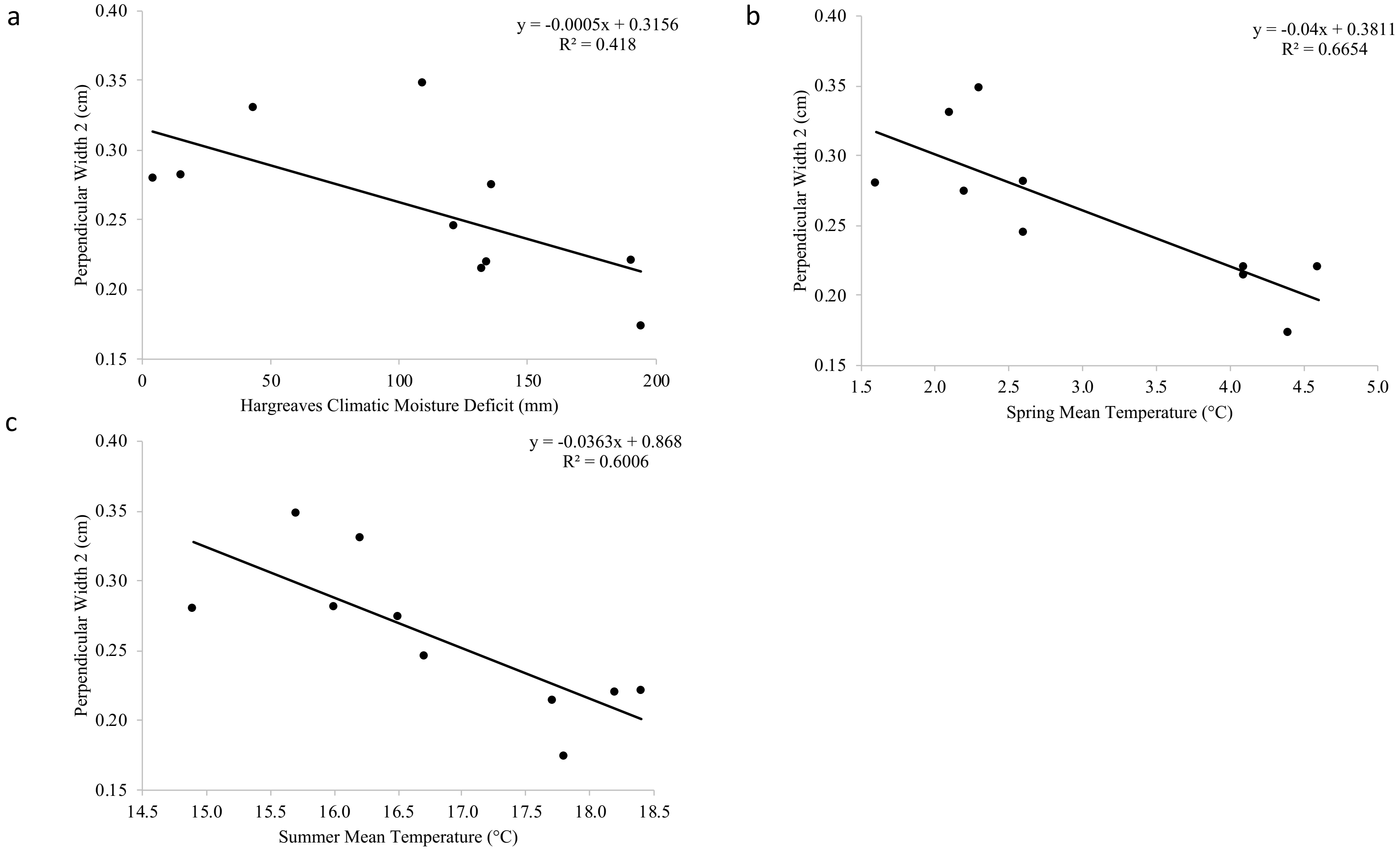

Figure 8. Simple linear regression analysis of perpendicular width 2 and (a) Hargreaves climate moisture deficit; (b) spring mean temperature; and (c) summer mean temperature.

MULTIPLE LINEAR REGRESSION

None of the multiple regression models were significant in explaining variations in provenance height, average horizontal width, horizontal width, or maximum perpendicular width. Climate variable DD5 was the strongest predictor for perpendicular width 1 (R^2=0.441, p=0.036) (Table 5), while the combination of climate variables of DD5, MAT, and AHM explained 71% of the variation in perpendicular width 2 (R^2=0.712, p=0.046) (Table 6). DD5 was a strong predictor for both models, showing a strong relationship between leaf shape and the number of growing degree days.

Table 5. Stepwise multiple regression analysis of perpendicular width 1 and climate variables with R square values and significance.

Model	Predictors		Sum of Squares	df	Mean Square	F	Sig.	R Square
1	DD5	Regression	0.007	1	0.007	6.303	0.036	0.441
Residual		0.009	8	0.001				
Total		0.016	9					
2	DD5, AHM	Regression	0.008	2	0.004	3.14	0.106	0.473
Residual		0.008	7	0.001				
Total		0.016	9					
3	DD5, AHM,	Regression	0.009	3	0.003	2.605	0.147	0.566
Residual	MAT	0.007	6	0.001				
Total		0.016	9					
4	DD5, AHM,	Regression	0.012	4	0.003	3.16	0.119	0.717
Residual	MAT, PPT_SM	0.005	5	0.001				
Total		0.016	9					

Table 6. Stepwise multiple regression analysis of perpendicular width 2 and climate variables with R square values and significance.

Model	Predictors		Sum of Squares	df	Mean Square	F	Sig.	R Square
1	DD5	Regression	0.018	1	0.018	16.662	0.004	0.676
Residual			0.009	8	0.001			
Total			0.027	9				
2	DD5, MAT	Regression	0.018	2	0.009	7.527	0.018	0.683
Residual			0.009	7	0.001			
Total			0.027	9				
3	DD5, MAT,	Regression	0.019	3	0.006	4.943	0.046	0.712
Residual	AHM		0.008	6	0.001			
Total			0.027	9				
4	DD5, MAT,	Regression	0.021	4	0.005	4.294	0.071	0.775
Residual	AHM, PPT_SM		0.006	5	0.001			
Total			0.027	9				

DISCUSSION

The results of this study support my first hypothesis that yellow birch would demonstrate significant intraspecific variation in measured variables. The finding of significant variation in height is consistent with other provenance studies of yellow birch (Clausen, 1967, 1980; Clausen & Garrett, 1968). Clausen and Garrett (1968) found significant variation among three-year-old seedlings originating from 55 provenances, with the best performing provenances measuring at least double the height as those from the poorest performing provenances. However, variation in height did not follow a specific clinal gradient or correlation with climate at seed origin, which remained consistent with the results of seedling height growth the year prior (Clausen, 1967). My study did find slight significant correlation ($p<0.1$) between height growth and the climate variable PPT_SM. While Clausen's study included a greater number and wider range of provenances, the climate and geographic data was limited to variables such as length of growing season, average July temperature, annual precipitation, latitude and longitude. Conversely, my study included 29 climate variables, providing a wider range of possible drivers of the genetic variation present within yellow birch. However, no other climate variables were significantly correlated with height growth in my study.

A recent review of provenance studies found that in some species, height was not significantly correlated with temperature-related climate variables (such as MAT) or precipitation-related variables (such as MSP) (Aitken & Bemmels, 2016). Therefore, variation in height growth may be driven by other factors not included in our analysis but remains an important trait for trees in terms of competition and survival. Height growth is a strong indicator for juvenile tree fitness. Typically, seedlings with faster growth rates are more efficient when competing for important resources such as light, gaps in the canopy, and moisture. For example, a recent review identified smaller and slower growing trees to be more vulnerable to drought-induced mortality in temperate forest regions (O'Brien et al., 2017).

The finding of significant differences in leaf measurements also supports my first hypothesis that significant variation would be present among functional traits. This was expected, as adjustments in leaf morphology are an efficient way for trees to adapt and acclimate to their environment (Xu et al., 2009). Moreover, intraspecific variation in leaf morphology has been documented in previous studies of yellow birch (Dancik & Barnes, 1975). Wider leaves are considered an adaptation to cool and wet environments as narrow leaves are typically found in more hot and dry climates, allowing rates of transpiration to decrease (Warren et al., 2005; Westoby et al., 2004; Wright et al., 2005). Narrow and low SLA leaves are better adapted to environments where resources may be limited and the ability to control water balance and resource retention is crucial (Westoby et al., 2004; Wright & Westoby, 1999). In my study, average horizontal width and perpendicular width 2 decreased with warmer and drier climate at seed origin. Conversely, log-transformed values of maximum perpendicular width, horizontal width, and perpendicular width 1 increased with warmer temperatures and heat moisture indices at seed origin. Therefore, the results of my study follow typical trends of leaf shape and climate (Westoby et al., 2004; Wright et al., 2005; Wright & Westoby, 1999).

Interestingly, characters such as total area, blade length, or SLA did not differ significantly among provenances, which have been previously shown to vary in yellow birch (Dancik & Barnes, 1975; Sharik & Barnes, 1979) as well as paper birch (Pyakurel

& Wang, 2013) populations. For example, Dancik and Barnes (1975) compared 13 leaf characteristics among 112 yellow birch populations from Michigan, Wisconsin, and Minnesota and found that blade length, blade width, and petiole length differed the most among populations. However, that study also reported high among-population and among-individual tree variation in leaf characters, which the authors suggested may have obscured between-population variance. They also found that populations originating from the Great Lakes area had larger leaves than those from the Appalachian Mountains. However, they found that site characteristics, such as drainage and acidity, influenced leaf morphology more than mean annual precipitation or geographic distance between populations. Another study of yellow birch populations in the Appalachian Mountains found a positive correlation between latitude and leaf blade length and width, as well as coarseness of leaf serration (Sharik & Barnes, 1979). The authors also found that as leaves grew larger, leaf venation grew farther apart. Pyakurel and Wang (2013) found significant variation in paper birch leaf morphology and most significant leaf characters had negative correlations with climate variables at seed origin. Unexpectedly, leaf characters such as SLA, maximum horizontal width, aspect ratio, and petiole size increased with warmer and drier conditions at seed origin, which is inconsistent with global trends and the results of the present study. The authors concluded that many factors (i.e. WUE, leaf life span, nutrient availability) are involved in determining traits such as SLA and the increase in petiole length and leaf width may be an adaptation to increase leaf cooling in drier climates.

No significant differences between carbon isotope values existed among the provenances in my study. It has been shown in ponderosa pine seedlings that greater differences in $\delta^{13}C$ occur under water-stressed conditions compared to those in well-watered conditions (Olivas-Garcia et al., 2000). My seedlings were grown in a uniform, well-watered environment, therefore significant variation may not be revealed in these conditions. Carbon isotopes, to my knowledge, have not been investigated in yellow birch trees, so whether our mean values are typical of this species remains unclear.

RELATIONSHIPS BETWEEN TREE TRAITS AND CLIMATE VARIABLES

Perpendicular width 2 had the greatest number of significant correlations with climate variables, suggesting that variation in overall leaf shape is associated with adaptation to local climate. These results are consistent with the literature that climate is a strong predictor of morphological variation within tree species (Leites et al., 2019; Moles et al., 2014; Warren et al., 2005; Wright et al., 2005). While most significant climatic predictor variables were temperature-related (i.e. MWMT, DD5, and DD18), two climate variables related to both precipitation and moisture were also significantly correlated with leaf traits. For example, AHM correlated with horizontal width and maximum perpendicular width ($p<0.1$) and SHM correlated with perpendicular width 1 ($p<0.1$). Provenances with higher AHM and SHM values tended to have greater leaf widths (i.e. Provenance 1 vs. Provenance 7). Seedlings from Provenance 7 originated from Hiawatha National Forest in Michigan, which tends to be much warmer and drier than Provenance 1, located in Black Brook, New Brunswick. These results are consistent with studies using global plant data that suggest temperature is a stronger predictor of functional trait values than precipitation, but climate variables which combine temperature and precipitation values (such as heat-moisture indices or evaporation) tend to significantly correlate as well (Aitken & Bemmels, 2016; Leites et al., 2019; Moles et al., 2014). Furthermore, these results suggest that the seedlings originating from drier and warmer climates expressed functional traits (i.e narrower leaves) that have been shown to assist trees in resisting drought (Westoby et al., 2004; Wright et al., 2005; Wright & Westoby, 1999).

The overall weak correlations between climate variables and phenotypic traits may be due to lack of statistical power associated with the small sample size of this study (n=4 per provenance). However, a recent study also found very weak geographic clines related to variation in traits of yellow birch and the authors concluded it was due to lack of statistical power and high within-population variation (Leites et al., 2019). This trend is in contrast to provenance studies on conifers such as white spruce (Lesser, 2005; Thomson, 2008), eastern white pine (*Pinus strobus* Linneaus) (Joyce & Rehfeldt, 2013; Lu et al., 2003), and lodgepole pine (*Pinus contorta* Engelmann) (Rehfeldt et al., 1999) that generally report strong clinal patterns of variation. The results of my study do not necessarily mean local adaptation is not present in traits such as height, diameter, biomass allocation, and stable carbon isotopes, but rather, the climate variables analyzed may not be the most important factor influencing variation in these traits. Other factors involved may include soil pH, site drainage, elevation, and other site characteristics (Dancik & Barnes, 1975; Moles et al., 2014).

MULTIPLE LINEAR REGRESSION

The results of the multiple linear regressions were not as expected, as only perpendicular width 1 and perpendicular width 2 resulted in significant models with the chosen climate variables DD5, AHM, MAT, and PPT_SM. The simple linear regressions resulted in significant correlations between each leaf character and a set of climate variables, therefore, it was unexpected that the multiple linear regressions did not result in a greater number of significant models. In the models that were significant, leaf width strongly correlated with DD5, indicating local adaptation to climate, especially temperature-related variables. MAT and AHM were also strongly correlated with perpendicular width 2 when combined with DD5 and explained 71% of variation in this trait. DD5 is a measure of the length of growing season and is strongly correlated with other temperature variables included in this study. Variables such as length of growing season as well as annual and seasonal temperature have been shown to significantly correlate with leaf phenology, flowering times, and growth cessation in yellow birch (Clausen, 1967, 1975, 1980; Clausen & Garrett, 1968). For example, growth cessation has been negatively correlated with latitude, positively correlated with average July temperature, and weakly correlated with length of growing season and annual temperature (Clausen, 1967). Similar to the results of my study, Clausen (1967) found second year height growth to have no (or very weak) correlations with geographic or climatic variables. In a later study, Clausen (1975) observed provenances from more northern locations produced seed earlier than the more southern provenances when grown in a uniform environment.

Temperature-related climate variables have been shown to be strong drivers of local adaptation in trees, generally more-so than precipitation-related variables (Aitken & Bemmels, 2016; Leites et al., 2019; Moles et al., 2014). Although extreme drought is caused by lack of rainfall, it appears that temperature is the strongest driving factor for local adaptation in drought-related traits (Aitken & Bemmels, 2016; Leites et al., 2019; Moles et al., 2014). Tree physiology relies on more variables than the amount of rainfall, causing measurements of precipitation to lack important characteristics of water availability such as soil depth, soil type, seasonal distribution of rainfall, and other hydrological factors (Moles et al., 2014). Therefore, precipitation-based climate variables such as MAP fall short in describing water availability, which may be why climate variables involving both temperature and precipitation tend to have stronger correlations to variation in tree variables. A recent review described global trends of forest decline and tree mortality due to the combination of drought and warmer temperatures (hotter drought) which is considered a more common reality of climate change (Allen et al., 2015).

Limitations of the stepwise regression model may explain why only two tree variables resulted in significant models with climate variables. Although multiple stepwise regression is widespread and commonly used in ecology and biology (Mac Nally, 2000), limitations are present and critiqued strongly across disciplines (Hurvich & Tsai, 1990; Whittingham et al., 2006; Wilkinson, 1979). Multiple regressions are typically used to test the impact a set of independent variables (X) upon a dependent variable (Y) and to find the strongest combinations of predictors for a model. One limitation is multicollinearity (or intercorrelation) among independent variables, which must be avoided in order to decrease excess "noise" in the model (Mac Nally, 2000). In the present study, significantly correlated climate variables were eliminated prior to the multiple regression analysis, resulting in four uncorrelated independent variables (DD5, MAT, AHM, and PPT_SM) tested as model predictors. However, these specific climate variables may not be strong predictors of variation within the dependent variables provided for the model. This is one possible explanation as to why seedling traits of height, average horizontal width, average horizontal width, and maximum perpendicular width did not result in significant multiple regression models. Further limitations include bias of parameter estimation (choosing a model based on parameter significance) and the effect of using different algorithms, order of data entry, and independent variables on the final model (Whittingham et al., 2006). In the present study, however, stepwise regression (vs. other algorithms such as enter, deletion, etc.) resulted in the strongest and most significant model for the dataset provided. This can be considered an inference of a “best-fit” model, which likely can be biased and insufficient for explaining all biological factors (Whittingham et al., 2006). Therefore, the use of multiple stepwise regressions for the analysis of the effect of climate at seed origin on yellow birch seedling traits may have resulted in “best” models for two traits, but I acknowledge alternative model selection methods may be more sufficient.

RESEARCH LIMITATIONS AND IMPROVEMENTS

Due to the widespread geographic range of tested populations, it was hypothesized that large variations would be present in growth characters such as diameter, biomass, and other leaf characteristics such as SLA or total leaf area. Furthermore, significant variation of growth characters has been reported in yellow birch by a variety of studies including height (Wearstler et al., 1977), diameter (Clausen & Garrett, 1968), and biomass (Rasheed & Delagrange, 2016). Interestingly, provenance height means (n=40) were the only growth trait that varied significantly (p=0.048) among populations in this study. Large sample sizes are generally required to detect among-population variations, especially in species with large within-population variations (Aitken & Bemmels, 2016; Lu et al., 2016; Matyas, 1996). However, in my study, the ANOVA comparing height and diameter of the 200 seedlings did not result in significant variation among provenances. This contradicts the expectation that a larger sample size would result in higher expressed levels of variation.

Yellow birch has been shown to exhibit high within-population variation (Clausen & Garrett, 1968; Dancik & Barnes, 1975; Leites et al., 2019), which may be a main factor for the weak statistical significance in my study. Species with large effective population sizes and long-distance pollen dispersal tend to have higher rates of within-population variation (Frankham et al., 2013). Furthermore, yellow birch is an allopolyploid (Clausen, 1973), which has been inferred to increase levels of variability within individuals and populations (Thomson, 2013). Yellow birch tends to grow in mesic site conditions (Erdmann, 1990), causing the species to have a relatively narrow ecological niche. In contrast to other birches that are generalists (i.e. paper birch), yellow birch typically do not inhabit dry environments (Erdmann, 1990). It has been shown that yellow x paper birch hybrids have the ability to grow and survive in drier sites than what is favorable of yellow birch (Clausen, 1972a). Paper birch has a much wider ecological niche than yellow birch, inhabiting a wide range of soil types and climates (Hutnik & Cunningham, 1961), therefore increasing the potential to express higher levels of genetic variation within the species. For example, a recent study on paper birch found significant variation in over 10 leaf morphological characters, including leaf size, SLA, blade length, maximum width, form coefficient, and petiole length (Pyakurel & Wang, 2013). In comparison, although my study included the same leaf characters in the original analysis, only leaf width measurements were significantly different among the yellow birch provenances. Therefore, the lack of statistical significance of morphological traits found in this study may be due to the low tolerance of unfavorable site conditions that is characteristic of yellow birch as a species. Further possible explanation for the lack of statistical significance could be due stress caused by aphids in the second growing season, as different provenances may have varying degrees of pest resistance, ultimately masking genetic differences in growth potential among provenances (Aitken & Bemmels, 2016).

Further research on genetic variation in yellow birch should measure multiple growing seasons to compare growth between seedling ages. Past research has shown that younger seedlings may express higher levels of plasticity than older seedlings of the same species (Clausen, 1980; Gaucher et al., 2005; Logan, 1965). Therefore, collecting multiple growing seasons of measurements in growth, biomass, leaf characteristics, and carbon isotope values may provide insight to plasticity of yellow birch seedlings at varying ages grown under greenhouse conditions. Including measurements of leaf phenology and growth cessation may also uncover patterns of local adaptation within yellow birch, as past research has shown significant differences among provenances within the species (Clausen, 1967) and other temperate hardwoods (Aitken & Bemmels, 2016; Aitken et al., 2008; Aspelmeier & Leuschner, 2006; Joyce & Rehfeldt, 2013). Furthermore, including more provenances would be beneficial for increasing statistical power and capturing a more robust view of the genetic variability among populations. Lastly, a soil moisture deficit study using seed from the same 10 provenances would provide greater knowledge regarding the responses that yellow birch seedlings have towards changes in their environment. Including measurements of photosynthesis, transpiration, and carbon assimilation would also add significant value to a study involving water use efficiency and resource usage. This study design would be time consuming and labor intensive. However, it is crucial for further understanding how yellow birch may respond to varying degrees of drought conditions.

CHAPTER III

MULTIVARIATE ANALYSIS OF PHENOTYPIC VARIATION IN RELATION TO CLIMATE

INTRODUCTION

Multivariate analyses such as principal component analysis (PCA) and canonical correlation analysis (CANCOR) are effective ways to summarize and describe one or more data sets (Tausz et al., 2002). They differ with univariate analyses in that they combine multiple steps into one complete analysis, providing a more robust biological view of relationships among variables. PCA is typically used to summarize many variables into groupings that can be described by one or more factor (Abdi & Williams, 2010). This analysis can be used to identify main patterns of variation within the data set. Furthermore, relationships between variables can be highlighted and visualized. Similar to PCA, CANCOR summarizes the data into main groupings, but also maximizes relationships between two data sets and the variation within them (Gittins, 1986; B. Thompson, 1987).

Past research has used both PCA and CANCORR to successfully describe and group multiple data sets in species such as white spruce (Lesser & Parker, 2006), paper birch (Pyakurel & Wang, 2013), white birch (Oke & Wang, 2013), trembling aspen (Thompson, 2014), and yellow birch (Dancik & Barnes, 1975; Sharik & Ford, 1984). The two data sets for used for this study included provenance trait means and climate measurements at seed origin. In Chapter 2, I used simple and multiple linear regression to relate climate variables to tree variables. However, PCA and CANCOR may provide a more thorough biological description of variation, as multivariate analyses includes all significant tree and climate variables, creates groups, and describes relationships among and between the two data sets (Tausz et al., 2002).

The objectives of this chapter were to use multivariate analyses to (i) identify the main components of variance within yellow birch populations and (ii) examine patterns of variation in tree variables in relation to climate variables and relationships between tree characteristics and climate at seed origin.

MATERIALS & METHODS

Significant measured tree variables identified in Chapter 2 were used for the principal component and canonical correlation analyses (Table 3). Both analyses were performed using SPSS statistics software (IBM SPSS, version 28). Leaf variables including horizontal width, maximum perpendicular width, and perpendicular width 1 were reflected and log-based 10 transformed for normal distribution. Highly correlated climate variables were not used for the analyses, resulting in MAT, DD5, AHM, and PPT_SM being retained for canonical correlation with tree variables. A detailed description of climate variables can be found in Appendix III, Table A. 2.

Kaieser-Meyer-Olkin (KMO) and Bartlett's tests were used to determine whether the results of the principal component analysis were sufficient for further interpretation (KMO >0.6; Bartlett's $p<0.001$). Principal components with Eigenvalues greater than one were extracted for further analysis, while those with Eigenvalues less than one were discarded. A visual review of the resulting scree plot was also used to determine which principal components were appropriate for analysis. A syntax MANOVA was used to carry out the canonical correlation analysis in SPSS. The Wilk's multivariate test of significance determined whether there was significant correlation within the two data sets of measured tree variables and climate variables ($p<0.05$).

RESULTS

PRINCIPAL COMPONENT ANALYSIS

The principal component analysis was determined to be appropriate to further analyze due to the KMO sampling adequacy (=0.740) and Bartlett's Test of Sphericity ($p<0.001$). The correlation matrix resulted in a determinant = $2.502E^{-6}$. Therefore, interpretation of results continued (KMO >0.6; Bartlett's $p<0.001$). The first two principal components explained a cumulative total of 90.7% of variance among the measured phenotypic variables (Table 5). Eigenvalues for PC1 and PC2 were 4.408 and 1.035, respectively (Table 7). The following principal components 3-6 had Eigenvalues less than one and accounted for less than 10% of the remaining variance among tree variables (Table 7). The resulting scree plot allowed for confirmation to retain the first two principal components, due to the shape of the curve and the associated Eigenvalues (<1) (Figure 9).

Table 7. Total variance explained by principal component analysis. Two principal components were extracted resulting in 90.718% variance described among provenance means.

Total Variance Explained						
	Initial Eigenvalues			Extraction Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.408	73.468	73.468	4.408	73.468	73.468
2	1.035	17.251	90.718	1.035	17.251	90.718
3	0.451	7.520	98.238			
4	0.077	1.288	99.526			
5	0.028	0.465	99.991			
6	0.001	0.009	100.000			

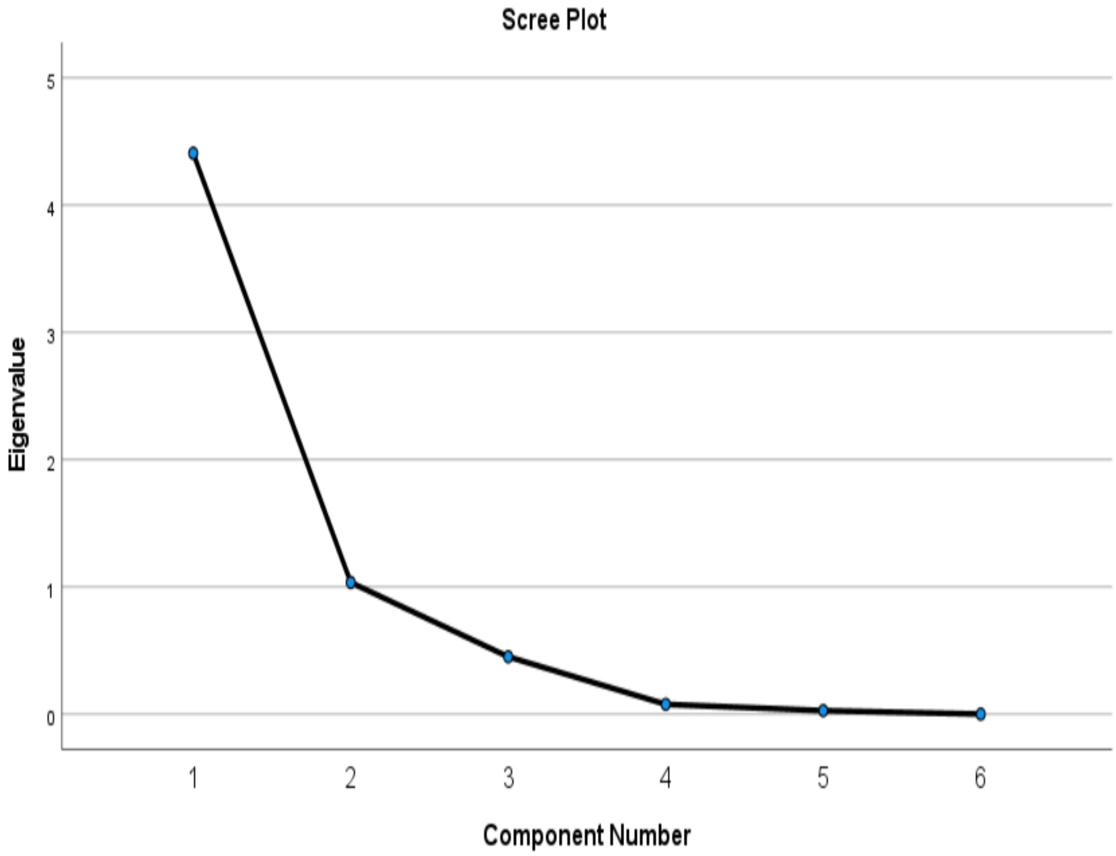

Figure 9. Principal component analysis scree plot with principal components one through six and their associated Eigenvalues.

The component matrix of the extracted principal components 1 and 2 resulted in values of loadings between the tree variables and principal components. Principal component 1 was strongly associated with all significant leaf characters whereas principal component 2 was strongly associated with height and slightly with perpendicular width 2 (Table 8). The component plot shows the strong relationship between the leaf characters horizontal width, maximum perpendicular width, and perpendicular width 1 as well as between average horizontal width and perpendicular width 2 (Figure 10).

Table 8. Component matrix of the two extracted principal components, measured variables, and their associated component loadings.

Component Matrix[a]

	Component	
	1	2
Height	0.048	0.985
Log10ReflectHorizontalWidth	0.974	-0.074
Log10ReflectMaxPerpWidth	0.972	-0.088
Log10ReflectPerpWidth1	0.972	-0.039
AvgHorizontalWidth	-0.979	0.026
PerpWidth2	-0.781	-0.223

Extraction Method: Principal Component Analysis.

a. 2 components extracted.

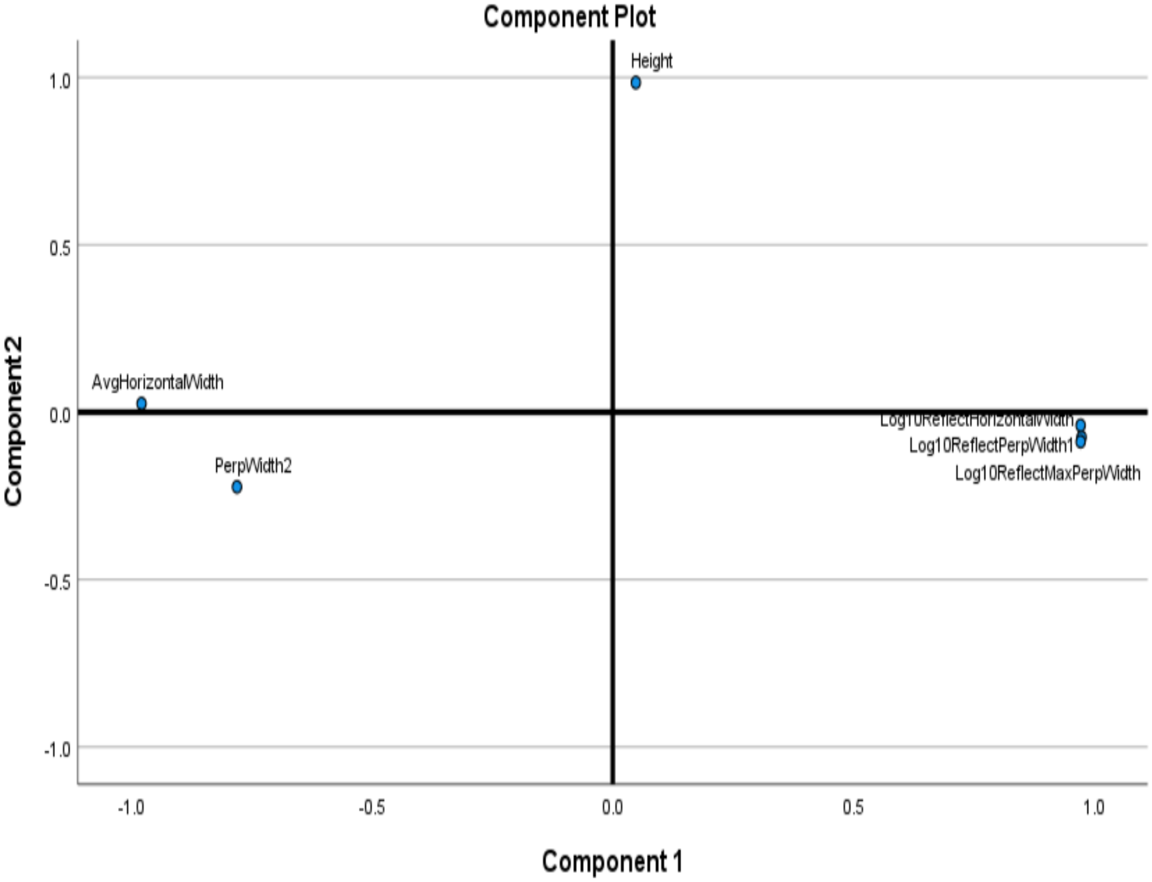

Figure 10. Principal component plot of component 1 (x axis) and component 2 (y axis) with the positive or negative loading values of measured tree variables.

CANONICAL CORRELATION ANALYSIS

The canonical correlation analysis resulted in a significant Wilks test (p=0.02) which determined significant relationships between the tree variable and climate variable data sets, allowing for further interpretation of the results. A total of four canonical variates (CV) were extracted from the data sets. CV1 accounted for 67.23% of total variance between the two data sets with an Eigenvalue of 1.09 and Pearson's correlation value of 0.72 (Table 9). CV2 explained 23.93% more variance between the two data sets with an Eigenvalue of 0.388 and Pearson's correlation value of 0.53 (Table

9). The first two canonical variates explained a cumulative 91.16% of variance between the two data sets (Table 9). CV3 and CV4 explained the remaining <10% of variance between tree variables and climate variables with Eigenvalues <0.1 (Table 9). CV1 is the only canonical variate with an Eigenvalue >1.

Table 9. Four canonical variates extracted with the associated Eigenvalues, percent of total variance explained (Pct.), cumulative percent of variance explained (Cum. Pct.), Pearson's correlation in data sets (Canon Cor.) and squared canonical correlation values (Sq. Cor).

Root No.	Eigenvalue	%	Cum %	Canon Cor.	Sq. Cor
1	1.09232	67.22781	67.22781	0.72254	0.52206
2	0.38884	23.93142	91.15922	0.52913	0.27997
3	0.08408	5.17491	96.33414	0.2785	0.07756
4	0.05956	3.66586	100	0.2371	0.05621

The dimension reduction analysis resulted in a significant Wilks test when comparing CV1 through CV4 (p=0.02) (Table 10). However, when CV1 is not involved in the comparison of canonical variates, the Wilks test is no longer significant (p=0.408). Therefore, further interpretation of CV2, CV3, and CV4 was not completed as the relationships with these variates and data sets are insignificant (Table 10).

Table 10. Dimension reduction analysis of canonical variates 1-4 and the Wilks Levene values, F statistic, hypothesis degrees freedom, error degrees freedom, and significance of F.

Roots	Wilks L.	F	Hypoth. DF	Error DF	Sig. of F
1 TO 4	0.29959	1.82051	24	105.87	0.02
2 TO 4	0.62684	1.05665	15	85.98	0.408
3 TO 4	0.87058	0.57402	8	64	0.795
4 TO 4	0.94379	0.65519	3	33	0.585

Leaf variables that were strongly positively correlated with CV1 include perpendicular width 1 (0.87), horizontal width (0.67), and maximum perpendicular width (0.66) (Table 11). Conversely, leaf variables perpendicular width 2 and average horizontal width had strong negative correlations with CV1, with values of -0.8 and -0.69, respectively. Height had the weakest correlation with CV1 (0.16).

Table 11. Structure matrix with resulting correlations between dependent and canonical variables 1-4. Dependent variables are significant tree variables. See Appendix for full names and definitions of measured tree variables.

	Function No.			
Variable	1	2	3	4
Height	0.15969	0.72078	-0.19788	-0.33232
AvgHoriz	-0.69079	-0.17592	-0.00421	-0.68063
HorizWidth	0.66972	0.13925	0.11003	0.70967
MaxPerpWidth	0.66452	0.1221	0.13754	0.71249
PerpWidth1	0.87031	0.01577	0.13939	0.46593
PerpWidth2	-0.79538	0.09962	0.49174	-0.2344

Climate variables DD5 (0.82), MAT (0.66), and AHM (0.552) had strong positive correlations with CV1 (Table 12). Conversely, PPT_SM was moderately negatively correlated with CV1 (-0.51). DD5, MAT and AHM are all temperature related climate variables whereas PPT_SM is solely a precipitation-based variable. CV1 explained a total of 46.3% of the variance among tree variables and 24.2% of the variance among climate variables (Table 13).

Table 12. Structure matrix with resulting correlations between covariates (climate variables) and canonical variables 1-4.

	Canonical Variables			
Covariate	1	2	3	4
MAT	0.6565	-0.39105	-0.1251	-0.63279
DD5	0.82415	-0.05829	-0.56102	0.05134
AHM	0.552	0.64244	-0.22458	0.48181
PPT_SM	-0.51451	-0.70877	0.43034	0.21847

Table 13. Variance in dependent variables and covariates explained by canonical variables. Dependent variables are significant tree variables. Covariates are climate variables. CanVar = canonical variate; %VarDep = % variance of dependent variables; Cum%Dep = cumulative % variance of dependent variables; %VarCov = % variance of covariates; Cum%Cov = cumulative % variance of covariates.

CanVar	%VarDep	Cum%Dep	%VarCov	Cum%Cov
1	46.3814	46.3814	24.21392	24.21392
2	9.91581	56.29721	2.77616	26.99009
3	5.52404	61.82125	0.42845	27.41854
4	30.95009	92.77134	1.73985	29.15838

DISCUSSION

PRINCIPAL COMPONENT ANALYSIS

The Principal Component 1 (PC1) which explained 73.4% of the variance could be interpreted as a leaf morphology component, as all leaf characters had strong positive or negative loadings with this component. Principal Component 2 (PC2) which explained an additional 17.3% of the variance could be interpreted as a fitness-related component since height had a strong, positive loading with this component. Height growth is a strong indicator for juvenile tree fitness. Typically, seedlings with faster growth rates are more efficient when competing for important resources such as light, gaps in the canopy, and moisture (O'Brien et al., 2017). The results of the PCA indicate that leaf morphology and height growth are independent functional traits and have the potential to respond to changes in climate simultaneously without causing an effect on the other. This is beneficial for yellow birch as a species, as it has been shown that correlated traits may work against one another in terms of selection (termed "antagonistic"), resulting in a constraint to adaptive evolution (Etterson & Shaw, 2001).

Strong correlations between leaf morphological characters and geographic or climate clines have been shown (Aitken et al., 2008; Clausen & Garrett, 1968; Dancik & Barnes, 1975; Sharik & Barnes, 1979). Wider leaves are considered an adaptation to cool and wet environments as narrow leaves are typically found in more hot and dry climates, allowing rates of transpiration to decrease (Warren et al., 2005; Westoby et al., 2004; Wright et al., 2005). Therefore, under drought conditions, it would be expected that leaf shape, especially width, would decrease. In Chapter 2, my study found that all leaf width characters decreased with warmer and drier climate at seed origin. The principal component plot shows this relationship between leaf characters as well. Therefore, the results from both chapters of my study follow typical trends of leaf shape and climate. Furthermore, these results suggest that the seedlings originating from drier and warmer climates expressed functional traits (i.e narrower leaves) that have been shown to assist trees in drought resistance or tolerance (Aubin et al., 2016; Westoby et al., 2004; Wright et al., 2005; Wright & Westoby, 1999). In a recent review, changes in leaf morphology and the ability to control water loss in warmer and drier conditions was determined as an important functional trait for temperate and boreal forest tree species in order to persist under climate change (Aubin et al., 2016). Other functional traits that may assist in individual tree persistence include stomatal sensitivity, rooting depth, leaf or branch shedding, and leaf mass area, to name a few. The authors conclude population persistence under climate change conditions is crucial for the short-term survival of tree species, whereas shifts in species distribution will play a role for long-term survival. The results from my study show that local adaptation is present among leaf morphology in yellow birch populations, and therefore has the potential to adapt to a variety of climates. Further studies investigating more functional traits (i.e. physiological or phenological) that have been shown to assist trees under environmental stress would be beneficial for the future conservation of this species.

CANONICAL CORRELATION ANALYSIS

The canonical correlation analysis resulted in a single significant canonical variable, explaining 67.2% of the variance within both data sets of tree variables and climate variables. Similar to the PCA results, strong positive correlations occurred between horizontal width, maximum perpendicular width, perpendicular width 1 and CV1. These variables were all reflected and log-transformed prior to analysis, which describes their inverse relationship with the other leaf variables (average horizontal width and perpendicular width 2). Furthermore, height had a slight positive correlation with CV1, supporting the conclusion that height is independent of leaf width, as shown with the PCA.

The climate variable DD5 had the strongest positive correlation with CV1 followed by MAT and AHM. PPT_SM had a neutral yet negative correlation with CV1. These results are consistent with the multiple regression analysis in Chapter 2, as the strongest model included DD5, MAT, and AHM, but not PPT_SM. Similar to the univariate analyses, DD5 was the strongest predictor of multiple leaf width characters. This climate variable is a measurement of degree-days above 5 ºC and influences key phenological events such as the timing of bud burst and bud set (Aitken et al., 2008). Previous studies on yellow birch have shown strong to moderate correlations between leaf phenology, flowering times, and growth cessation with geographic (i.e. latitude) and climatic variables (i.e. average July temperature, length of growing season and annual temperature) (Clausen, 1967, 1975, 1980; Clausen & Garrett, 1968). The present study did not measure phenological variables, but it does provide evidence that the number of degree-days above 5ºC may have an impact on overall leaf shape for this species.

Precipitation variables were stronger predictors of variation in provenance height, although the correlations were only slightly significant or not significant at all. Previous studies on yellow birch have also found height to be independent of geographic and climatic variables (Clausen, 1967, 1974; Clausen & Garrett, 1968; Leites et al., 2019). A recent review of provenance studies found that in some species, height was not significantly correlated with temperature-related climate variables (MAT) or precipitation-related variables (MSP) (Aitken & Bemmels, 2016). The authors conclude that this does not mean local adaptation is not present at all in these species (as they are widely distributed) but rather, MAT or MSP may not the driving force for variation in a specific species, population, or measured trait. Therefore, it may be possible that local adaptation in yellow birch is more strongly expressed in traits such as leaf morphology, rather than height, diameter, or biomass allocation. The present study did not include certain yellow birch traits that have been shown to correlate with geographic or climatic variables such as such as leaf venation, tooth serration, fruit characteristics, and bract size (Sharik & Barnes, 1979), as well as growth cessation, flowering times, and survival (Clausen, 1968, 1975, 1980). Other site variables may have strong effects on yellow birch traits such as soil pH and drainage (Dancik & Barnes, 1975), soil moisture (Rasheed & Delagrange, 2016), and light availability (Beaudet & Messier, 1998; Gaucher et al., 2005; Messier & Nikinmaa, 2000).

Much within-provenance variation has been consistently reported in yellow birch research (Clausen, 1967, 1972b, 1980; Clausen & Garrett, 1968; Dancik & Barnes, 1975; Sharik & Barnes, 1979). Therefore, collecting data from larger numbers of provenances and individuals may be essential in order to fully show trends in local adaptation of functional traits within the species (Leites et al., 2019; Sharik & Barnes, 1979). Furthermore, including provenances from the extremes of the species range has been argued to be crucial for true expression of clinal variation as excluding the extremes can underestimate the steepness of regression clines (linear) or curves (nonlinear) (Leites et al., 2019). Further research including both of these improvements is necessary in order to fully describe intraspecific genetic variation within yellow birch, ultimately aiding in the conservation and protection of this prominent North American hardwood species.

MULTIVARIATE VERSUS UNIVARIATE ANALYSES

Based on the combined results from Chapter 2 and Chapter 3, I would recommend the use of both multivariate and univariate analyses. I believe that the simple linear regression resulted in the strongest univariate analysis. The simple linear regressions showed distinct correlations between seedling characteristics and climate variables. Although the majority of significant seedling characteristics were related to leaf width, they were all strongly correlated with different climate variables. Therefore, I believe this analysis provides the strongest view regarding the relationships between climate at seed origin and variation among yellow birch leaf traits. However, simple linear regression cannot explain the relationships among seedling characteristics as well as climate variables. I would recommend the use of multivariate analyses in order to further describe these relationships. The PCA showed that height and leaf characters are independent of one another and have the potential to respond to climate independently and simultaneously. This is an important factor for yellow birch as a species and the multivariate analyses revealed this relationship.

Other than this finding, PCA and CANCOR did not explain new information regarding the data sets, but they further supported and summarized the results from the univariate analyses. One benefit of using multivariate analyses would be the reduction of steps in the statistical process with the ability to attain very similar results. However, PCA only explained the relationships among seedling characteristics. Although this is valuable information, whether local adaptation is present remains unknown. In order to relate this variation to climate, further regressions of climate variables would need to be performed.

On the other hand, CANCOR has the ability to summarize variation among seedling traits and climate variables simultaneously. Only one canonical variable was significant for this study (CV1), which strongly correlated to leaf characteristics and temperature-related climate variables. Further studies involving phenological or physiological functional traits of yellow birch may reveal more significant canonical variables. The CANCOR results also showed which combination of climate variables were strongly correlated with variation in tree variables. This is unique to the multivariate approach, as simple linear regressions only analyse one climate variable at a time. Therefore, I would recommend the combination of univariate and multivariate analyses in the form of simple linear regression and CANCOR for the strongest and most concise analysis of this study.

CONCLUSIONS

Significant variation was shown among 10 yellow birch provenances in functional traits including: height, leaf horizontal width, average horizontal width, maximum perpendicular width, perpendicular width 1, and perpendicular width 2. Simple linear regressions resulted in the strongest correlations, with 12 out of 29 climate variables significantly correlating with variation in seedling traits. Multiple linear regressions did not necessarily result in stronger analyses, as only perpendicular width 1 and 2 had significant stepwise regression models with climate variables such as DD5, MAT, and AHM. Variation in height was independent of climate and geographic variables in both simple and multiple regression analyses. Multivariate analyses (PCA and CANCOR) further suggested strong relationships between significant leaf characters and temperature-related climate variables (DD5, MAT, and AHM). Height had a negative correlation with climate variable PPT_SM and was independent of leaf characters.

The results of this study indicate significant variation is present in height growth among yellow birch populations, but the driving factor for this trait remains unknown. Past research on this species has also resulted in weak or insignificant correlations of height growth to climate or geographic clines (Clausen, 1967, 1974, 1980; Leites et al., 2019; Sharik & Barnes, 1979). The lack of significant correlation may be due to high within-population variation or lack of statistical power to detect such trends. Furthermore, due to the relatively narrow ecological niche of yellow birch, there may be an overall lack of genetic variation differentiating provenances, due to the low tolerance of non-mesic site conditions. However, adaptive variation of leaf shape was expressed, strongly driven by temperature-related variables (i.e. DD5, MAT, MWMT, DD18, TAVE_SP, TAVE_SM) and heat-moisture indices (i.e. AHM, SHM, EREF, and CMD). Precipitation-related variables resulted in the weakest or least amount of correlations to variation among leaf traits. Temperature-related variables have been shown to be a strong driver of genetic variation in leaf morphology and phenology in species such as yellow birch (Dancik & Barnes, 1975; Sharik & Barnes, 1979), paper birch (Pyakurel & Wang, 2013), and trembling aspen (Thompson, 2014).

Global and regional reviews have also determined temperature to be a stronger predictor of variation among tree species (Aitken & Bemmels, 2016; Aitken et al., 2008; Leites et al., 2019; Moles et al., 2014). This is especially intriguing in light of climate change, as more consistent and extreme droughts have been predicted to increase tree mortality and global forest decline (Allen et al., 2015, 2010; McDowell et al., 2008). However, periods of drought following above-average temperatures have been shown to be more detrimental to forest health and productivity, and may be a stronger driver of drought-induced mortality (Allen et al., 2015). Therefore, understanding patterns of adaptive variation among yellow birch populations is crucial for the conservation and health of this species in future climate change conditions.

This study has shown temperature to be a strong driver of genetic variation among yellow birch provenances, despite high within-population variation. In order to acquire a stronger understanding of the variation within this species, further common garden studies should include (i) larger numbers of provenances and individuals sampled from, (ii) provenances from the extremes of the species range, and (iii) multiple-year data collections of other functional traits including germination, survival, leaf phenology, and flowering times.

As this and previous studies have shown, height growth of yellow birch is seemingly independent of climate or geographic variables (Clausen, 1967, 1980; Leites et al., 2019; Sharik & Barnes, 1979). Thus, the inclusion of a greater variety of measured functional traits (i.e. growth cessation, leaf phenology, and rooting depth) as well as physiological traits (i.e. photosynthesis rates, stomatal conductance, and WUE) may reveal stronger trends of local adaptation in relation to climate at seed origin. Ultimately, describing these patterns of intraspecific variation is crucial for understanding the effects of climate change on North American hardwood forests, improving future species range shifts, and conserving genetic diversity among yellow birch populations.

LITERATURE CITED

Abdi, H., & Williams, L. J. (2010). Principal component analysis. *Wiley Interdisciplinary Reviews: Computational Statistics*, *2*(4), 433–459. https://doi.org/10.1002/wics.101

Aitken, S. N., & Bemmels, J. B. (2016). Time to get moving: Assisted gene flow of forest trees. *Evolutionary Applications*, *9*(1), 271–290. https://doi.org/10.1111/eva.12293

Aitken, S. N., Yeaman, S., Holliday, J. A., Wang, T., & Curtis-McLane, S. (2008). Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications*, *1*(1), 95–111. https://doi.org/10.1111/j.1752-4571.2007.00013.x

Allen, C. D., Breshears, D. D., & McDowell, N. G. (2015). On underestimation of global vulnerability to tree mortality and forest die-off from hotter drought in the Anthropocene. *Ecosphere*, *6*(8), 1–55. https://doi.org/10.1890/ES15-00203.1

Allen, C. D., Macalady, A. K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., … Cobb, N. (2010). A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management*, *259*(4), 660–684. https://doi.org/10.1016/j.foreco.2009.09.001

Aspelmeier, S., & Leuschner, C. (2006). Genotypic variation in drought response of silver birch (Betula pendula Roth): Leaf and root morphology and carbon partitioning. *Trees - Structure and Function*, *20*(1), 42–52. https://doi.org/10.1007/s00468-005-0011-9

Aubin, I., Munson, A. D., Cardou, F., Burton, P. J., Isabel, N., Pedlar, J. H., … McKenney, D. (2016). Traits to stay, traits to move: A review of functional traits to assess sensitivity and adaptive capacity of temperate and boreal trees to climate change. *Environmental Reviews*, *24*(2), 164–186. https://doi.org/10.1139/er-2015-0072

Beaudet, M., & Messier, C. (1998). Growth and morphological responses of yellow birch, sugar maple, and beech seedlings growing under a natural light gradient. *Canadian Journal of Forest Research*, *28*, 1007–1015.

Bréda, N., & Badeau, V. (2008). Forest tree responses to extreme drought and some biotic events: Towards a selection according to hazard tolerance? *Comptes Rendus - Geoscience*, *340*(9–10), 651–662. https://doi.org/10.1016/j.crte.2008.08.003

Calmé, S., Bigras, F. J., Margolis, H. A., & Hébert, C. (1994). Frost tolerance and bud dormancy of container-grown yellow birch, red oak and sugar maple seedlings. *Tree Physiology*, *14*, 1313–1325. Retrieved from https://academic.oup.com/treephys/article-abstract/14/12/1313/1645014

Campbell, R. K., & Sugano, A. I. (1975). Phenology of Bud Burst in Douglas-Fir Related to Provenance, Photoperiod, Chilling, and Flushing Temperature. In *Source: Botanical Gazette* (Vol. 136).

Cheng, S., Widden, P., & Messier, C. (2005). Light and tree size influence belowground development in yellow birch and sugar maple. *Plant and Soil*, *270*(1), 321–330.

Clausen, K. E. (1967). *Variation in height growth and growth cessation of 55 yellow birch seed sources*. Rhinelander, Wisconsin.

Clausen, K. E. (1968). *Natural variation in catkin and fruit characteristics of yellow birch*. Rhinelander, Wisconsin.

Clausen, K. E. (1972a). *The yellow x paper birch hybrid - a potential substitute for yellow birch on problem sites*.

Clausen, K. E. (1972b). *Within-provenance variation in yellow birch*.

Clausen, K. E. (1973). *Genetics of Yellow Birch*.

Clausen, K. E. (1974). *Variation in early growth and survival of yellow birch provenances*. Rhinelander, Wisconsin.

Clausen, K. E. (1975). *Early flowering and seed production in a yellow birch progeny test*.

Clausen, K. E. (1980). Survival, growth, and flowering of yellow birch progenies in an open-field test. *Silvae Genetica*, *29*(3–4), 108–114.

Clausen, K. E., & Garrett, P. W. (1968). *Progress in birch genetics and tree improvement*.

Craig, H. (1953). The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*, *3*(2–3), 53–92. https://doi.org/10.1016/0016-7037(53)90001-5

Craig, H. (1954). Carbon 13 in plants and the relationship between Carbon 13 and Carbon 14 variations in nature. *Journal of Geology*, *62*(2), 115–149.

Dale, V. H., Joyce, L. A., McNulty, S., Neilson, R. P., Ayres, M. P., Flannigan, M. D., … Wotton, B. M. (2001). Climate Change and Forest Disturbances: Climate change can affect forests by altering the frequency, intensity, duration, and timing of fire, drought, introduced species, insect and pathogen outbreaks, hurricanes, windstorms, ice storms, or landslides. *BioScience*, *51*(9), 723–734. https://doi.org/10.1641/0006-3568(2001)051[0723:CCAFD]2.0.CO;2

Dancik, B., & Barnes, B. (1975). Leaf variability in yellow birch (Betula alleghaniensis) in relation to environment. *Canadian Journal of Forest Research*, *5*(2), 149–159. Retrieved from www.nrcresearchpress.com

Dawson, T. E., Mambelli, S., Plamboeck, A. H., Templer, P. H., & Tu, K. P. (2002). Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics*, *33*, 507–559. https://doi.org/10.1146/annurev.ecolsys.33.020602.095451

Delagrange, S. (2011). Light- and seasonal-induced plasticity in leaf morphology, N partitioning and photosynthetic capacity of two temperate deciduous species. *Environmental and Experimental Botany*, *70*(1), 1–10. https://doi.org/10.1016/j.envexpbot.2010.05.008

Delagrange, S., Messier, C., Lechowicz, M. J., & Dizengremel, P. (2004). Physiological, morphological and allocational plasticity in understory deciduous trees: Importance of plant size and light availability. *Tree Physiology*, *24*(7), 775–784. https://doi.org/10.1093/treephys/24.7.775

Diefendorf, A. F., Mueller, K. E., Wing, S. L., Koch, P. L., & Freeman, K. H. (2010). Global patterns in leaf 13C discrimination and implications for studies of past and future climate. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(13), 5738–5743. https://doi.org/10.1073/pnas.0910513107

Dillen, S. Y., Marron, N., Koch, B., & Ceulemans, R. (2008). Genetic variation of stomatal traits and carbon isotope discrimination in two hybrid popular families (Populus deltoides “S9-2” X P. nigra “Ghoy” and P. deltoides “S9-2” X P.

trichocarpa “V24.” *Annals of Botany*, *102*(1), 399–407. https://doi.org/10.1093/ITNOW/BWAA110

Donovan, L. A., & Ehleringer, J. R. (1994). Potential for selection on plants for water-use efficiency as estimated by carbon isotope discrimination. *American Journal of Botany*, *81*(7), 927–935. https://doi.org/10.2307/2445774

Dudley, S. A. (1996). Differing selection on plant physiological traits in response to environmental water availability: A test of adaptive hypotheses. *Evolution*, *50*(1), 92–102. https://doi.org/10.1111/j.1558-5646.1996.tb04475.x

Erdmann, G. G. (1990). Betula alleghaniensis Britton yellow birch. In B. H. Burns, Russell M.; Honkala (Ed.), *Silvics of North America: 2 Hardwoods; Agriculture Handbook 654* (pp. 133–147). Retrieved from https://www.srs.fs.usda.gov/pubs/misc/ag_654/volume_2/betula/alleghaniensis.htm

Etterson, J. R., & Shaw, R. G. (2001). Constraint to adaptive evolution in response to global warming. *Science*, *294*, 151–154.

Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon Isotope Discrimination And Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, *40*, 503–537. Retrieved from www.annualreviews.org/aronline

Ferguson, J. N., Humphry, M., Lawson, T., Brendel, O., & Bechtold, U. (2018). Natural variation of life-history traits, water use, and drought responses in Arabidopsis. *Plant Direct*, *2*(1). https://doi.org/10.1002/pld3.35

Frankham, R., Ballou, J. D., & Briscoe, D. A. (2013). *Introduction to Conservation Genetics* (2nd Editio). Retrieved from https://ocul-lhd.primo.exlibrisgroup.com/permalink/01OCUL_LHD/544bqc/alma9910983031505155

Gao, R., Shi, X., & Wang, J. R. (2017). Comparative studies of the response of larch and birch seedlings from two origins to water deficit. *New Zealand Journal of Forestry Science*, *47*(1). https://doi.org/10.1186/S40490-017-0095-1

Gaucher, C., Gougeon, S., Mauffette, Y., & Messier, C. (2005). Seasonal variation in biomass and carbohydrate partitioning of understory sugar maple (Acer saccharum) and yellow birch (Betula alleghaniensis) seedlings. *Tree Physiology*, *25*, 93–100. https://doi.org/10.1093/treephys/25.1.93

Gittins, R. (1986). Canonical Analysis; a review with applications in ecology. *Biomathematics*, *12*, 135–136.

Guet, J., Fabbrini, F., Fichot, R., Sabatti, M., Bastien, C., & Brignolas, F. (2015). Genetic variation for leaf morphology, leaf structure and leaf carbon isotope discrimination in European populations of black poplar (Populus nigra L.). *Tree Physiology*, *35*(8), 850–863. https://doi.org/10.1093/treephys/tpv056

Gunderson, C. A., Edwards, N. T., Walker, A. V., O’Hara, K. H., Campion, C. M., & Hanson, P. J. (2012). Forest phenology and a warmer climate - growing season extension in relation to climatic provenance. *Global Change Biology*, *18*(6), 2008–2025. https://doi.org/10.1111/j.1365-2486.2011.02632.x

Hovenden, M. J., & Vander Schoor, J. K. (2004). Nature vs nurture in the leaf morphology of Southern beech, Nothofagus cunninghamii (Nothofagaceae). *New Phytologist*, *161*(2), 585–594. https://doi.org/10.1046/j.1469-8137.2003.00931.x

Howe, G. T., Aitken, S. N., Neale, D. B., Jermstad, K. D., Wheeler, N. C., & Chen, T. H. H. (2003). From genotype to phenotype: Unraveling the complexities of cold adaptation in forest trees. *Canadian Journal of Botany*, *81*(12), 1247–1266.

https://doi.org/10.1139/b03-141

Hurvich, C. M., & Tsai, C. (1990). The Impact of Model Selection on Inference in Linear Regression. *The American Statistician*, *44*(3), 214–217.

Hutnik, R. J., & Cunningham, F. E. (1961). *Silvical characteristics of paper birch (*Betula papyrifera*). Station Paper No. 141*. 24.

Jones, E., Reed, D., Mroz, G., Liechty, H., & Cattelino, P. (1993). Climate stress as a precursor to forest decline: paper birch in northern Michigan, 1985-1990. *Canadian Journal of Forest Research*, *23*, 229–233.

Joyce, D. G., & Rehfeldt, G. E. (2013). Climatic niche, ecological genetics, and impact of climate change on eastern white pine (Pinus strobus L.): Guidelines for land managers. *Forest Ecology and Management*, *295*, 173–192. https://doi.org/10.1016/j.foreco.2012.12.024

Juncus, L. (1998). *Plant Guide*. (Figure 3), 5–8.

Kondo, M., Pablico, P. P., Aragones, D. V., & Agbisit, R. (2004). Genotypic variations in carbon isotope discrimination, transpiration efficiency, and biomass production in rice as affected by soil water conditions and N. *Plant and Soil*, *267*(1–2), 165–177. https://doi.org/10.1007/s11104-005-4884-6

Lamont, B. B., Groom, P. K., & Cowling, R. M. (2002). High leaf mass per area of related species assemblages may reflect low rainfall and carbon isotope discrimination rather than low phosphorus and nitrogen concentrations. *Functional Ecology*, *16*(3), 403–412. https://doi.org/10.1046/j.1365-2435.2002.00631.x

Leavitt, S. W., & Long, A. (1986). Stable-Carbon Isotope Variability in Tree Foliage and Wood. *Ecology*, *67*(4), 1002–1010.

Leites, L. P., Rehfeldt, G. E., & Steiner, K. C. (2019). Adaptation to climate in five eastern North America broadleaf deciduous species: Growth clines and evidence of the growth-cold tolerance trade-off. *Perspectives in Plant Ecology, Evolution and Systematics*, *37*, 64–72. https://doi.org/10.1016/j.ppees.2019.02.002

Lesser, M. R. (2005). *Genecology, patterns of adaptive variation and a comparison of focal point seed zone development methodologies for white spruce (Picea glauca)*.

Lesser, M. R., & Parker, W. H. (2006). Comparison of canonical correlation and regression based focal point seed zones of white spruce. *Canadian Journal of Forest Research*, *36*, 1572–1586.

Logan, K. T. (1965). Growth of tree seedlings as affected by light intensity. *Department of Forestry*, *1121*(1121), 1–16.

Lu, P., Joyce, D. G., & Sinclair, R. W. (2003). Geographic variation in cold hardiness among eastern white pine (Pinus strobus L.) provenances in Ontario. *Forest Ecology and Management*, *178*(3), 329–340. https://doi.org/10.1016/S0378-1127(02)00481-4

Lu, P., Parker, W. C., Colombo, S. J., & Man, R. (2016). Restructuring tree provenance test data to conform to reciprocal transplant experiments for detecting local adaptation. *Journal of Applied Ecology*, *53*(4), 1088–1097. https://doi.org/10.1111/1365-2664.12647

Mac Nally, R. (2000). Regression and model-building in conservation biology, biogeography and ecology: The distinction between – and reconciliation of – “predictive” and “explanatory” models. *Biodiversity and Conservation*, *9*, 655–671.

Matyas, C. (1996). Climatic adaptation of trees: rediscovering provenance tests. In *Euphytica* (Vol. 92).

McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., … Yepez, E. A. (2008). Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytologist*, *178*(4), 719–739. https://doi.org/10.1111/j.1469-8137.2008.02436.x

Messier, C., & Nikinmaa, E. (2000). Effects of light availibility and sapling size on the growth, biomass allocation, and crown morphology of understory sugar maple, yellow birch, and beech. *Ecoscience*, *7*(3), 345–356.

Moles, A. T., Perkins, S. E., Laffan, S. W., Flores-Moreno, H., Awasthy, M., Tindall, M. L., … Bonser, S. P. (2014). Which is a better predictor of plant traits: Temperature or precipitation? *Journal of Vegetation Science*, *25*(5), 1167–1180. https://doi.org/10.1111/jvs.12190

O'Brien, M. J., Engelbrecht, B. M. J., Joswig, J., Pereyra, G., Schuldt, B., Jansen, S., … Macinnis-Ng, C. (2017). A synthesis of tree functional traits related to drought-induced mortality in forests across climatic zones. *Journal of Applied Ecology*, *54*(6), 1669–1686. https://doi.org/10.1111/1365-2664.12874

O'Leary, M. H. (1988). Carbon Isotopes in Photosynthesis. *BioScience*, *38*(5), 328–336. https://doi.org/10.2307/1310735

Oke, O. A. (2009). *Adaptive and phenotypic responses of white birch provenances to climate variations*. Lakehead University.

Oke, O. A., & Wang, J. R. (2013). Assessing effects of seed source and transfer potential of white birch populations using transfer functions. *Open Journal of Ecology*, *03*(05), 359–369. https://doi.org/10.4236/oje.2013.35041

Olivas-Garcia, J. M., Cregg, B. M., & Hennessey, T. C. (2000). Genotypic variation in carbon isotope discrimination and gas exchange of ponderosa pine seedlings under two levels of water stress. *Canadian Journal of Forest Research*, *30*(10), 1581–1590. https://doi.org/10.1139/x00-080

Osmond, C. B., Björkman, O., & Anderson, D. J. (1980). *Physiological processes in plant ecology*. New York: Springer.

Parker, W. H., Riddell, C. L., & Lesser, M. R. (2004). *Identification of black spruce seed sources to compensate for loss of growth resulting from climate change*. Thunder Bay, Ontario.

Picon, C., Guehl, J. M., & Ferhi, A. (1996). Leaf gas exchange and carbon isotope composition response? to drought in a drought-avoiding (Pinus pinaster) and a drought-tolerant (Quercus petraea) species under present and elevated atmospheric CO2 concentrations. *Plant, Cell and Environment*, *19*(2), 182–190. https://doi.org/10.1111/j.1365-3040.1996.tb00239.x

Prunier, J., Pelgas, B., Gagnon, F., Desponts, M., Isabel, N., Beaulieu, J., & Bousquet, J. (2013). The genomic architecture and association genetics of adaptive characters using a candidate SNP approach in boreal black spruce. *BMC Genomics*, *14*(1). https://doi.org/10.1186/1471-2164-14-368

Pyakurel, A., & Wang, J. R. (2013). Leaf morphological variation among paper birch (Betula papyrifera Marsh.) genotypes across Canada. *Open Journal of Ecology*, *03*(04), 284–295. https://doi.org/10.4236/oje.2013.34033

Rasheed, F., & Delagrange, S. (2016). Acclimation of Betula alleghaniensis Britton to moderate soil water deficit: small morphological changes make for important consequences in crown display. *Tree Physiology*, *36*, 1320–1329. https://doi.org/10.1093/treephys/tpw064

Rehfeldt, G. E., Leites, L. P., Joyce, D. G., & Weiskittel, A. R. (2018). Role of population genetics in guiding ecological responses to climate. *Global Change Biology*, *24*(2), 858–868. https://doi.org/10.1111/gcb.13883

Rehfeldt, G. E., Ying, C. C., Spittlehouse, D. L., & Hamilton, D. A. (1999). Genetic responses to climate in Pinus contorta: Niche breadth, climate change, and reforestation. *Ecological Monographs*, *69*(3), 375–407. https://doi.org/10.2307/2657162

Robson, T. M., Sánchez-Gómez, D., Cano, F. J., & Aranda, I. (2012). Variation in functional leaf traits among beech provenances during a Spanish summer reflects the differences in their origin. *Tree Genetics and Genomes*, *8*(5), 1111–1121. https://doi.org/10.1007/s11295-012-0496-5

Seibt, U., Rajabi, A., Griffiths, H., & Berry, J. A. (2008). Carbon isotopes and water use efficiency: Sense and sensitivity. *Oecologia*, *155*(3), 441–454. https://doi.org/10.1007/s00442-007-0932-7

Sharik, T. L., & Barnes, B. V. (1979). Natural variation in morphology among diverse populations of yellow birch (Betula alleghaniensis) and sweet birch (B. lenta) . *Canadian Journal of Botany*, *57*(18), 1932–1939. https://doi.org/10.1139/b79-242

Sharik, T. L., & Ford, R. H. (1984). Variation and taxonomy of Betula uber, B. Lenta, and B. alleghaniensis. *Brittonia*, *36*(3), 307–316. https://doi.org/10.2307/2806532

Siegwolf, R. T. W., Matyssek, R., Saurer, M., Maurer, S., Günthardt-Goerg, M. S., Schmutz, P., & Bucher, J. B. (2001). Stable isotope analysis reveals differential effects of soil nitrogen and nitrogen dioxide on the water use efficiency in hybrid poplar leaves. *New Phytologist*, *149*(2), 233–246. https://doi.org/10.1046/j.1469-8137.2001.00032.x

Tausz, M., Wonisch, A., Ribarič-Lasnik, C., Batič, F., & Grill, D. (2002). Multivariate analyses of tree physiological attributes - Applications in field studies. *Phyton - Annales Rei Botanicae*, *42*(3), 215–221.

Thompson, B. (1987). *Fundamentals of Canonical Correlation Analysis: Basics and Three Common Fallacies in*. Retrieved from https://files.eric.ed.gov/fulltext/ED282904.pdf

Thompson, M. (2014). *Adaptive variation of Trembling Aspen in Northwestern Ontario* (Lakehead University). Retrieved from http://knowledgecommons.lakeheadu.ca/bitstream/2453/656/1/ThompsonM2014m-1b.pdf

Thomson, A. M. (2008). *Development of optimal breeding zones for white spruce in Ontario under current, past, and anticipated future climate change*. Lakehead University.

Thomson, A. M. (2013). *Phylogeography , introgression , and population structure of the eastern North American birches Betula alleghaniensis , B . papyrifera , and B . lenta*. Concordia University.

Thomson, A. M., Crowe, K. A., & Parker, W. H. (2010). Optimal white spruce breeding zones for ontario under current and future climates. *Canadian Journal of Forest Research*, *40*(8), 1576–1587. https://doi.org/10.1139/X10-112

Thomson, A. M., Dick, C. W., & Dayanandan, S. (2015). A similar phylogeographical structure among sympatric North American birches (Betula) is better explained by introgression than by shared biogeographical history. *Journal of Biogeography*, *42*(2), 339–350. https://doi.org/10.1111/jbi.12394

Thomson, A. M., Dick, C. W., Pascoini, A. L., & Dayanandan, S. (2015). Despite introgressive hybridization, North American birches (Betula spp.) maintain strong differentiation at nuclear microsatellite loci. *Tree Genetics and Genomes*, *11*(5). https://doi.org/10.1007/s11295-015-0922-6

Thomson, A. M., & Parker, W. H. (2008). Boreal forest provenance tests used to predict optimal growth and response to climate change. 1. Jack pine. *Canadian Journal of Forest Research*, *38*(1), 157–170. https://doi.org/10.1139/X07-122

Vitasse, Y., Delzon, S., Dufrêne, E., Pontailler, J. Y., Louvet, J. M., Kremer, A., & Michalet, R. (2009). Leaf phenology sensitivity to temperature in European trees: Do within-species populations exhibit similar responses? *Agricultural and Forest Meteorology*. https://doi.org/10.1016/j.agrformet.2008.10.019

Wang, J. R., Hawkins, C. D. B., & Letchford, T. (1998). Relative growth rate and biomass allocation of paper birch (*Betula papyrifera*) populations under different soil moisture and nutrient regimes. *Canadian Journal of Forest Research*, *28*(1), 44–55. https://doi.org/10.1139/cjfr-28-1-44

Wang, T., Hamann, A., Spittlehouse, D., & Carroll, C. (2016). Locally Downscaled and Spatially Customizable Climate Data for Historical and Future Periods for North America. *PLOS ONE*, *11*(6), e0156720. https://doi.org/10.1371/journal.pone.0156720

Warren, C. R., Tausz, M., & Adams, M. A. (2005). Does rainfall explain variation in leaf morphology and physiology among populations of red ironbark (Eucalyptus sideroxylon subsp. tricarpa) grown in a common garden? *Tree Physiology*, *25*(11), 1369–1378. https://doi.org/10.1093/treephys/25.11.1369

Wearstler, K. A., Barnes, B. V, & Barnes, B. V. (1977). Genetic diversity of yellow birch seedlings in Michigan. *Canadian Journal of Botany*, *55*, 2778–2788. Retrieved from www.nrcresearchpress.com

Westoby, M., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Diemer, M., … Cornelissen, J. H. . (2004). The worldwide leaf economics spectrum. *Nature*, *428*, 821–827. Retrieved from www.nature.com/nature%0Ahttps://idp.nature.com/authorize/casa?redirect_uri=https://www.nature.com/articles/nature02403&casa_token=CtYdn6u63BcAAAAA:cNAkoHi669d547DoKaM3keUixX15dhKLnmyUD_oX2GBEB1VTe9mtWcMdFoXauJPFpb4iQItw5D0oAvb3

Westoby, M., Reich, P. B., & Wright, I. J. (2013). Understanding ecological variation across species: Area-based vs mass-based expression of leaf traits. *New Phytologist*, *199*(2), 322–323. https://doi.org/10.1111/nph.12345

Whittingham, M. J., Stephens, P. A., Bradbury, R. B., & Freckleton, R. P. (2006). Why do we still use stepwise modelling in ecology and behaviour? *Journal of Animal Ecology*, *75*(5), 1182–1189. https://doi.org/10.1111/j.1365-2656.2006.01141.x

Wigle, R. D., Wiersma, Y. F., Arsenault, A., & McMullin, R. T. (2021). Drivers of arboreal lichen community structure and diversity on Abies balsamea and Betula alleghaniensis in the Avalon Forest Ecoregion, Newfoundland. *Botany*, *99*(1), 43–54. https://doi.org/10.1139/cjb-2020-0061

Wilkinson, L. (1979). Tests of significance in stepwise regression. *Psychological Bulletin*, *86*(1), 168–174. https://doi.org/10.1037/0033-2909.86.1.168

Wilkonsin, S., & Davies, W. (2010). Drought, ozone, ABA, and ethylene: new insights from cell to plant to community. *Plant Cell and Environment*, *33*, 510–525.

Wright, I. J., Reich, P. B., Cornelissen, J. H. C., Falster, D. S., Groom, P. K., Hikosaka, K., … Westoby, M. (2005). Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography*, *14*(5), 411–421. https://doi.org/10.1111/j.1466-822x.2005.00172.x

Wright, I. J., Reich, P. B., Cornelissen, J. H. C., Falster, D. S., Hikosaka, K., Lamont, B. B., … May, N. (2018). Assessing the Generality of Global Leaf Trait Relationships. *New Phytologist*, *166*(2), 485–496.

Wright, I. J., & Westoby, M. (1999). Differences in seedling growth behaviour among species: Trait correlations across species, and trait shifts along nutrient compared to rainfall gradients. *Journal of Ecology*, *87*(1), 85–97. https://doi.org/10.1046/j.1365-2745.1999.00330.x

Wu, C. A., Lowry, D. B., Nutter, L. I., & Willis, J. H. (2010). Natural variation for drought-response traits in the Mimulus guttatus species complex. *Oecologia*, *162*(1), 23–33. https://doi.org/10.1007/s00442-009-1448-0

Xu, F., Guo, W., Xu, W., Wei, Y., & Wang, R. (2009). Leaf morphology correlates with water and light availability: What consequences for simple and compound leaves. *Progress in Natural Science*, *19*(12), 1789–1798. https://doi.org/10.1016/j.pnsc.2009.10.001

Zhang, J., Marshall, J. D., & Jaquish, B. C. (1993). Genetic differentiation in carbon isotope discrimination and gas exchange in Pseudotsuga menziesii - A common-garden experiment. *Oecologia*, *93*(1), 80–87. https://doi.org/10.1007/BF00321195

Zhang, X., Zang, R., & Li, C. (2004). Population differences in physiological and morphological adaptations of Populus davidiana seedlings in response to progressive drought stress. *Plant Science*, *166*(3), 791–797. https://doi.org/10.1016/j.plantsci.2003.11.016

Zierl, B. (2004). A simulation study to analyse the relations between crown condition and drought in Switzerland. *Forest Ecology and Management*, *188*(1–3), 25–38. https://doi.org/10.1016/j.foreco.2003.07.019

APPENDICES

APPENDIX I

Table A. 1. Provenance number, seedlot number, name, geographic location, elevation, collection type and date (if applicable), and germination rate.

Provenance	Seedlot #	Location	State/Province	Country	Coll. Type	Latitude	Longitude	Elev. (m)	Germ %	Test Year
P1.1	20041112.0	Black Brook	NB	CAN	S	47.45000	-67.45000	300	72.5	2015
P1.2	20041113.0	Black Brook	NB	CAN	S	47.45000	-67.45000	300	51.0	2015
P1.3	20041114.0	Black Brook	NB	CAN	S	47.45000	-67.45000	300	63.0	2015
P1.4	20041115.0	Black Brook	NB	CAN	S	47.45000	-67.45000	300	59.5	2015
P1.5	20041116.0	Black Brook	NB	CAN	S	47.45000	-67.45000	300	70.5	2015
P1.6	20041117.0	Black Brook	NB	CAN	S	47.45000	-67.45000	300	72.0	2015
P1.7	20041118.0	Black Brook	NB	CAN	S	47.45000	-67.45000	300	58.0	2015
P1.8	20041121.0	Black Brook	NB	CAN	S	47.45000	-67.45000	300	50.0	2015
P1.9	20041122.0	Black Brook	NB	CAN	S	47.45000	-67.45000	300	58.5	2015
P1.10	20041124.0	Black Brook	NB	CAN	S	47.45000	-67.45000	300	56.5	2015
P2.1	20070115.0	Gallants	NL	CAN	S	48.67692	-58.19208	145	82.0	2018
P2.2	20070116.0	Gallants	NL	CAN	S	48.67657	-58.18987	161	84.0	2018
P2.3	20070117.0	Gallants	NL	CAN	S	48.67660	-58.19062	160	56.0	2018
P2.4	20070118.0	Gallants	NL	CAN	S	48.67568	-58.20485	146	64.5	2018
P2.5	20070119.0	Gallants	NL	CAN	S	48.67844	-58.18196	123	67.5	2018
P3.1	20091134.0	Big Pond	NL	CAN	S	45.95879	-60.42612	169	81.5	2010
P3.2	20091135.0	Big Pond	NL	CAN	S	45.93525	-60.46489	196	63.5	2010
P3.3	20091136.0	Big Pond	NL	CAN	S	45.91672	-60.50623	40	82.0	2010
P3.4	20091137.0	Big Pond	NL	CAN	S	45.93173	-60.47038	189	68.5	2010
P3.5	20091138.0	Big Pond	NL	CAN	S	45.94134	-60.45244	184	54.0	2010
P3.6	20091139.0	Big Pond	NL	CAN	S	45.93961	-60.45601	193	78.0	2010
P3.7	20091141.0	Big Pond	NL	CAN	S	45.93830	-60.45858	211	86.0	2010
P3.8	20091142.0	Big Pond	NL	CAN	S	45.94161	-60.45198	185	97.0	2010
P3.9	20091143.0	Big Pond	NL	CAN	S	45.93813	-60.46236	178	60.5	2010
P3.10	20091146.0	Big Pond	NL	CAN	S	45.94713	-60.44260	201	83.5	2010
P4.1	7434400.0	Petawawa R.F	ON	CAN	S	46.00000	-77.43333	150	75.0	2009
P4.2	8930038.0	Petawawa R.F	ON	CAN	S	46.00000	-77.43333	150	54.0	2009
P4.3	8930040.0	Petawawa R.F	ON	CAN	S	46.00000	-77.43333	150	58.5	2009
P4.4	9130055.0	Petawawa R.F	ON	CAN	S	46.00000	-77.41666	n/a	45.0	2009
P4.5	9130056.0	Petawawa R.F	ON	CAN	S	46.00000	-77.40000	n/a	49.5	2012
P5.1	201930338.0	Greenwood	ON	CAN	S	48.39344	-90.75198	497	n/a	Coll. 10/10/19
P5.2	201930340.0	Greenwood	ON	CAN	S	48.39224	-90.71440	495	n/a	Coll. 10/10/19
P5.3	201930342.0	Greenwood	ON	CAN	S	48.28065	-90.75352	501	n/a	Coll. 10/10/19
P5.4	201930344.0	Greenwood	ON	CAN	S	48.39579	-90.75356	502	n/a	Coll. 10/10/19
P5.5	201930345.0	Greenwood	ON	CAN	S	48.39383	-90.75204	497	n/a	Coll. 10/10/19
P6.1	201930339.0	Squaretop Mt	ON	CAN	S	48.28006	-89.39645	402	n/a	Coll. 10/10/19
P6.2	201930341.0	Squaretop Mt	ON	CAN	S	48.39546	-89.39546	398	n/a	Coll. 10/10/19
P6.3	201930343.0	Squaretop Mt	ON	CAN	S	48.39422	-89.39674	401	n/a	Coll. 10/10/19
P6.4	201930346.0	Squaretop Mt	ON	CAN	S	48.28037	-89.39666	404	n/a	Coll. 10/10/19
P6.5	201930350.0	Squaretop Mt	ON	CAN	S	48.28034	-89.34616	398	n/a	Coll. 10/10/19
7.1	T17113	Hiawatha NF	MI	USA	B	46.233036	-86.50807	247	n/a	2019
8.2	T17129	Superior NF	MN	USA	B	47.895205	-91.01247	555	n/a	2017
9.1	T1133 (1137B)	Chippewa NF	MN	USA	B	47.583782	-93.89938	435	n/a	2017
10.1	201980727.0	Ottawa NF	MI	USA	S	46.3587	-89.81761	503	n/a	Coll. 10/27/19
10.2	201980728.0	Ottawa NF	MI	USA	S	46.36995	-89.78534	490	n/a	Coll. 10/27/19
10.3	201980729.0	Ottawa NF	MI	USA	S	46.37221	-89.82616	484	n/a	Coll. 10/27/19
10.4	201980730.0	Ottawa NF	MI	USA	S	46.37706	-89.82825	480	n/a	Coll. 10/27/19
10.5	201980731.0	Ottawa NF	MI	USA	S	46.38989	-89.83968	490	n/a	Coll. 10/27/19

APPENDIX II

CARBON AND NITROGEN ISOTOPE ANALYSIS PROTOCOL AND DESCRIPTION

(Provided by Environmental Isotope Laboratory, University of Waterloo)

^{13}C and ^{15}N Isotope Analysis: Equipment, data report guide and precision details

The analysis of solid materials for ^{13}C and ^{15}N isotope measurements was determined through combustion conversion of sample material to gas through a 4010 Elemental Analyzer (Costech Instruments, Italy) coupled to a Delta Plus XL (Thermo-Finnigan, Germany) continuous flow isotope ratio mass spectrometer (CFIRMS).

Results report and column guide;
All samples that arrive at EIL (Sample column) are assigned unique Lab numbers (Lab # column) the total of which (# column) is grouped within a unique EIL ISO file number (2018XXX).

The sample weight (Weight column) used in analysis along with the measured N_2 and CO_2 signal (Major Peak Area column) may or may not be included in the final report. This is usually of interest to researchers and clients weighing out their own samples and who may require the information to adjust the sample target weight for sample repeat submission; these details are available upon request. The ‘range’ details for the run are found at the bottom of the Major Peak Area column.

The %N and %C element content (Total % columns) is a bulk measurement based on the sample weight against known certified elemental standard materials.
The $\delta^{13}C$ data ($\delta^{13}C$ IRMEA / VPDB column) is the corrected delta value, reported in per mil (‰) units, against the primary reference scale of Vienna Pee Dee Belemnite (VPDB).
The $\delta^{15}N$ data ($\delta^{15}N$ IRMEA / AIR column) is the corrected delta value, reported in per mil (‰) units, against the primary reference scale of Atmospheric Air.

General Precision details;
Data quality control is monitored and corrections made using an array of international reference material and in-house EIL standards that are calibrated using certified international reference materials (i.e. IAEA-N1 + N2, IAEA-CH3 + CH6, USGS-40 + 41) with values provided through CIAAW. A mix of EIL in-house (i.e. EIL-72, EGC-3 and JSEC-01) and international materials are analyzed in each run.

A ‘like’ material is also used when possible; usually a NIST material like NIST-1577b (Bovine Liver) is used when tissues or invertebrates are analyzed and NIST 2704 (River

sediment) is used when analyzing soils and sediments. This provides a post-correction check throughout the analysis run for both isotopic and elemental percent data.
In the case of ^{15}N analysis in ammonia, three in-house ammonium sulphate materials were created and calibrated using the certified international reference materials IAEA-N1 + N2. These are incorporated within the ammonia disk sample preparation of each run, in duplicate to provide the information required to correct for any offset within the disk preparation technique.

Of the total sample number dropped in an analytical run, no less than 20% are Std/Ref materials. These Std/Ref measurements are used in data normalization and to ensure daily mass spec precision and accuracy; also to assess linearity issues or mass spec drift throughout the duration of the run. With these QA/QC checks an error of 0.2‰ $\delta^{13}C$ and 0.3‰ $\delta^{15}N$ are required for reportable data.

APPENDIX III CLIMATE DATA DESCRIPTIONS AND VALUES

Table A. 2. Climate Variables with their definitions, range maximum and minimum values, units, and abbreviations.

Definition	Range Min	Range Max	Units	Abbreviation
mean annual temperature	2.4	5.7	°C	MAT
mean annual precipitation	684	1510	mm	MAP
mean summer precipitation	402	535	mm	MSP
mean warmest month temperature	16.4	19.7	°C	MWMT
mean coldest month temperature	-15.4	-5.8	°C	MCMT
temperature difference between MWMT and MCMT	23.5	33.3	°C	TD
annual heat-moisture index	10.4	20.5	((MAT+10)/(MAP/1000))	AHM
summer heat-moisture index	30.8	47.6	((MWMT)/(MSP/1000))	SHM
degree-days below 0°C, chilling degree days	632	1574	degree days	DD_0
degree-days above 5°C, growing degree days	1361	1906	degree days	DD5
degree-days below 18°C, heating degree days	4526	5724	degree days	DD_18
degree-days above 18°C, cooling degree days	49	173	degree days	DD18
the number of frost free days	134	173	days	NFFD
frost free period	109	136	days	FFP
precipitation as snow	114	393	mm	PAS
extreme minimum temperature over 30 years	-42.9	-29.5	°C	EMT
extreme maximum temperature over 30 years	32	37.5	°C	EXT
Hogg's climate moisture index	16.2	113.36	mm	CMI
Hargreaves reference evaporation	515	706	mm	EREF
Hargreaves climatic moisture deficit	4	194	mm	CMD
mean annual relative humidity	56	73	%	RH
winter mean temperature	-13.2	-4.3	°C	TAVE_WT
spring mean temperature	1.6	4.6	°C	TAVE_SP
summer mean temperature	14.9	18.4	°C	TAVE_SM
autumn mean temperature	4.1	8.7	°C	TAVE_AT
winter precipitation	56	423	mm	PPT_WT
spring precipitation	136	349	mm	PPT_SP
summer precipitation	239	322	mm	PPT_SM
autumn precipitation	187	430	mm	PPT_AT
longitude	-58.19	-93.89	decimal degrees	LONG
latitude	45.9	48.6	decimal degrees	LAT

APPENDIX IV

SIMPLE LINEAR REGRESSION ANALYSES (p<0.1)

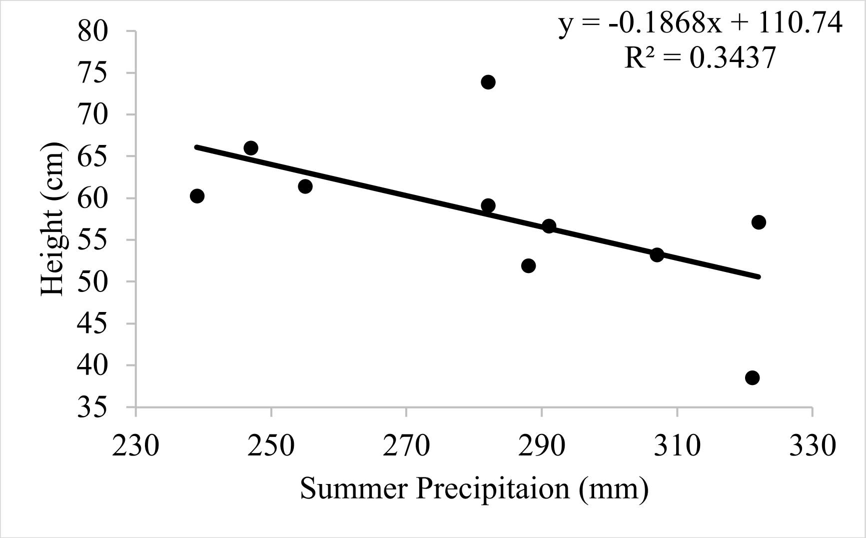

Figure A. 1. Simple linear regression analysis of height and summer precipitation.

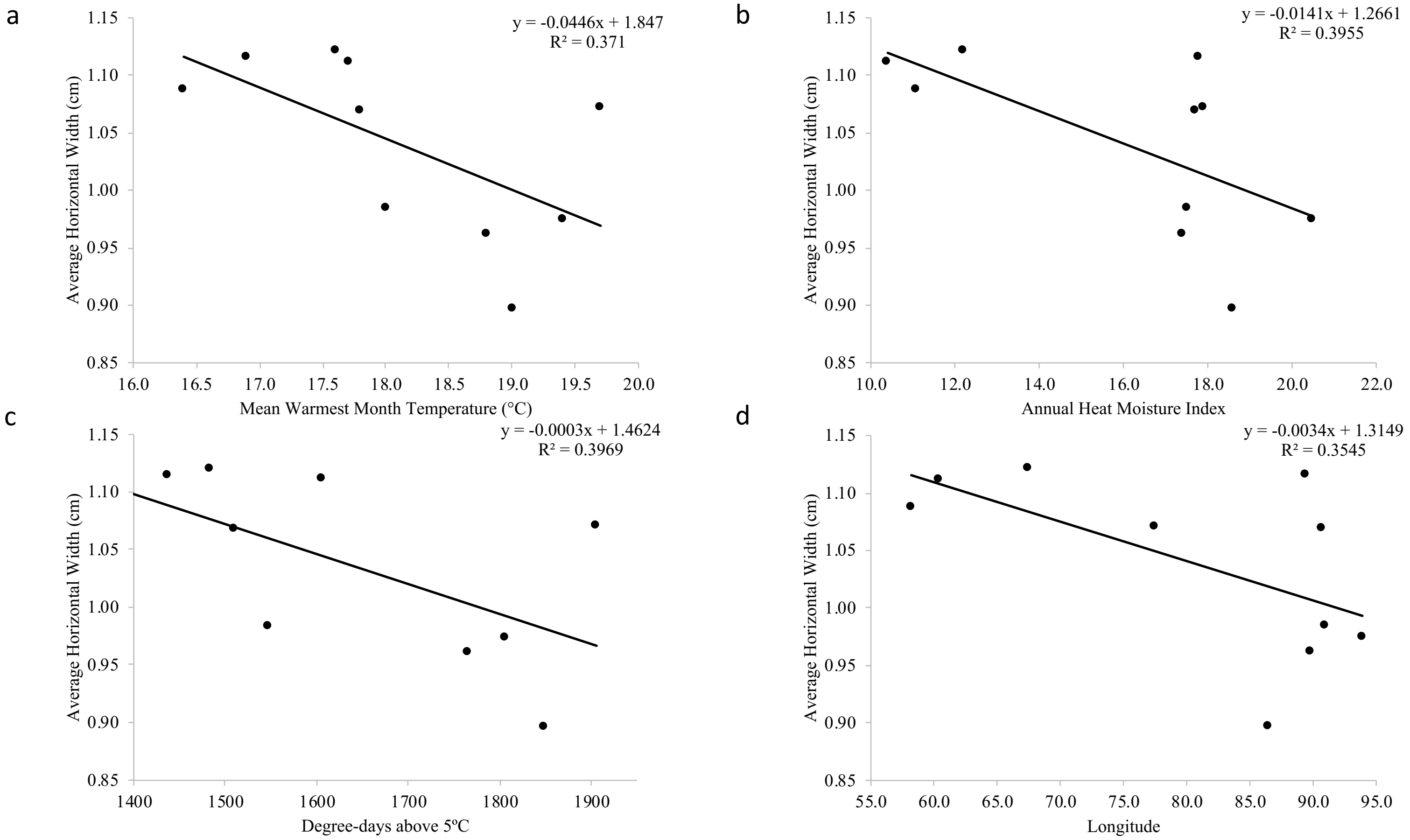

Figure A. 2. Simple linear regression analysis of average horizontal width and (a) mean warmest month temperature; (b) annual heat moisture index; (c), degree-days above 5ºC; and (d) longitude.

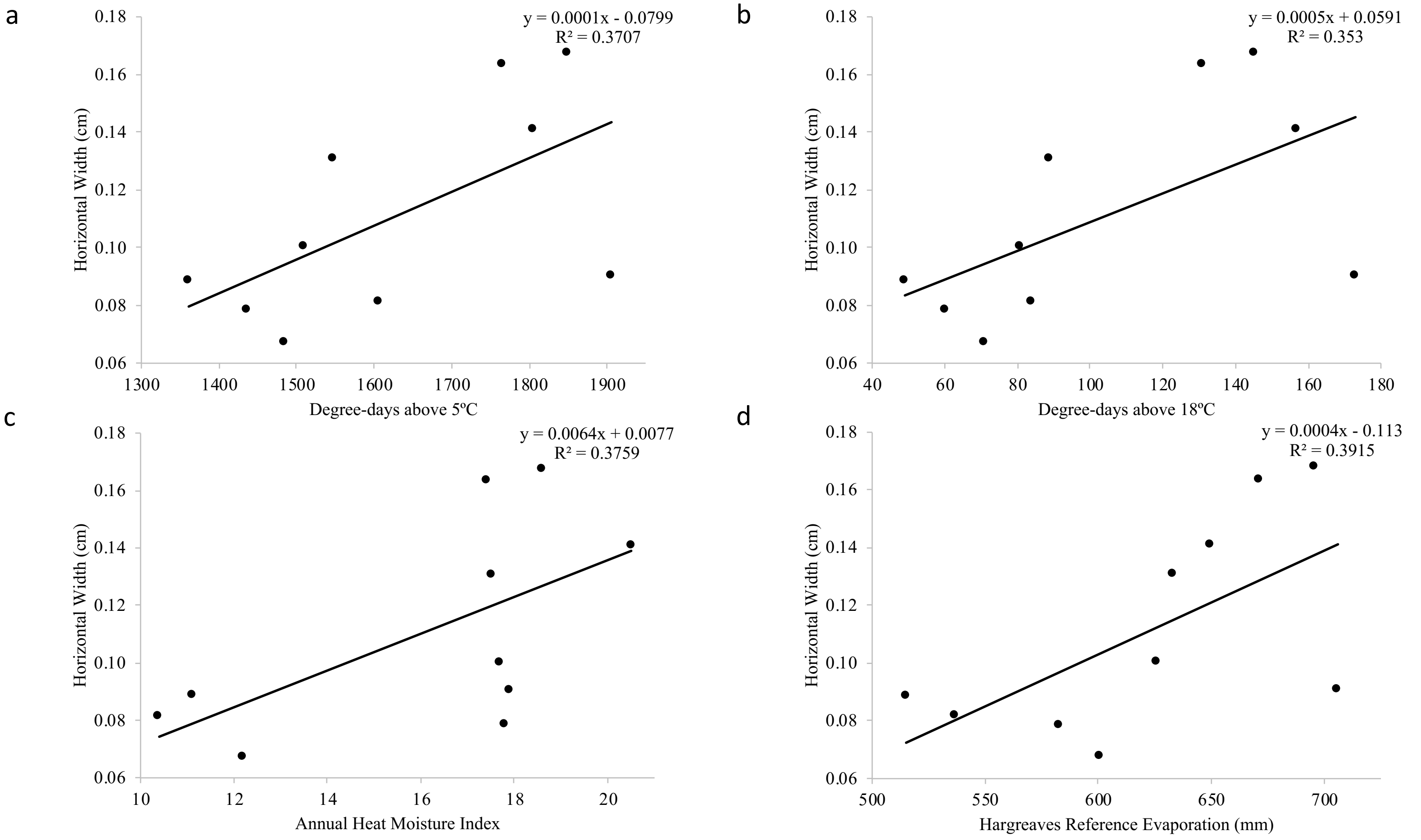

Figure A. 3. Simple linear regression analysis of horizontal width and (a) degree-days above 5ºC; (b) degree-days above 18ºC; (c) annual heat moisture index; and (d) Hargreaves reference evaporation.

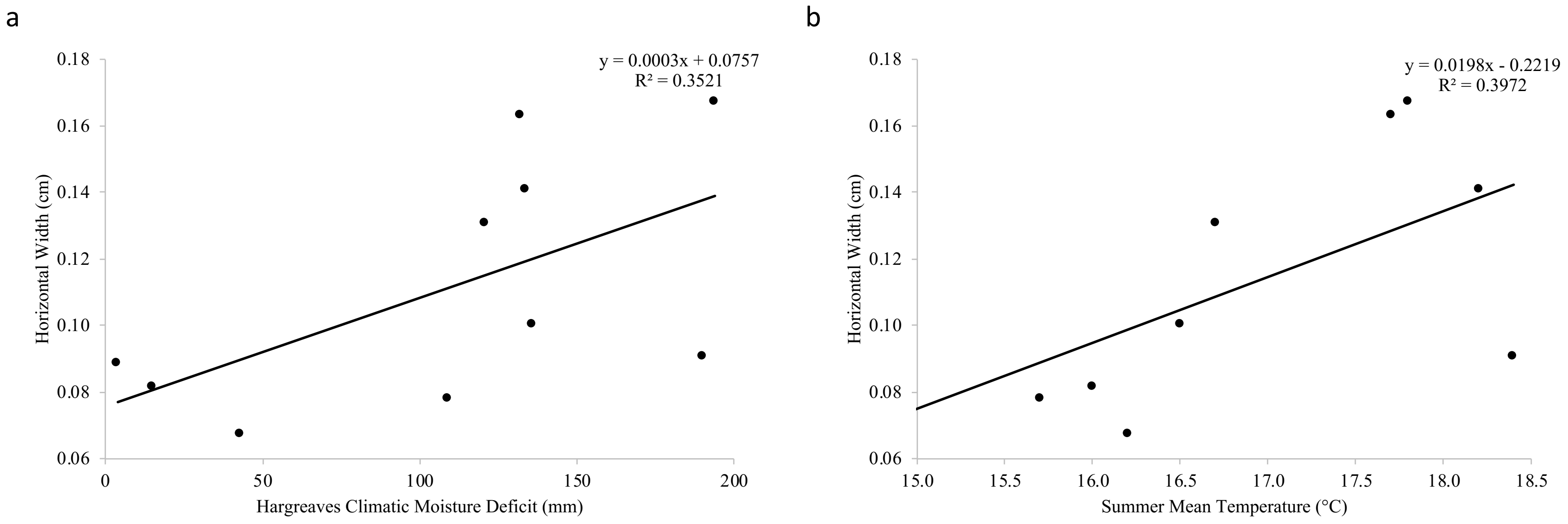

Figure A. 4. Simple linear regression analysis of horizontal width and (a) Hargreaves climatic moisture deficit and (b) summer mean temperature.

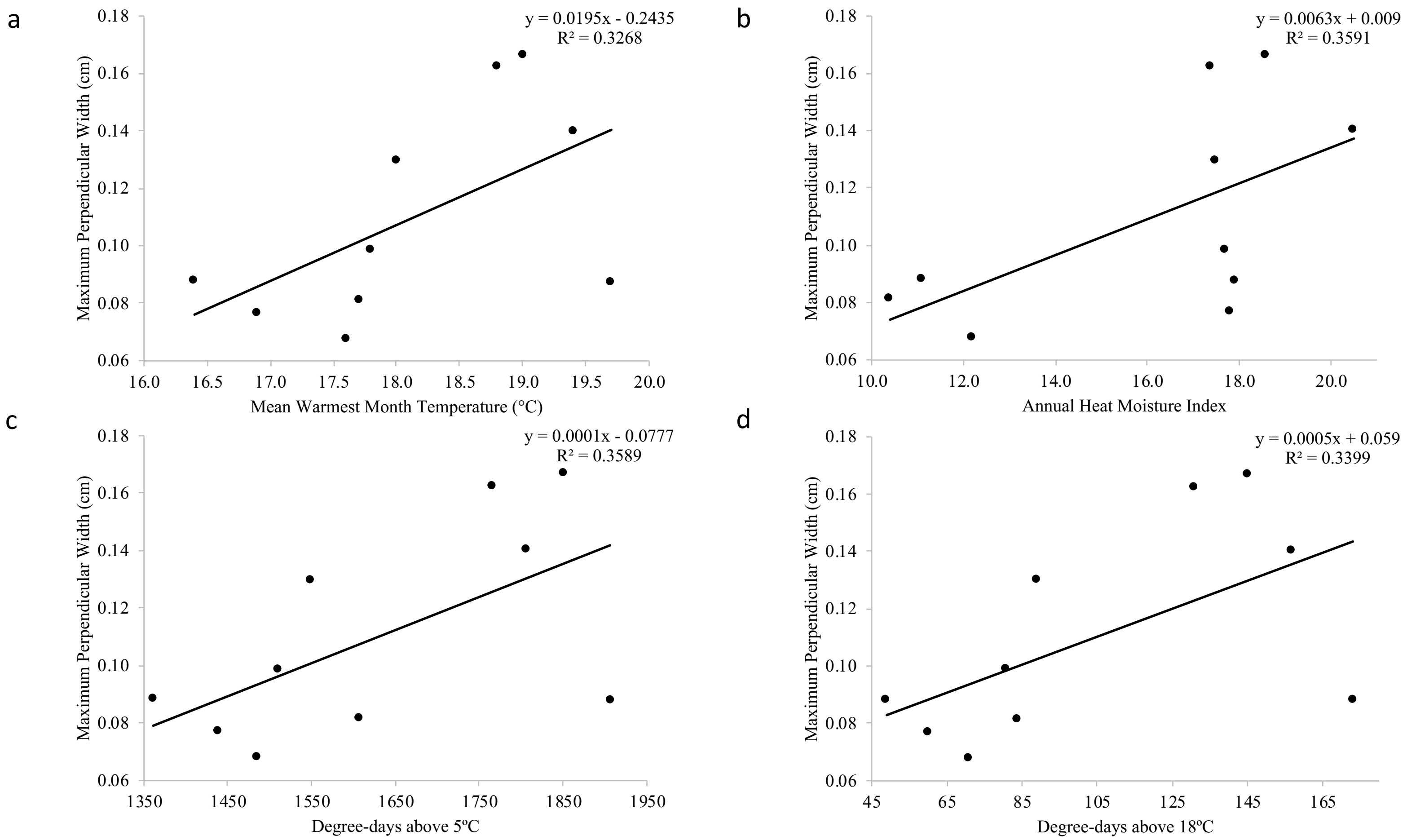

Figure A. 5. Simple linear regression analysis of maximum perpendicular width and (a) mean warmest month temperature; (b) annual heat moisture index; (c) degree-days above 5ºC; and (d) degree-days above 18ºC.

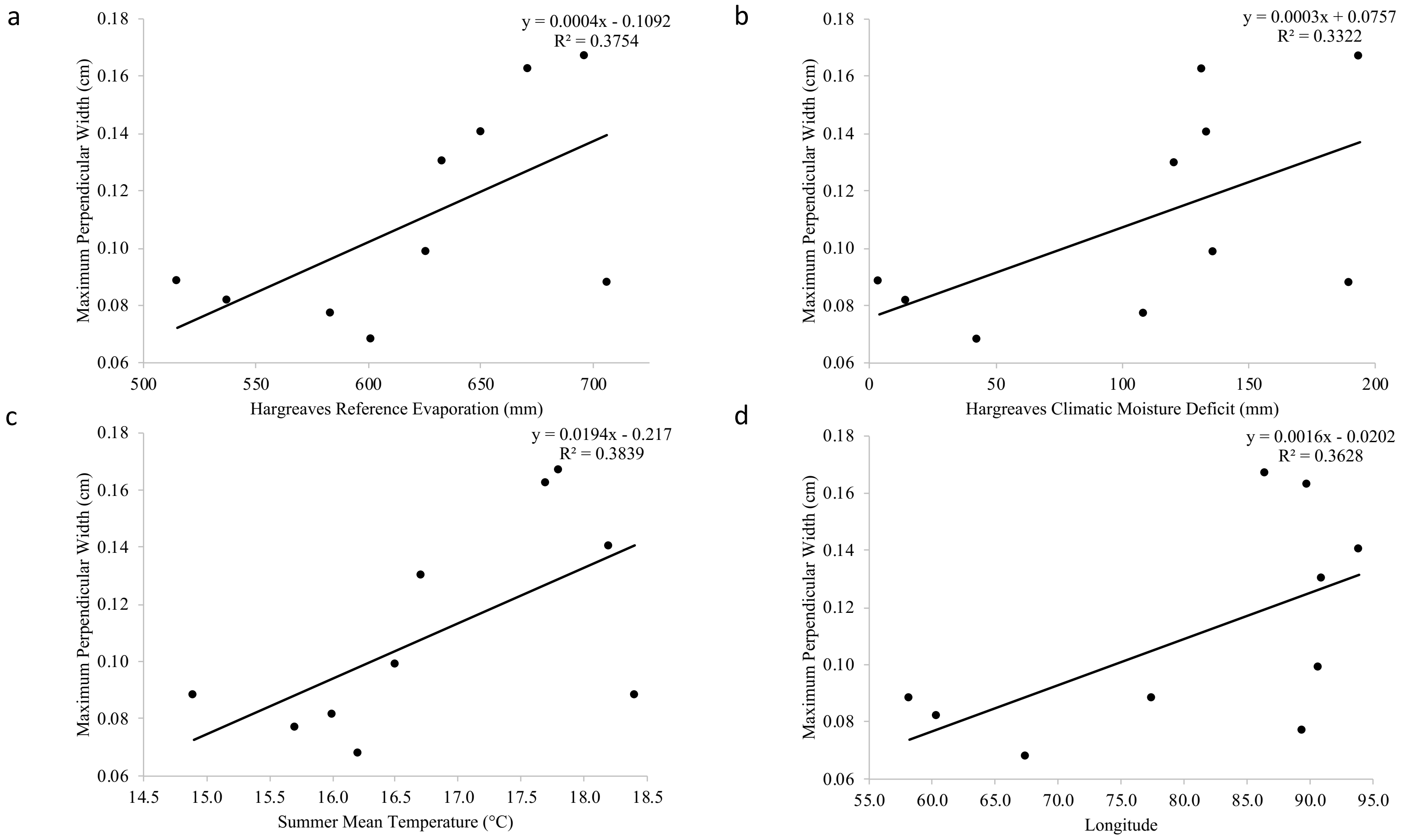

Figure A. 6. Simple linear regression analysis of maximum perpendicular width and (a) Hargreaves reference evaporation; (b) Hargreaves climatic moisture deficit; (c) summer mean temperature; and (d) longitude.

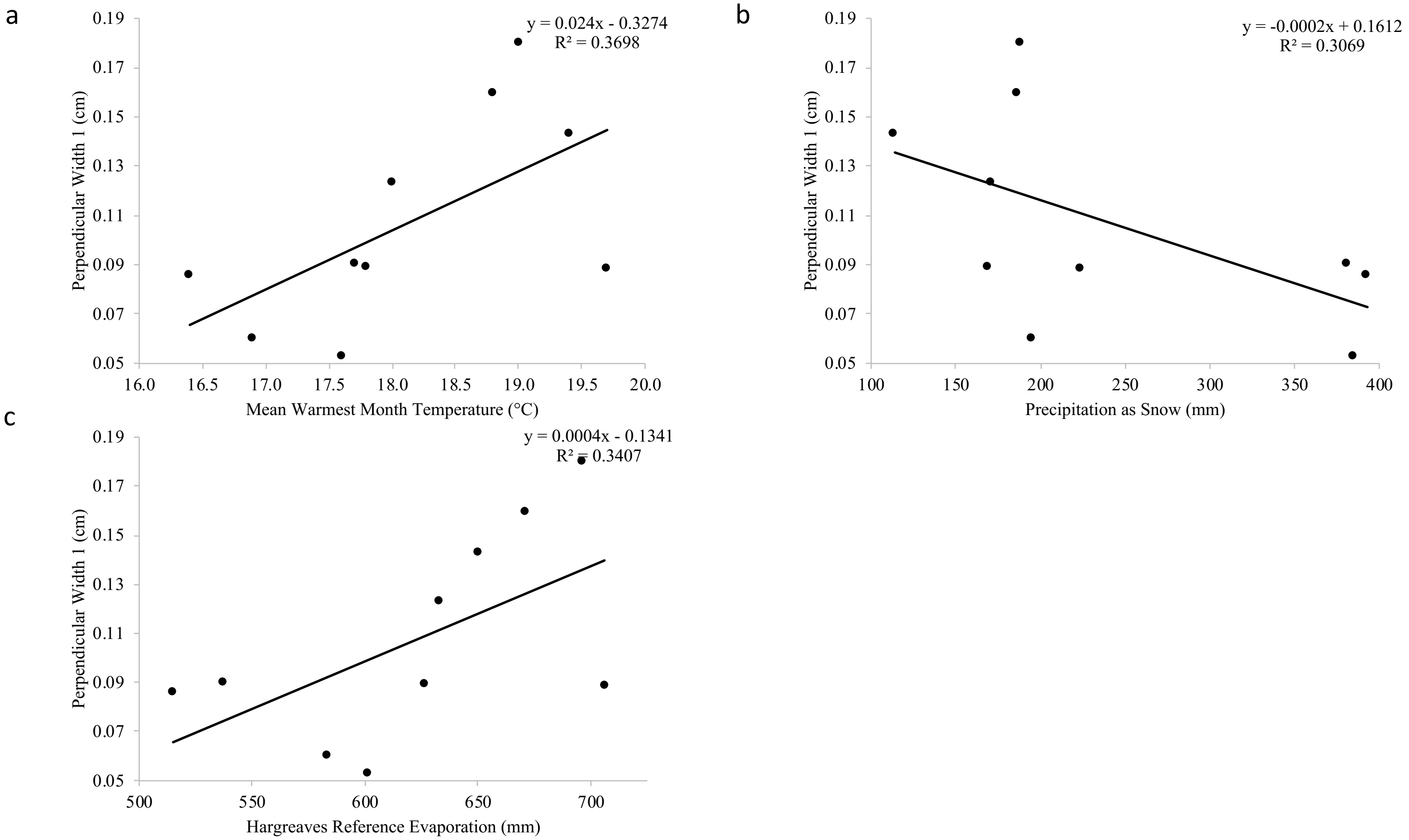

Figure A. 7. Simple linear regression analysis of perpendicular width 1 and (a) mean warmest month temperature; (b) precipitation as snow; and (c) Hargreaves reference evaporation.

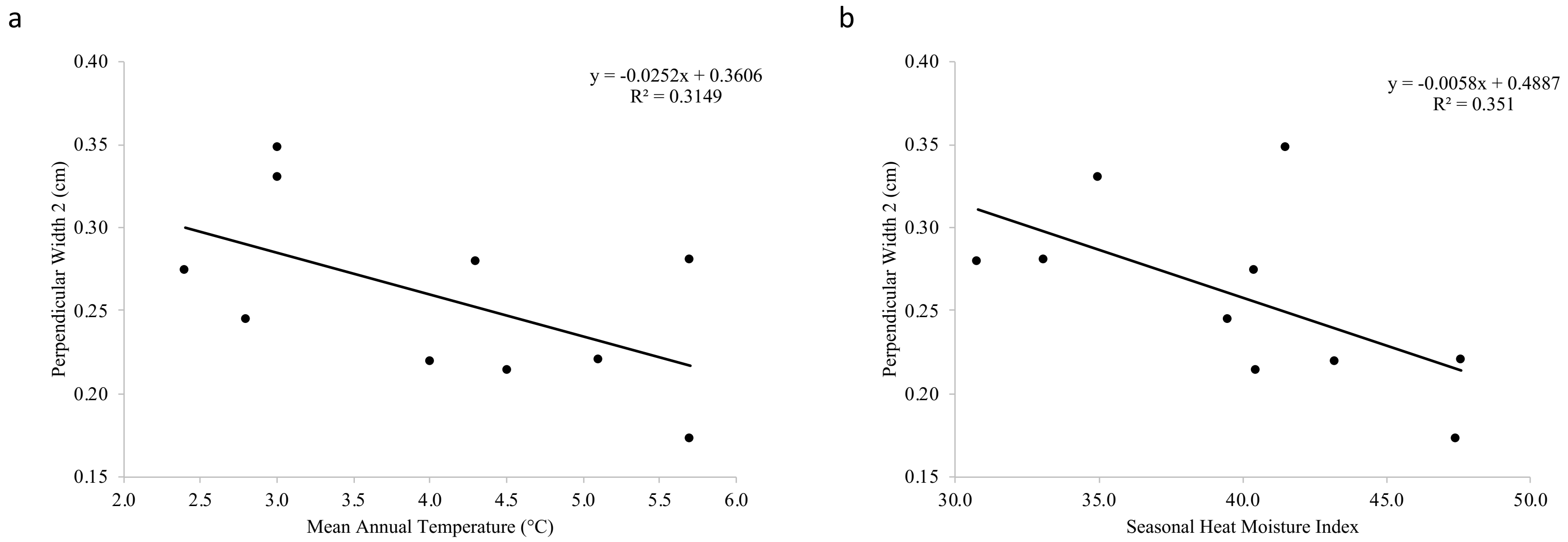

Figure A. 8. Simple linear regression analysis of perpendicular width 2 and (a) mean annual temperature and (b) seasonal heat moisture index.

APPENDIX V

SIGNIFICANT ANOVA AND POST-HOC RESULTS

Table A. 3. ANOVA results of measured provenance height (cm) p=0.049.

Height Provenance	N	Mean	Std. Deviation	Std. Error
1	4	38.5	20.23	10.11
2	4	57.1	12.04	6.02
3	4	53.3	2.63	1.32
4	4	66.0	8.57	4.29
5	4	73.9	7.09	3.54
6	4	61.4	21.88	10.94
7	4	60.3	12.31	6.16
8	4	56.6	5.11	2.55
9	4	51.9	10.62	5.31
10	4	59.1	10.08	5.04
Total	40	57.8	14.12	2.23

Table A. 4. ANOVA results of leaf horizontal width (cm) p=0.048.

Log10ReflectHorizontalWidth Provenance	N	Mean	Std. Deviation	Std. Error
1	4	0.067	0.038	0.019
2	4	0.089	0.014	0.007
3	4	0.082	0.035	0.018
4	4	0.091	0.038	0.019
5	4	0.100	0.018	0.009
6	4	0.078	0.052	0.026
7	4	0.168	0.089	0.045
8	4	0.131	0.034	0.017
9	4	0.141	0.061	0.030
10	4	0.163	0.061	0.031
Total	40	0.111	0.056	0.009

Table A. 5. ANOVA results of measured leaf average horizontal width (cm) p=0.028.

AvgHorizontalWidth				
Provenance	N	Mean	Std. Deviation	Std. Error
1	4	1.121	0.056	0.028
2	4	1.087	0.056	0.028
3	4	1.112	0.100	0.050
4	4	1.071	0.058	0.029
5	4	1.069	0.062	0.031
6	4	1.115	0.096	0.048
7	4	0.897	0.164	0.082
8	4	0.984	0.093	0.046
9	4	0.974	0.133	0.066
10	4	0.961	0.110	0.055
Total	40	1.039	0.115	0.018

Table A. 6. ANOVA results of leaf maximum perpendicular width (cm) p=0.048.

Log10ReflectMaxPerpWidth				
Provenance	N	Mean	Std. Deviation	Std. Error
1	4	0.068	0.040	0.020
2	4	0.088	0.013	0.007
3	4	0.081	0.036	0.018
4	4	0.088	0.038	0.019
5	4	0.098	0.021	0.011
6	4	0.077	0.051	0.026
7	4	0.167	0.090	0.045
8	4	0.130	0.033	0.017
9	4	0.140	0.060	0.030
10	4	0.162	0.060	0.030
Total	40	0.110	0.056	0.009

Table A. 7. ANOVA results of leaf perpendicular width 1 (cm) p=0.001.

Log10ReflectPerpWidth1 Provenance	N	Mean	Std. Deviation	Std. Error
1	4	0.053	0.046	0.023
2	4	0.086	0.024	0.012
3	4	0.090	0.016	0.008
4	4	0.089	0.025	0.013
5	4	0.089	0.025	0.013
6	4	0.060	0.040	0.020
7	4	0.180	0.074	0.037
8	4	0.123	0.041	0.021
9	4	0.143	0.045	0.023
10	4	0.160	0.033	0.017
Total	40	0.107	0.054	0.009

Table A. 8. ANOVA results of leaf perpendicular width 2 (cm) p=0.016.

PerpWidth2 Provenance	N	Mean	Std. Deviation	Std. Error
1	4	0.331	0.057	0.029
2	4	0.280	0.063	0.032
3	4	0.281	0.044	0.022
4	4	0.220	0.045	0.023
5	4	0.274	0.069	0.034
6	4	0.348	0.089	0.044
7	4	0.173	0.045	0.022
8	4	0.245	0.095	0.047
9	4	0.220	0.078	0.039
10	4	0.214	0.037	0.018
Total	40	0.259	0.077	0.012

Table A. 9. Tukey HSD post-hoc test results for height (n=40), showing significant differences of height between provenances (p<0.05**).

Height		Tukey HSD			95% Confidence Interval	
Prov (I)	Prov (J)	MeanDiff (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
1	2	-18.625	8.8187	0.534	-48.707	11.457
	3	-14.75	8.8187	0.802	-44.832	15.332
	4	-27.5	8.8187	0.096	-57.582	2.582
	5	-35.375	8.8187	0.012**	-65.457	-5.293
	6	-22.875	8.8187	0.264	-52.957	7.207
	7	-21.75	8.8187	0.325	-51.832	8.332
	8	-18.125	8.8187	0.57	-48.207	11.957
	9	-13.375	8.8187	0.875	-43.457	16.707
	10	-20.625	8.8187	0.395	-50.707	9.457
2	1	18.625	8.8187	0.534	-11.457	48.707
	3	3.875	8.8187	1	-26.207	33.957
	4	-8.875	8.8187	0.99	-38.957	21.207
	5	-16.75	8.8187	0.669	-46.832	13.332
	6	-4.25	8.8187	1	-34.332	25.832
	7	-3.125	8.8187	1	-33.207	26.957
	8	0.5	8.8187	1	-29.582	30.582
	9	5.25	8.8187	1	-24.832	35.332
	10	-2	8.8187	1	-32.082	28.082
3	1	14.75	8.8187	0.802	-15.332	44.832
	2	-3.875	8.8187	1	-33.957	26.207
	4	-12.75	8.8187	0.902	-42.832	17.332
	5	-20.625	8.8187	0.395	-50.707	9.457
	6	-8.125	8.8187	0.994	-38.207	21.957
	7	-7	8.8187	0.998	-37.082	23.082
	8	-3.375	8.8187	1	-33.457	26.707
	9	1.375	8.8187	1	-28.707	31.457
	10	-5.875	8.8187	1	-35.957	24.207
4	1	27.5	8.8187	0.096	-2.582	57.582
	2	8.875	8.8187	0.99	-21.207	38.957
	3	12.75	8.8187	0.902	-17.332	42.832
	5	-7.875	8.8187	0.996	-37.957	22.207
	6	4.625	8.8187	1	-25.457	34.707
	7	5.75	8.8187	1	-24.332	35.832
	8	9.375	8.8187	0.985	-20.707	39.457
	9	14.125	8.8187	0.837	-15.957	44.207
	10	6.875	8.8187	0.998	-23.207	36.957
5	1	35.3750*	8.8187	0.012**	5.293	65.457
	2	16.75	8.8187	0.669	-13.332	46.832
	3	20.625	8.8187	0.395	-9.457	50.707
	4	7.875	8.8187	0.996	-22.207	37.957
	6	12.5	8.8187	0.912	-17.582	42.582
	7	13.625	8.8187	0.863	-16.457	43.707
	8	17.25	8.8187	0.634	-12.832	47.332
	9	22	8.8187	0.311	-8.082	52.082
	10	14.75	8.8187	0.802	-15.332	44.832

Table A. 9. Ctd. Tukey HSD post-hoc test results for height (n=40).

Height		Tukey HSD			95% Confidence Interval	
Prov (I)	Prov (J)	MeanDiff (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
6	1	22.875	8.8187	0.264	-7.207	52.957
	2	4.25	8.8187	1	-25.832	34.332
	3	8.125	8.8187	0.994	-21.957	38.207
	4	-4.625	8.8187	1	-34.707	25.457
	5	-12.5	8.8187	0.912	-42.582	17.582
	7	1.125	8.8187	1	-28.957	31.207
	8	4.75	8.8187	1	-25.332	34.832
	9	9.5	8.8187	0.983	-20.582	39.582
	10	2.25	8.8187	1	-27.832	32.332
7	1	21.75	8.8187	0.325	-8.332	51.832
	2	3.125	8.8187	1	-26.957	33.207
	3	7	8.8187	0.998	-23.082	37.082
	4	-5.75	8.8187	1	-35.832	24.332
	5	-13.625	8.8187	0.863	-43.707	16.457
	6	-1.125	8.8187	1	-31.207	28.957
	8	3.625	8.8187	1	-26.457	33.707
	9	8.375	8.8187	0.993	-21.707	38.457
	10	1.125	8.8187	1	-28.957	31.207
8	1	18.125	8.8187	0.57	-11.957	48.207
	2	-0.5	8.8187	1	-30.582	29.582
	3	3.375	8.8187	1	-26.707	33.457
	4	-9.375	8.8187	0.985	-39.457	20.707
	5	-17.25	8.8187	0.634	-47.332	12.832
	6	-4.75	8.8187	1	-34.832	25.332
	7	-3.625	8.8187	1	-33.707	26.457
	9	4.75	8.8187	1	-25.332	34.832
	10	-2.5	8.8187	1	-32.582	27.582
9	1	13.375	8.8187	0.875	-16.707	43.457
	2	-5.25	8.8187	1	-35.332	24.832
	3	-1.375	8.8187	1	-31.457	28.707
	4	-14.125	8.8187	0.837	-44.207	15.957
	5	-22	8.8187	0.311	-52.082	8.082
	6	-9.5	8.8187	0.983	-39.582	20.582
	7	-8.375	8.8187	0.993	-38.457	21.707
	8	-4.75	8.8187	1	-34.832	25.332
	10	-7.25	8.8187	0.998	-37.332	22.832
10	1	20.625	8.8187	0.395	-9.457	50.707
	2	2	8.8187	1	-28.082	32.082
	3	5.875	8.8187	1	-24.207	35.957
	4	-6.875	8.8187	0.998	-36.957	23.207
	5	-14.75	8.8187	0.802	-44.832	15.332
	6	-2.25	8.8187	1	-32.332	27.832
	7	-1.125	8.8187	1	-31.207	28.957
	8	2.5	8.8187	1	-27.582	32.582
	9	7.25	8.8187	0.998	-22.832	37.332

Table A. 10. Tukey HSD post-hoc test results for perpendicular width 1 (reflected and log-based 10 transformed), showing significant differences of leaf morphology between provenances (p<0.05**).

Log10ReflectPerpWidth1			Post-Hoc Tukey		95% Confidence Interval	
Prov (I)	Prov (J)	Mean Diff (I-J)	Std. Error	Sig	Lower Bound	Upper Bound
1	2	-0.03333	0.02842	0.971	-0.1303	0.0636
	3	-0.0374	0.02842	0.942	-0.1344	0.0595
	4	-0.03587	0.02842	0.955	-0.1328	0.0611
	5	-0.03628	0.02842	0.951	-0.1332	0.0607
	6	-0.00749	0.02842	1	-0.1044	0.0895
	7	-.12708*	0.02842	0.004**	-0.224	-0.0301
	8	-0.07038	0.02842	0.32	-0.1673	0.0266
	9	-0.09016	0.02842	0.085	-0.1871	0.0068
	10	-.10687*	0.02842	0.022**	-0.2038	-0.0099
2	1	0.03333	0.02842	0.971	-0.0636	0.1303
	3	-0.00407	0.02842	1	-0.101	0.0929
	4	-0.00253	0.02842	1	-0.0995	0.0944
	5	-0.00295	0.02842	1	-0.0999	0.094
	6	0.02584	0.02842	0.995	-0.0711	0.1228
	7	-0.09374	0.02842	0.065	-0.1907	0.0032
	8	-0.03705	0.02842	0.945	-0.134	0.0599
	9	-0.05682	0.02842	0.606	-0.1538	0.0401
	10	-0.07354	0.02842	0.266	-0.1705	0.0234
3	1	0.0374	0.02842	0.942	-0.0595	0.1344
	2	0.00407	0.02842	1	-0.0929	0.101
	4	0.00154	0.02842	1	-0.0954	0.0985
	5	0.00112	0.02842	1	-0.0958	0.0981
	6	0.02991	0.02842	0.986	-0.067	0.1269
	7	-0.08967	0.02842	0.088	-0.1866	0.0073
	8	-0.03298	0.02842	0.973	-0.1299	0.064
	9	-0.05275	0.02842	0.696	-0.1497	0.0442
	10	-0.06947	0.02842	0.337	-0.1664	0.0275
4	1	0.03587	0.02842	0.955	-0.0611	0.1328
	2	0.00253	0.02842	1	-0.0944	0.0995
	3	-0.00154	0.02842	1	-0.0985	0.0954
	5	-0.00042	0.02842	1	-0.0974	0.0965
	6	0.02837	0.02842	0.99	-0.0686	0.1253
	7	-0.09121	0.02842	0.079	-0.1882	0.0057
	8	-0.03452	0.02842	0.964	-0.1315	0.0624
	9	-0.05429	0.02842	0.663	-0.1512	0.0427
	10	-0.07101	0.02842	0.309	-0.168	0.0259
5	1	0.03628	0.02842	0.951	-0.0607	0.1332
	2	0.00295	0.02842	1	-0.094	0.0999
	3	-0.00112	0.02842	1	-0.0981	0.0958
	4	0.00042	0.02842	1	-0.0965	0.0974
	6	0.02879	0.02842	0.989	-0.0682	0.1257
	7	-0.0908	0.02842	0.081	-0.1877	0.0062
	8	-0.0341	0.02842	0.967	-0.1311	0.0629
	9	-0.05387	0.02842	0.672	-0.1508	0.0431
	10	-0.07059	0.02842	0.316	-0.1675	0.0264

Table A. 10. ctd. Tukey HSD post-hoc test results for perpendicular width 1.

Log10ReflectPerpWidth1			Post-Hoc Tukey		95% Confidence Interval	
Prov (I)	Prov (J)	Mean Diff (I-J)	Std. Error	Sig	Lower Bound	Upper Bound
6	1	0.00749	0.02842	1	-0.0895	0.1044
	2	-0.02584	0.02842	0.995	-0.1228	0.0711
	3	-0.02991	0.02842	0.986	-0.1269	0.067
	4	-0.02837	0.02842	0.99	-0.1253	0.0686
	5	-0.02879	0.02842	0.989	-0.1257	0.0682
	7	-.11959*	0.02842	0.007**	-0.2165	-0.0226
	8	-0.06289	0.02842	0.47	-0.1598	0.0341
	9	-0.08266	0.02842	0.147	-0.1796	0.0143
	10	-.09938*	0.02842	0.041**	-0.1963	-0.0024
7	1	.12708*	0.02842	0.004**	0.0301	0.224
	2	0.09374	0.02842	0.065	-0.0032	0.1907
	3	0.08967	0.02842	0.088	-0.0073	0.1866
	4	0.09121	0.02842	0.079	-0.0057	0.1882
	5	0.0908	0.02842	0.081	-0.0062	0.1877
	6	.11959*	0.02842	0.007	0.0226	0.2165
	8	0.0567	0.02842	0.609	-0.0403	0.1536
	9	0.03692	0.02842	0.946	-0.06	0.1339
	10	0.0202	0.02842	0.999	-0.0767	0.1172
8	1	0.07038	0.02842	0.32	-0.0266	0.1673
	2	0.03705	0.02842	0.945	-0.0599	0.134
	3	0.03298	0.02842	0.973	-0.064	0.1299
	4	0.03452	0.02842	0.964	-0.0624	0.1315
	5	0.0341	0.02842	0.967	-0.0629	0.1311
	6	0.06289	0.02842	0.47	-0.0341	0.1598
	7	-0.0567	0.02842	0.609	-0.1536	0.0403
	9	-0.01977	0.02842	0.999	-0.1167	0.0772
	10	-0.03649	0.02842	0.95	-0.1334	0.0605
9	1	0.09016	0.02842	0.085	-0.0068	0.1871
	2	0.05682	0.02842	0.606	-0.0401	0.1538
	3	0.05275	0.02842	0.696	-0.0442	0.1497
	4	0.05429	0.02842	0.663	-0.0427	0.1512
	5	0.05387	0.02842	0.672	-0.0431	0.1508
	6	0.08266	0.02842	0.147	-0.0143	0.1796
	7	-0.03692	0.02842	0.946	-0.1339	0.06
	8	0.01977	0.02842	0.999	-0.0772	0.1167
	10	-0.01672	0.02842	1	-0.1137	0.0802
10	1	.10687*	0.02842	0.022**	0.0099	0.2038
	2	0.07354	0.02842	0.266	-0.0234	0.1705
	3	0.06947	0.02842	0.337	-0.0275	0.1664
	4	0.07101	0.02842	0.309	-0.0259	0.168
	5	0.07059	0.02842	0.316	-0.0264	0.1675
	6	.09938*	0.02842	0.041**	0.0024	0.1963
	7	-0.0202	0.02842	0.999	-0.1172	0.0767
	8	0.03649	0.02842	0.95	-0.0605	0.1334
	9	0.01672	0.02842	1	-0.0802	0.1137

Table A. 11. Tukey HSD post-hoc test results for perpendicular width 2, showing significant differences of leaf morphology between provenances (p<0.05**).

PerpWidth2		Post-Hoc Tukey			95% Confidence Interval	
Prov (I)	Prov (J)	Mean Diff (I-J)	Std. Error	Sig	Lower Bound	Upper Bound
1	2	0.050833	0.045975	0.98	-0.106	0.20766
	3	0.0495	0.045975	0.983	-0.10733	0.20633
	4	0.110183	0.045975	0.363	-0.04665	0.26701
	5	0.056475	0.045975	0.961	-0.10035	0.2133
	6	-0.017542	0.045975	1	-0.17437	0.13929
	7	.157400*	0.045975	0.049**	0.00057	0.31423
	8	0.085367	0.045975	0.696	-0.07146	0.2422
	9	0.110833	0.045975	0.355	-0.046	0.26766
	10	0.116467	0.045975	0.292	-0.04036	0.2733
2	1	-0.050833	0.045975	0.98	-0.20766	0.106
	3	-0.001333	0.045975	1	-0.15816	0.1555
	4	0.05935	0.045975	0.948	-0.09748	0.21618
	5	0.005642	0.045975	1	-0.15119	0.16247
	6	-0.068375	0.045975	0.887	-0.2252	0.08845
	7	0.106567	0.045975	0.407	-0.05026	0.2634
	8	0.034533	0.045975	0.999	-0.1223	0.19136
	9	0.06	0.045975	0.945	-0.09683	0.21683
	10	0.065633	0.045975	0.909	-0.0912	0.22246
3	1	-0.0495	0.045975	0.983	-0.20633	0.10733
	2	0.001333	0.045975	1	-0.1555	0.15816
	4	0.060683	0.045975	0.941	-0.09615	0.21751
	5	0.006975	0.045975	1	-0.14985	0.1638
	6	-0.067042	0.045975	0.898	-0.22387	0.08979
	7	0.1079	0.045975	0.39	-0.04893	0.26473
	8	0.035867	0.045975	0.998	-0.12096	0.1927
	9	0.061333	0.045975	0.937	-0.0955	0.21816
	10	0.066967	0.045975	0.898	-0.08986	0.2238
4	1	-0.110183	0.045975	0.363	-0.26701	0.04665
	2	-0.05935	0.045975	0.948	-0.21618	0.09748
	3	-0.060683	0.045975	0.941	-0.21751	0.09615
	5	-0.053708	0.045975	0.972	-0.21054	0.10312
	6	-0.127725	0.045975	0.189	-0.28455	0.0291
	7	0.047217	0.045975	0.988	-0.10961	0.20405
	8	-0.024817	0.045975	1	-0.18165	0.13201
	9	0.00065	0.045975	1	-0.15618	0.15748
	10	0.006283	0.045975	1	-0.15055	0.16311
5	1	-0.056475	0.045975	0.961	-0.2133	0.10035
	2	-0.005642	0.045975	1	-0.16247	0.15119
	3	-0.006975	0.045975	1	-0.1638	0.14985
	4	0.053708	0.045975	0.972	-0.10312	0.21054
	6	-0.074017	0.045975	0.833	-0.23085	0.08281
	7	0.100925	0.045975	0.481	-0.0559	0.25775
	8	0.028892	0.045975	1	-0.12794	0.18572
	9	0.054358	0.045975	0.97	-0.10247	0.21119
	10	0.059992	0.045975	0.945	-0.09684	0.21682

Table A. 11. Ctd. Tukey HSD post-hoc results for perpendicular width 2.

PerpWidth2		Post-Hoc Tukey			95% Confidence Interval	
Prov (I)	Prov (J)	Mean Diff (I-J)	Std. Error	Sig	Lower Bound	Upper Bound
6	1	0.017542	0.045975	1	-0.13929	0.17437
	2	0.068375	0.045975	0.887	-0.08845	0.2252
	3	0.067042	0.045975	0.898	-0.08979	0.22387
	4	0.127725	0.045975	0.189	-0.0291	0.28455
	5	0.074017	0.045975	0.833	-0.08281	0.23085
	7	.174942*	0.045975	0.019**	0.01811	0.33177
	8	0.102908	0.045975	0.455	-0.05392	0.25974
	9	0.128375	0.045975	0.184	-0.02845	0.2852
	10	0.134008	0.045975	0.145	-0.02282	0.29084
7	1	-.157400*	0.045975	0.049**	-0.31423	-0.00057
	2	-0.106567	0.045975	0.407	-0.2634	0.05026
	3	-0.1079	0.045975	0.39	-0.26473	0.04893
	4	-0.047217	0.045975	0.988	-0.20405	0.10961
	5	-0.100925	0.045975	0.481	-0.25775	0.0559
	6	-.174942*	0.045975	0.019**	-0.33177	-0.01811
	8	-0.072033	0.045975	0.853	-0.22886	0.0848
	9	-0.046567	0.045975	0.989	-0.2034	0.11026
	10	-0.040933	0.045975	0.996	-0.19776	0.1159
8	1	-0.085367	0.045975	0.696	-0.2422	0.07146
	2	-0.034533	0.045975	0.999	-0.19136	0.1223
	3	-0.035867	0.045975	0.998	-0.1927	0.12096
	4	0.024817	0.045975	1	-0.13201	0.18165
	5	-0.028892	0.045975	1	-0.18572	0.12794
	6	-0.102908	0.045975	0.455	-0.25974	0.05392
	7	0.072033	0.045975	0.853	-0.0848	0.22886
	9	0.025467	0.045975	1	-0.13136	0.1823
	10	0.0311	0.045975	0.999	-0.12573	0.18793
9	1	-0.110833	0.045975	0.355	-0.26766	0.046
	2	-0.06	0.045975	0.945	-0.21683	0.09683
	3	-0.061333	0.045975	0.937	-0.21816	0.0955
	4	-0.00065	0.045975	1	-0.15748	0.15618
	5	-0.054358	0.045975	0.97	-0.21119	0.10247
	6	-0.128375	0.045975	0.184	-0.2852	0.02845
	7	0.046567	0.045975	0.989	-0.11026	0.2034
	8	-0.025467	0.045975	1	-0.1823	0.13136
	10	0.005633	0.045975	1	-0.1512	0.16246
10	1	-0.116467	0.045975	0.292	-0.2733	0.04036
	2	-0.065633	0.045975	0.909	-0.22246	0.0912
	3	-0.066967	0.045975	0.898	-0.2238	0.08986
	4	-0.006283	0.045975	1	-0.16311	0.15055
	5	-0.059992	0.045975	0.945	-0.21682	0.09684
	6	-0.134008	0.045975	0.145	-0.29084	0.02282
	7	0.040933	0.045975	0.996	-0.1159	0.19776
	8	-0.0311	0.045975	0.999	-0.18793	0.12573
	9	-0.005633	0.045975	1	-0.16246	0.1512

Table A. 12. Two-way ANOVA results (n=200 seedlings) of height and diameter, including mean, standard deviation, minimum, maximum, and significance.

Measured Variable	Mean	Std. Deviation	Min	Max	Sig.
Height*Prov	58.912	13.4764	14.5	89	0.182
Height*Block	58.912	13.4764	14.5	89	0.852
Diameter*Prov	7.1151	1.20591	3.89	9.94	0.215
Diameter*Block	7.1151	1.20591	3.89	9.94	0.002**

Table A. 13. Two-way ANOVA results of height by provenance (n=200 seedlings), including mean, standard deviation, minimum and maximum values.

Provenance	Mean	N	Std. Deviation	Minimum	Maximum
1	55.4	20	18.4837	14.5	85.5
2	53.05	20	13.2237	23.5	74.5
3	61.275	20	9.4569	50	76.5
4	58.2	20	12.4851	37.5	84.5
5	67.6	20	13.1565	36.5	83.5
6	60.52	20	14.268	35.5	89
7	56.1	20	13.3826	31.5	80
8	58.2	20	11.501	32.5	77.5
9	58.225	20	12.1693	38.5	76
10	60.55	20	12.4212	36.5	84.5
Total	58.912	200	13.4764	14.5	89

Table A. 14. Two-way ANOVA results of height by block (n=200 seedlings), including mean, sample size, standard deviation, minimum and maximum values.

Block	Mean	N	Std. Deviation	Minimum	Maximum
1	58.53	50	14.6505	23.5	83.5
2	58.998	50	13.4315	37.5	89
3	60.29	50	11.6379	30	85.5
4	57.83	50	14.2759	14.5	83.5
Total	58.912	200	13.4764	14.5	89

Table A. 15. Two-way ANOVA results of diameter by provenance (n=200 seedlings), including mean, sample size, standard deviation, minimum and maximum values.

Provenance	Mean	N	Std. Deviation	Minimum	Maximum
1	6.9905	20	1.37098	3.89	8.7
2	6.966	20	1.36827	4.77	9.53
3	6.927	20	1.19231	4.29	9.09
4	6.8105	20	1.07115	4.57	8.69
5	7.451	20	1.41056	4.75	9.94
6	7.491	20	1.14125	5.23	9.7
7	7.35	20	1.0499	4.73	9.15
8	6.6935	20	1.00027	5	8.92
9	7.146	20	1.08153	4.63	9.09
10	7.325	20	1.2692	3.98	9.48
Total	7.1151	200	1.20591	3.89	9.94

Table A. 16. Two-way ANOVA results of diameter by block (n=200 seedlings), including mean, sample size, standard deviation, minimum and maximum values.

Block	Mean	N	Std. Deviation	Minimum	Maximum
1	6.7328	50	1.14923	4.49	9.15
2	7.2844	50	1.31613	4.29	9.67
3	7.548	50	1.20805	3.89	9.94
4	6.895	50	0.98718	3.98	8.92
Total	7.1151	200	1.20591	3.89	9.94

APPENDIX VI

SCANNED LEAF IMAGES BY PROVENANCE

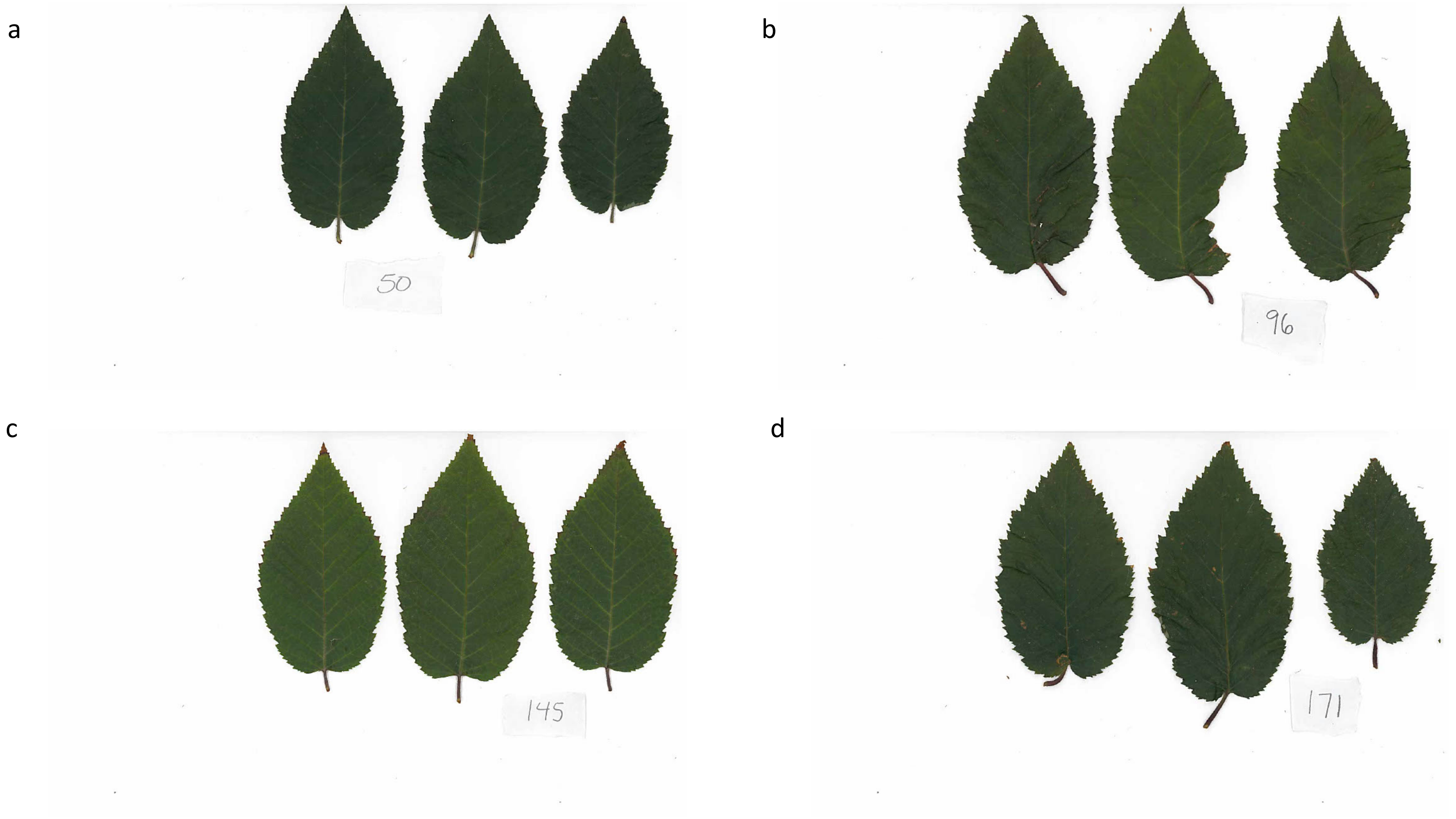

Figure A. 9. Scanned leaf images from Provenance 1 (Black Brook, NB).

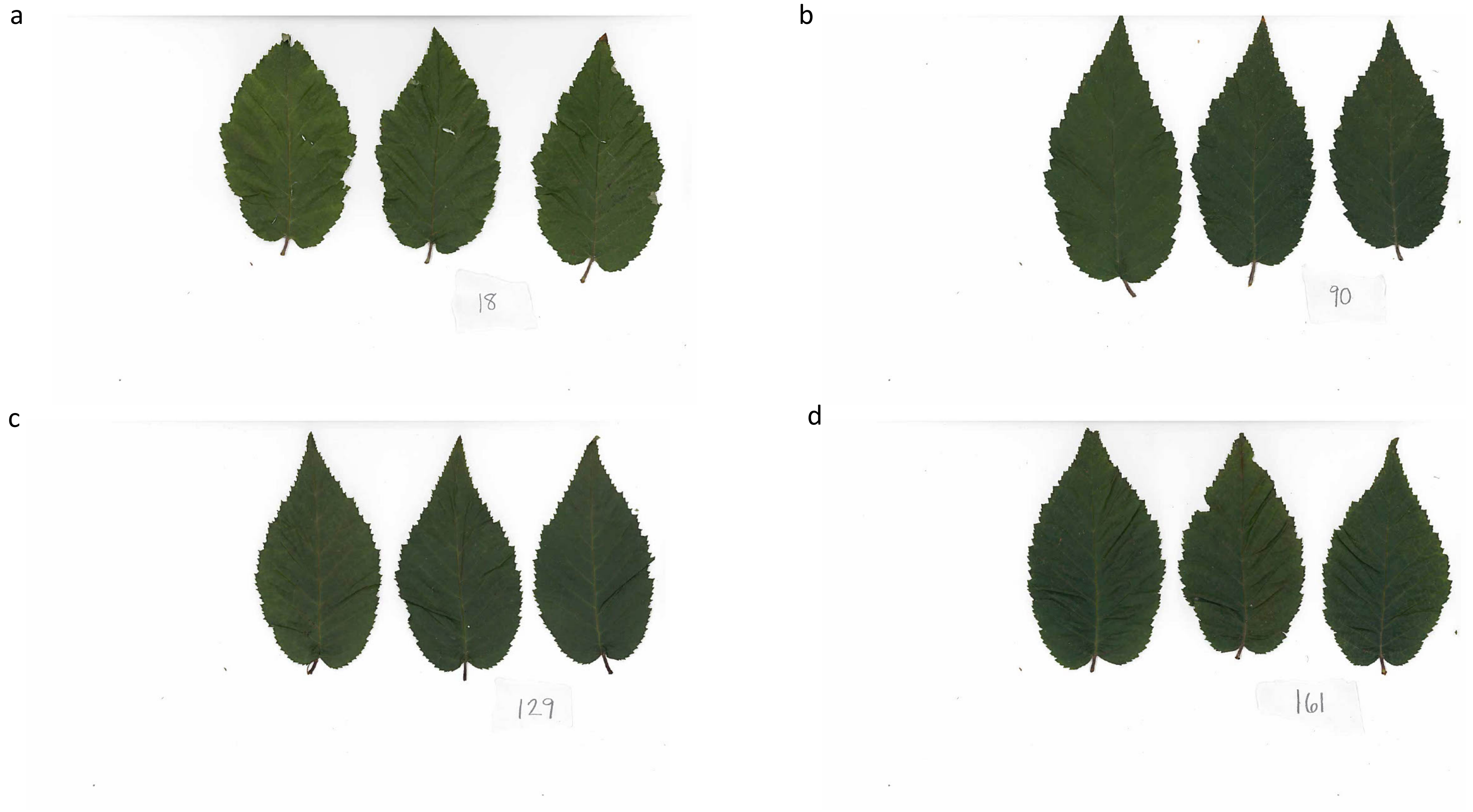

Figure A. 10. Scanned leaf images from Provenance 2 (Gallants, NL).

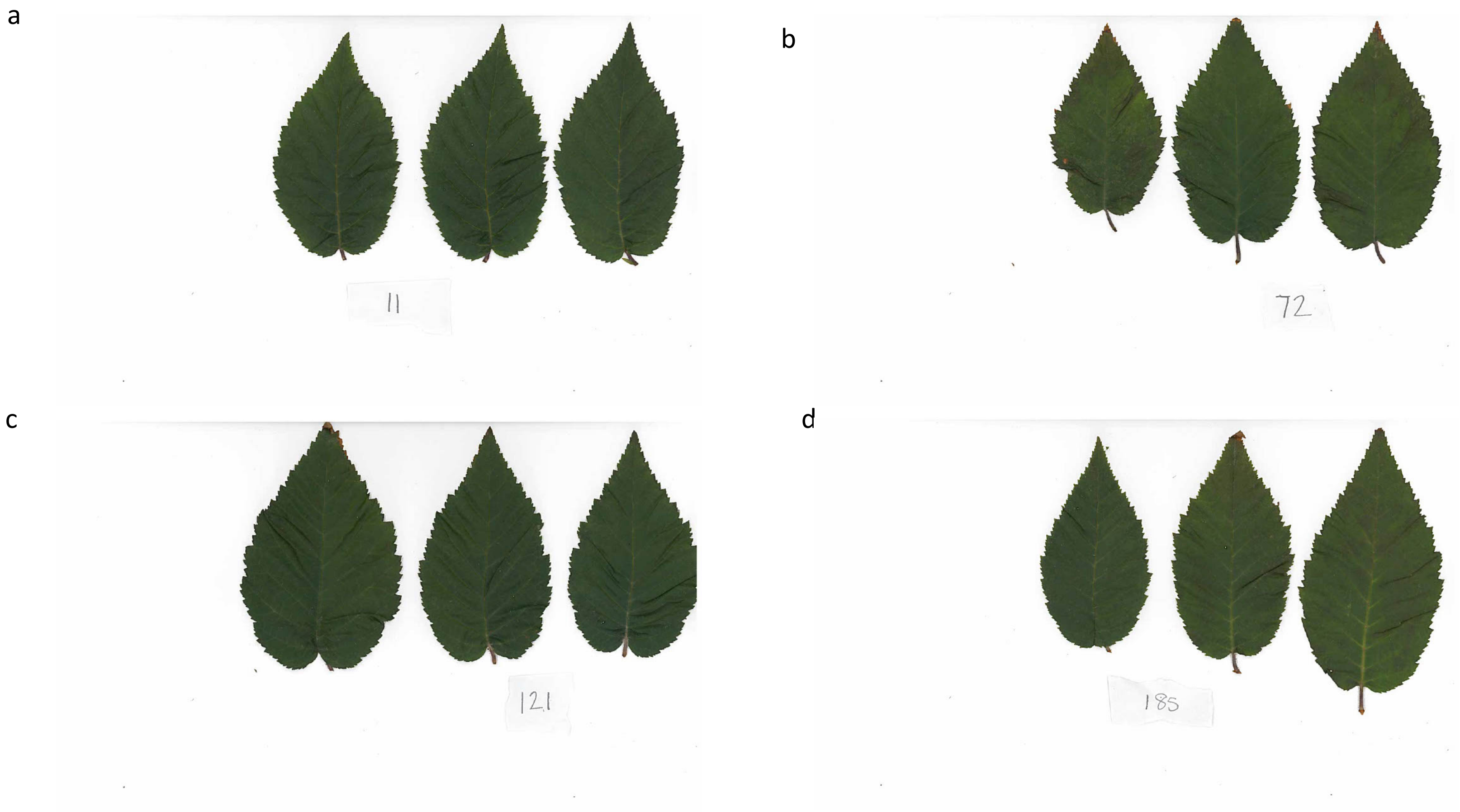

Figure A. 11. Scanned leaf images from Provenance 3 (Big Pond, NL).

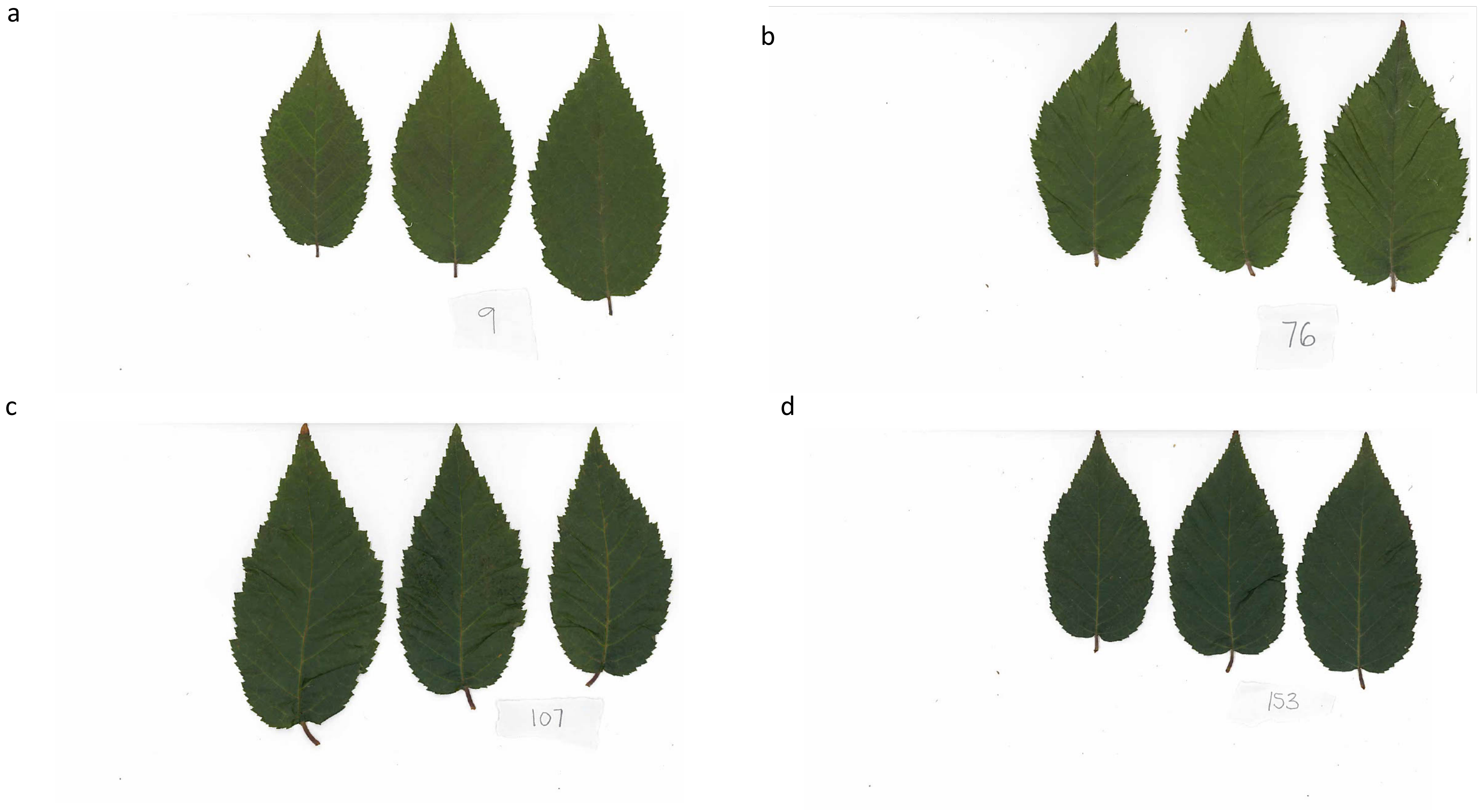

Figure A. 12. Scanned leaf images from Provenance 4 (Petawawa, ON).

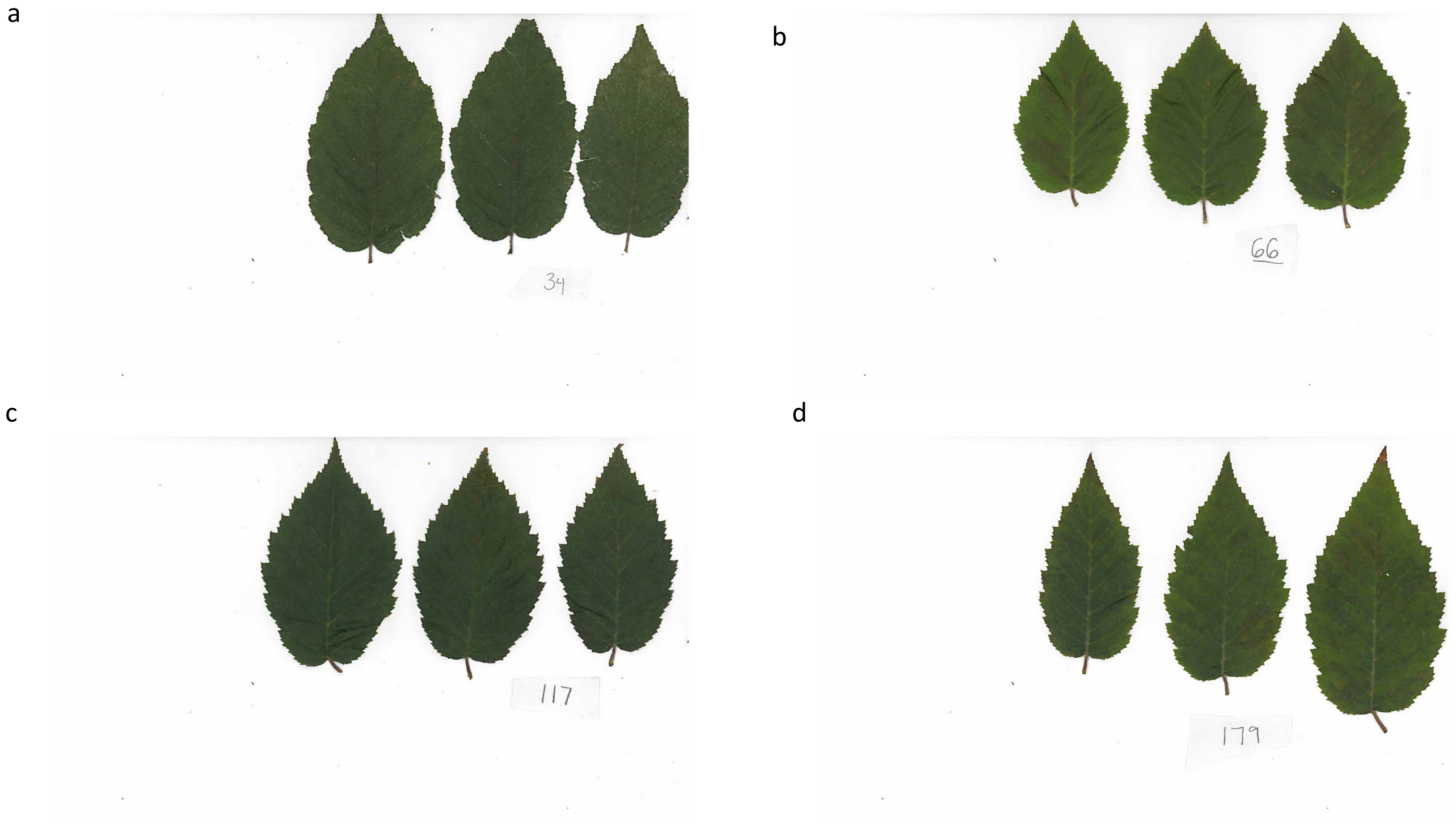

Figure A. 13. Scanned leaf images from Provenance 5 (Greenwood, ON).

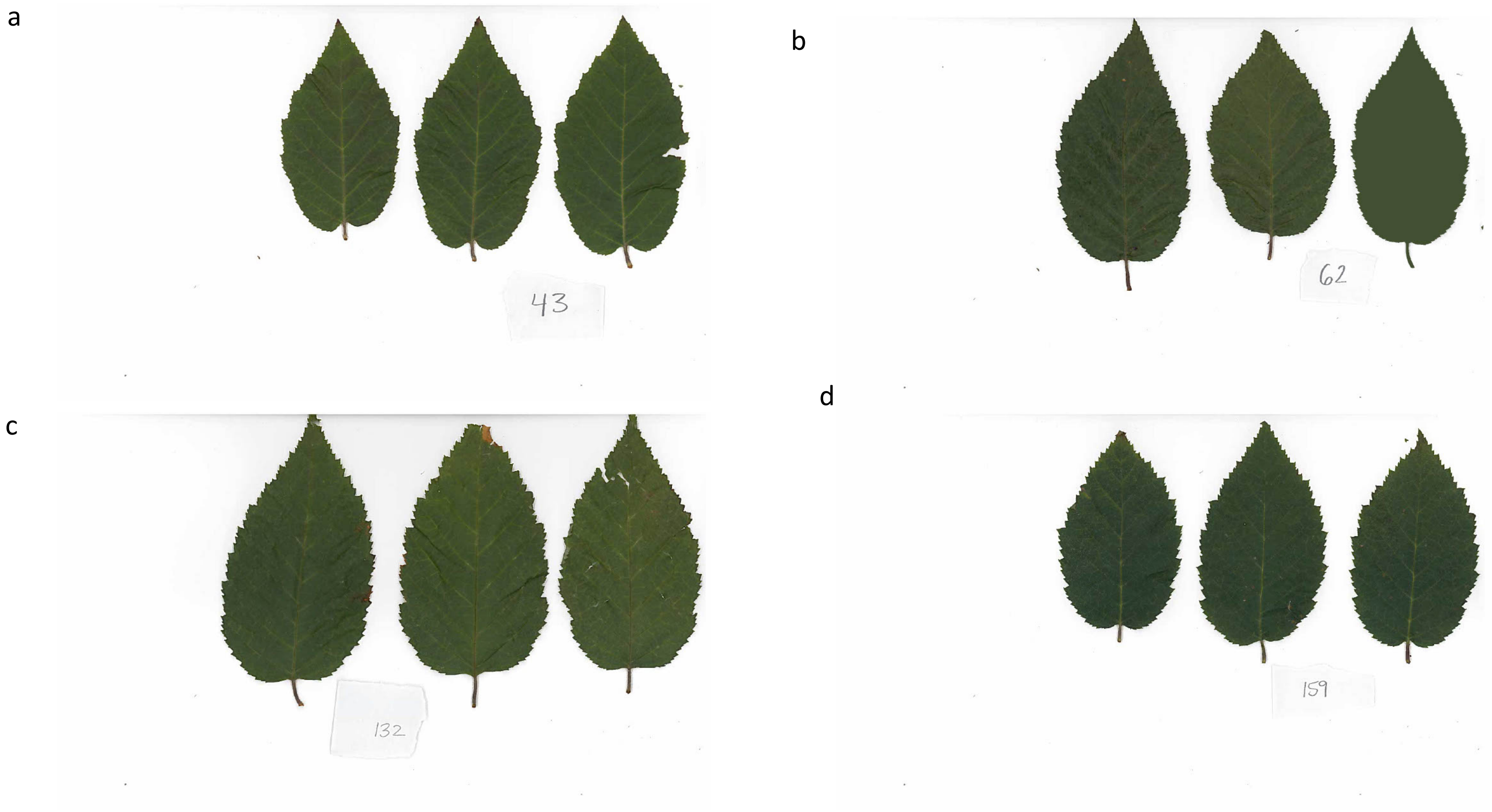

Figure A. 14. Scanned leaf images from Provenance 6 (Squaretop Mt., ON).

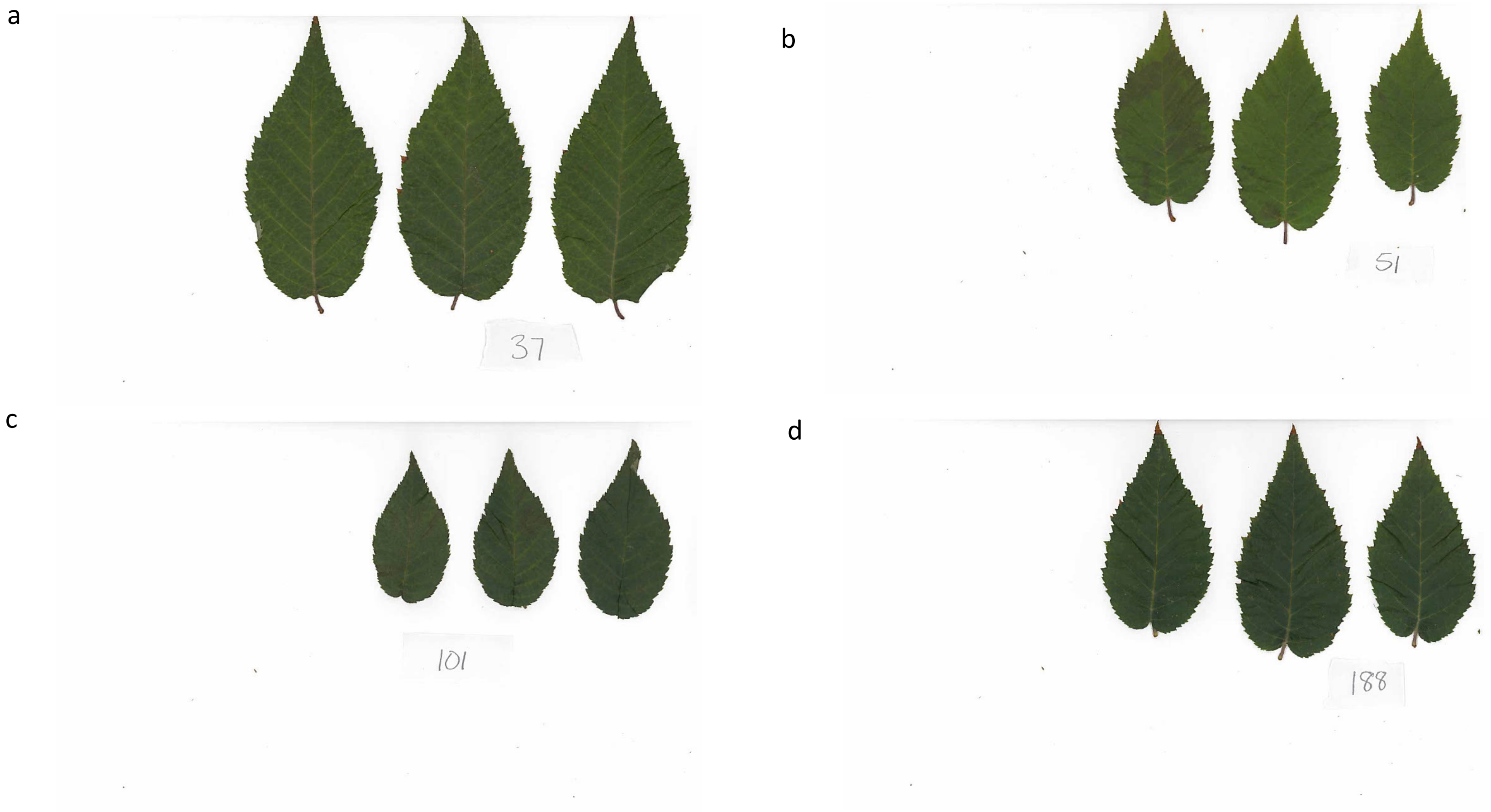

Figure A. 15. Scanned leaf images from Provenance 7 (Hiawatha, MI).

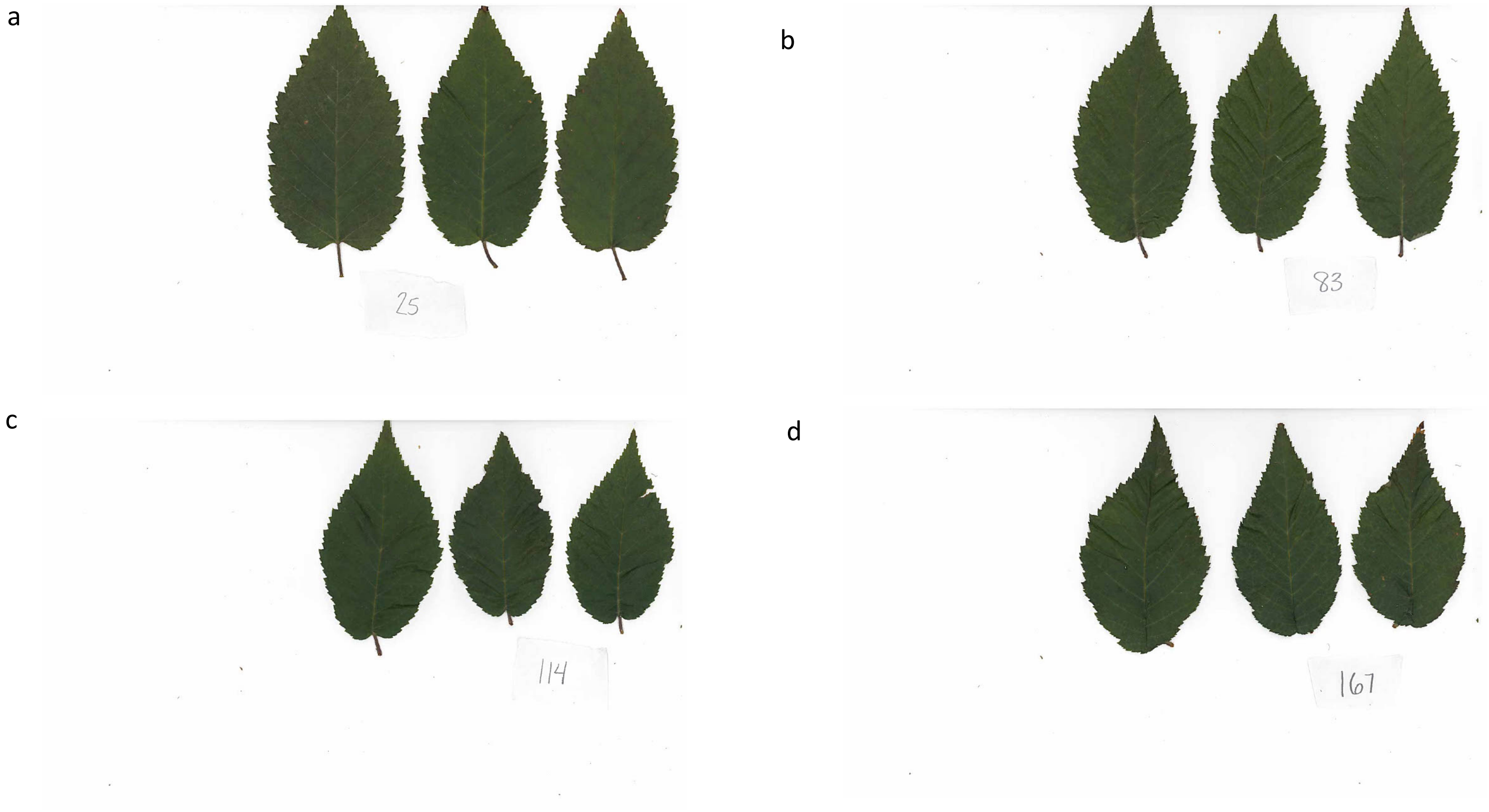

Figure A. 16. Scanned leaf images from Provenance 8 (Superior, MN).

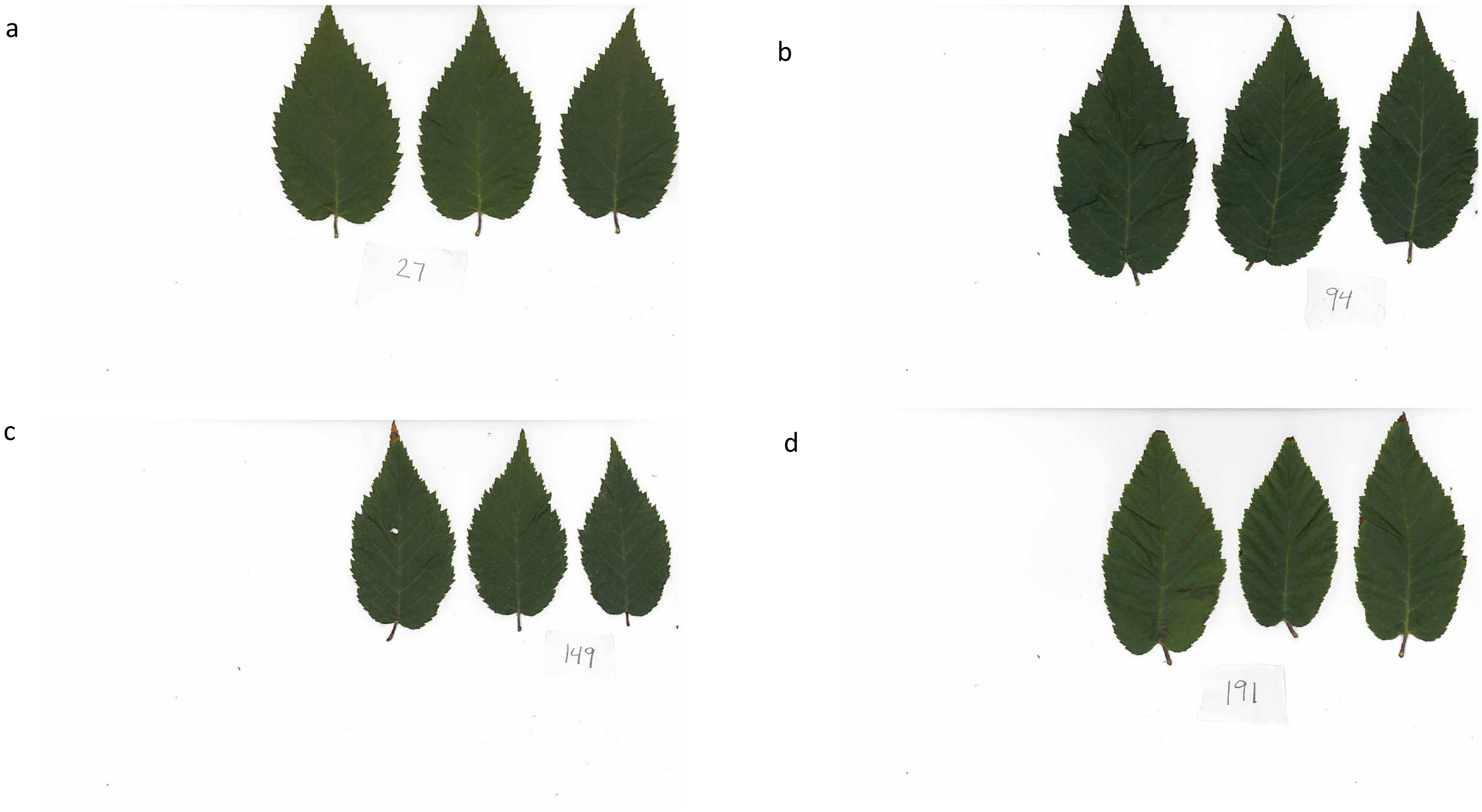

Figure A. 17. Scanned leaf images from Provenance 9 (Chippewa, MN).

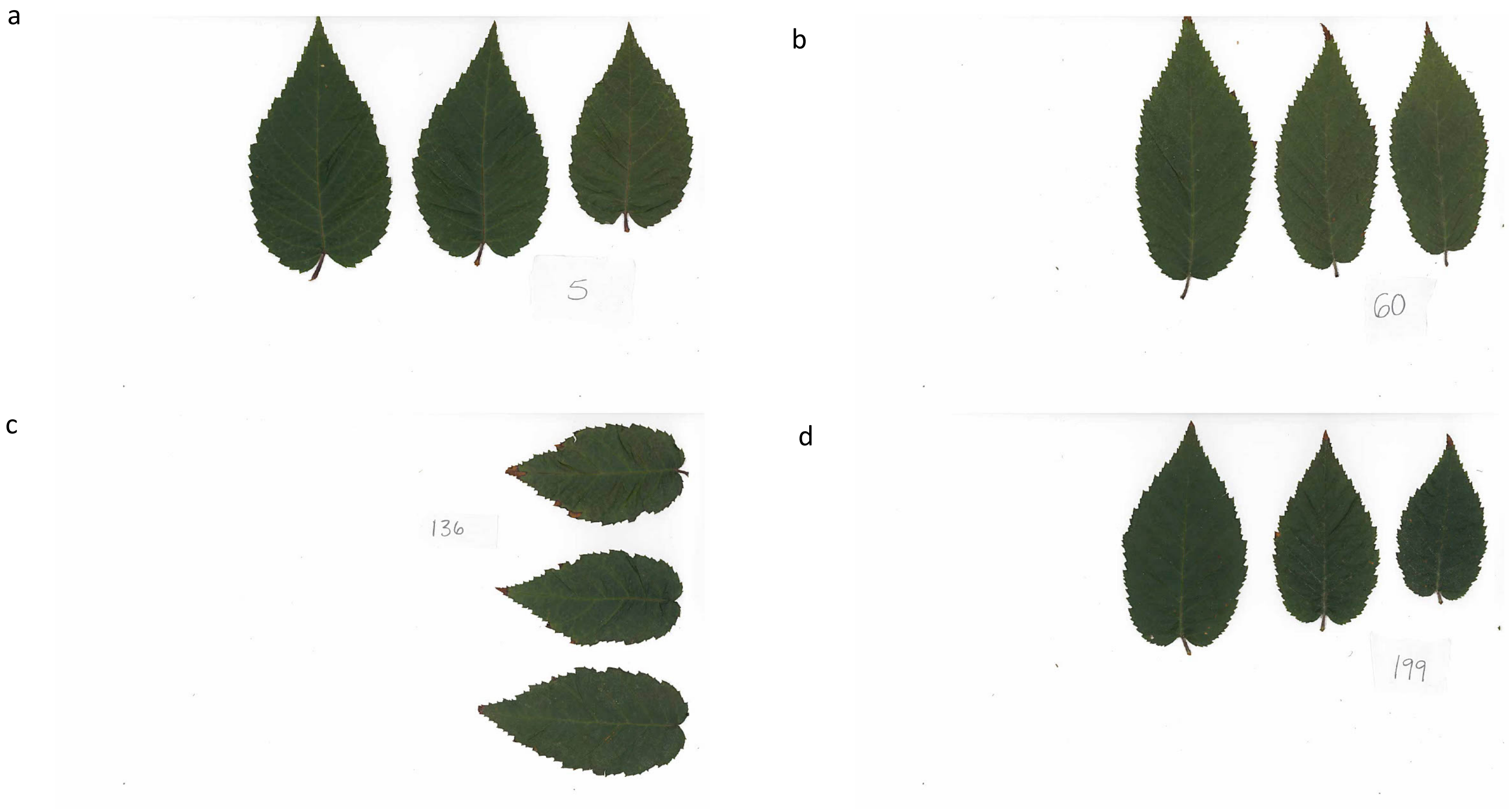

Figure A. 18. Scanned leaf images from Provenance 10 (Ottawa, MI).

APPENDIX VII

WINFOLIA PARAMETERS

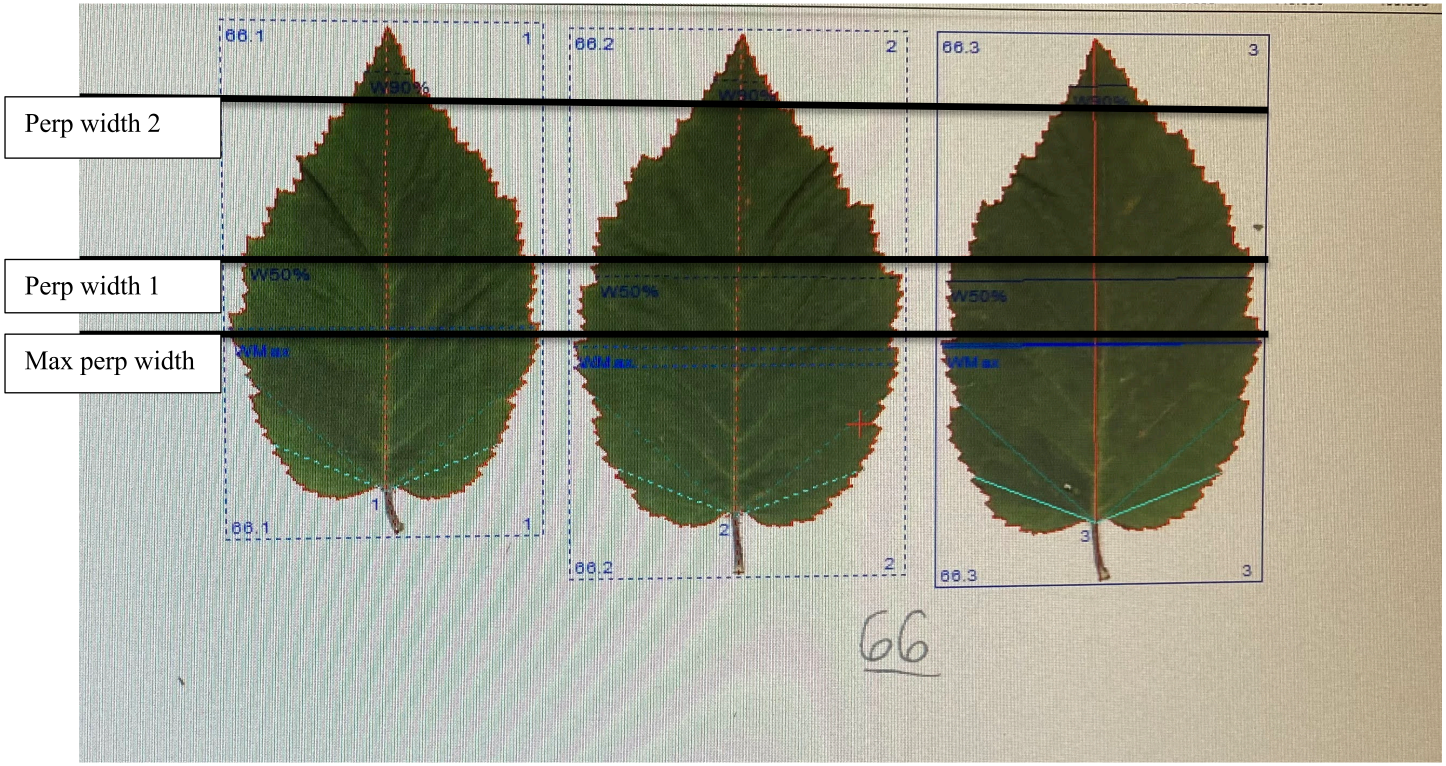

Figure A. 19. Image of important width characters analyzed with WinFolia software. Maximum perpendicular width was measured as the maximum width perpendicular to blade length. Perpendicular width 1 was measured as the width at 50% of the blade length. Perpendicular width 2 was measured as the width at 90% of the blade length.