

PHYSIOLOGY AND GENETIC STUDIES OF THE HARD MAPLE GROUP

by

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ABSTRACT OF THE DISSERTATION

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The hard maple group is comprised of several taxonomically ambiguous, phenotypically plastic, and genetically variable taxa, native (mainly) to the United States. Northern Sugar Maple, *Acer saccharum* Marsh, the most recognizable group member, is culturally, ecologically, and economically important in that region. Southern hard maples have adapted to climates defined by increasing thermal loading and extended periods of drought. Understanding southern hard maple behavioral response is needed in planning future efforts on developing strategies to sustain the role of northeastern sugar maple. I provide brief overview of hard maple taxonomy, silviculture, and relevant studies. I then describe a greenhouse study in which I compare the quantified mechanical drought response of hard maple populations from range extremes. I found differential drought response to be higher among northern trees than southern trees. Southern hard maples modified stomatal aperture earlier than did northern trees, and water use efficiency was highly correlated with provenance of study groups. Next, I describe a study to better understand environmental boundaries of southern hard maples, where I quantify processes associated with plant biochemistry to determine differential drought response among southern hard maples exposed to extreme neglect during a summer heat wave. Southern trees did not exhibit significant differentiation in biochemical study parameters among taxa, but population means were influenced by time, suggesting a tight genetic link to

seasonality in the southern cohort. I also undertook a genotyping analysis of hard maple germplasm tissue that I collected from field campaigns and the drought studies to determine the correlation between drought response and genotype among taxa. Relative genetic variance was highest for individuals within provenances, followed by variance among taxa, with provenances within taxa showing the least variation. All populations were shown to be significantly different genetically. Groupings resulting from population pairwise comparisons from the drought study (Tukey's HSD) were compared to those for the genotype AMOVA (Φ_{PT}). Groupings were not consistent between the drought study and the genotype analysis. These results suggest that in terms of breeding and selection programs for improving sugar maple industry stock, it is important to consider provenance location as an important indicator of plant performance. Genetic affinity is only part of the story.

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perseverance, and love. You deserve anything you ask the universe to provide, all you need to do is ask. I truly believe that I would not have been able to get through all life has put us through without you. You have made me the best mom I can be, which for me means that I am the best me I can be. The state of change that COVID-19 has put at your feet is not what you deserve. But I know that, like the sword of Gryffindor, that you only take on that which makes you stronger. You are strong, funny, caring, and kind. You see the goodness in everything. Things seem unfair at the moment, but this is temporary, and you are a master (though you do not know it yet) at making friends wherever you go. I cannot thank you enough here. I could write another 100 pages about you and you know it!

Dedication

This document and all of the work I did to see it through, is dedicated to my parents. Both of whom would have been so proud and not at all surprised. Love you both.

Table of Contents

Abstract	ii
Acknowledgements and Dedication.....	iv
Table of Contents.....	viii
List of Figures.....	ix
List of Tables.....	xi
Chapter 1: An overview of the sugar maple species complex with focus on environmental limitations of the inclusive taxa.....	1
Chapter 2: the influence of hard maple provenance on stomatal conductance, carbon assimilation transpiration rate, and water use efficiency under different water regimes...	32
Chapter 3 biochemical response of photosynthetic processes in southern hard maple taxa under water treatments.....	70
Chapter 4: Exploring genetic affinities of hard maple taxa.....	93
Chapter 5: Implications of Hard Maple Research and Future Foci.....	115
Literature Cited.....	129

List of Figures

Figure 1.1a-b. Native range and dominant soil orders for <i>Acer barbatum</i> Michx.....	24
Figure 1.2a-b. Native range map and dominant soil orders for <i>Acer leucoderme</i> Small...	25
Figure 1.3a-b. Native range map and dominant soil orders for <i>Acer grandidentatum</i> Nuttall	26
Figure 1.4a-b. Native range and dominant soil order maps for <i>Acer nigrum</i> Michx, f.....	27
Figure 1.5a-b. Native range map and dominant soil orders for <i>Acer saccharum</i>	28
Figure 1.6. Native range map and dominant soil orders for of <i>Acer saccharum</i> subsp <i>skutchii</i>	29
Figure 1.7. USDA Hardiness Zone Map published in 2012.....	30
Figure 1.8. USDA Hardiness Zone Map published in 1990.....	31
Figure 2.1. Germplasm collection range maps.....	56
Figure 2.2 a-d. Interaction plot graph for fixed effects of Population and Treatment for population means.....	61
Figure 2.3. Photo of hard maple germplasm collection held on landscape fabric on Cook Campus at Rutgers University, New Brunswick, New Jersey.....	64
Figure 2.4. Overwintering potted germplasm collection-first year on campus at Rutgers greenhouse.....	65
Figure 2.5. Photograph showing potted study trees with ID labels.....	66
Figure 2.6. Northern plot map for the greenhouse drought study.....	67
Figure 2.7. Southern plot map for the greenhouse drought study.....	68

Figure 2.8. Photo of gas-exchange measurement procedure.....	69
Figure 3.1. Using the LI6400XT portable photosynthesis system.....	85
Figure 3.2. Plot map of study trees for controlled greenhouse drought study.....	86
Figure 4.1. Pie diagram, showing relative variances within hard maple study groups....	108
Figure 4.2. Principal Coordinate Analysis results for AMOVA.....	112
Figure 4.3. Suggested tree based on Tukey HSD pairwise groupings from Chapter 2....	114
Figure 5.1. Modeled forest type changes under climate change models of high and low carbon emissions scenarios.....	127

List of Tables

Table 1.1 Regional categories of hard maple species.....	23
Table 2.1. List of hard maple study populations, their provenance, and suppliers.....	54
Table 2.2. List of study population provenance location, elevation, water source, soils, and temperature.....	55
Table 2.3. Full model analysis of variance (ANOVA) results for population means for study variables.....	57
Table 2.4. Tukey's HSD post hoc test significance of treatment effect on population means.....	58
Table 2.5. Tukey's HSD post hoc test significance of population effect on variable means	59
Table 2.6. ANOVA results for fixed effects of Treatment, population, and their interaction on population means.....	60
Table 2.7. Average percent (%) change in northern population mean response.....	62
Table 2.8. Average percent (%) change in southern population mean response.....	63
Table 3.1. Taxonomic designations, provenance, and supplier.....	84
Table 3.2. Provenance averages and population means for maximum rate of carboxylation	87
Table 3.3. Provenance averages and population means are provided for electron transport rate.....	88

Table 3.4. Provenance averages and population means are provided for triose-phosphate utilization.....	89
Table 3.5. Summary for fixed effects of Provenance, Treatment, and their interaction....	90
Table 3.6 Summary for fixed effects of Provenance, drought length, and their interaction	91
Table 3.7. Tukey HSD test results for treatment means by time without water.....	92
Table 4.1. Table listing of hard maple species, their sources, latitude and longitude of sources, and number of individuals submitted for genotyping analysis.....	106
Table 4.2. Molecular markers (microsatellites) used for genotype analysis.....	107
Table 4.3. AMOVA results of genotype analysis.....	109
Table 4.4. Statistical constructs statistics estimated by AMOVA.....	110
Table 4.5. AMOVA Pairwise population Φ_{ij} pairwise comparisons.....	111
Table 4.6 . Principal Coordinates Analysis variance by axes.....	113

CHAPTER 1

AN OVERVIEW OF THE SUGAR MAPLE SPECIES COMPLEX WITH FOCUS ON ENVIRONMENTAL LIMITATIONS OF THE INCLUSIVE TAXA

ABSTRACT

Sugar Maple, *Acer saccharum* Marsh., is the group's best-known member and shows sensitivity to moisture and temperature changes in the local environment. Close ties to the economic base of the northeastern United States have contributed to decades of research based on sugar sap concentration and quality of the northeastern sugar maple. Investigations of behavioral trait response to variable environmental conditions favor *A. saccharum* for the same reason, but there is a dearth of literature on the remaining hard maple group members. Addressing and organizing gaps in hard maple research can provide opportunities for improving species fitness, while potentially abating the negative impacts projected by climate change models for the sensitive sugar maple. I present the need for revitalizing research of this vulnerable forest species that includes other hard maple species, due to the recent identification of sugar maple as a species susceptible to negative impacts of rapidly changing climates. Understanding environmental limitations of sugar maple allies can broaden our understanding of hard maple biology and reveal behavioral traits useful in the maple sugar and timber industries.

INTRODUCTION

The sugar maple species complex, also known as the hard maple group, is comprised of several closely related species: *Acer barbatum* Michx f. (Southern Sugar Maple), *Acer grandidentatum* Nutt (Bigtooth Maple), *Acer leucoderme* Small (Chalk Maple), *Acer nigrum* Michx (Black Maple), *Acer saccharum* Marsh, (Sugar Maple), *Acer skutchii* Murray (Mexican Maple). Hard maple species occupy a broad geographical range in the United States and southern Canada over varying soil conditions, climates, and elevations (Godman et al. 1990). Sugar maples present a high level of genetic variation that is well documented and taxonomic discrepancies are well known (Desmarais 1952, Kriebel 1957). *Acer saccharum* has close ties to the economies of New England and is used as a symbol of cultural identity among those states and in others (Shea et al. 2001). Sugar maple *sensu strictu* is sensitive to drought, heat, and urbanized conditions (Kriebel 1957, Dirr 1990, Godman et al. 1990, Graves 1994). The United States Forest Service Climate Change Tree Atlas models a reduction in suitable habitat for forest populations of *A. saccharum*, *A. barbatum*, and *A. nigrum* over the next 60 years (Prasad et al. 2007). In the northeastern region of the U.S., changes in local climates are influencing a northward shift of natural populations of *A. saccharum* into Canada and probable shifts in the same direction for the hard maples in general (Woodall et al. 2009). Given the dearth of information regarding southern members of the complex, it is timely to assess and record their genetic profiles, both improving taxonomic distinctions and recognition, while better understanding their performance across environmental gradients. An overview of the sugar maple species complex is presented, focusing on species distribution, climate, and identifying characteristics. Relevant literature focusing on genetic variation and environmental stress

response in *A. saccharum* is discussed, as well as the paucity of literature on the other hard maple taxa. Voids in current research are organized and presented as a series of research questions.

HARD MAPLE TAXONOMY

Taxonomic treatments of the hard maple species complex are inconsistent across electronic databases and published texts. In the interests of simplicity, this paper will adhere to the treatment of these taxa species, as published in *The Flora of North America* (Committee 1993). A new version of *The Flora of North America* is available online and some volumes are also available in print. In 2005 a genetic study by Harrington et al. spawned the reassignment of the genus *Acer* to the Sapindaceae (Harrington et al. 2005). The new release of Sapindaceae has not been announced but will reflect the change in taxonomic revision of the maple family. For my purposes, hard maple group members will be discussed as individual species.

DISTRIBUTION AND ECOLOGY

Hard maple species, though closely related genetically, have evolved to exploit distinct ecological niches (van Gelderen et al. 1994). Success for each species is limited by local hydrology and soil type. Descriptions of species native range, occurrence, and habitat are presented here by adapting range maps and site descriptions from the Silvics Manual Volume 2: Hardwoods (Burns et al. 1990), originally sourced to Elbert Little Jr. (Little 1971, Little 1976, 1977, 1978). Elbert Little drafted range maps published in this widely respected document and they are useful, since they are presented at the county level. In cases where a range map could not be found in the Silvics Manual, USDA Plants Database county level entries were used for species native ranges. The following section discusses

native range distributions and/or niche characteristics, average annual temperatures and precipitation, soil preferences and relevance of hard maple complex species. Due to the nature of the content, the attempt to restate and paraphrase as much as possible has been made, but it is difficult to reiterate direct information at this level of specificity. Multiple citations are used when available.

***Acer barbatum* Michx (Florida Maple, Southern Sugar Maple)**

Native range, precipitation, temperature, and soils

The native range map and associated soil orders of *A. barbatum* are provided in Figures 1.1a-b. *Acer barbatum* is reported throughout the southeastern United States. Populations occur discontinuously from Virginia south to Florida and west across the states of Alabama, Georgia, and Arkansas into eastern Oklahoma. One population was reported farther west into central Iowa, while others are found halfway down the gulf side of Florida (Jones, 1990). The average annual precipitation for Florida maple is 1120 to 1630 mm and falls consistently during the growth season. During the dry season, minimum precipitation is 50 mm (Jones 1990). Winter temperatures have a maximum range of 11 to 18 °C and lows from - 2 to 7 °C. Warm season temperatures often fall between 29 to 33 °C with lows of 21 to 24 °C. The frost-free season is approximately 200 to 270 days, on average (Jones 1990). *Acer floridanum* (Chap) Pax shares a native range and ecology with *A. barbatum* but is said to prefer coastal plains of the southeast, and some populations have been noted in Mexico. Florida maple is often growing on soil order categories of Inceptisols, Entisols, and Ultisols. Florida maple is considered tolerant to drought conditions and high temperatures, compared with *A. saccharum* (Dirr 1990, Jones 1990). The tree can be found listed at species, subspecies, and variety classification levels in the literature and online

databases (Jones 1990, van Gelderen et al. 1994, USDA 2016, Tropicos.org 2020). Often, the common names associated with *A. floridanum* are also associated with *A. barbatum* (USDA 2016). The similarity and lack of conclusive classification between these two taxa illustrate the common characteristic of ambiguity within hard maple taxonomy and the lack of thorough observation of these species in terms of environmental niche fidelity or opportunity for breeding development. If shifts in climatic zones occur as predicted, adaptive traits already in place may make this species a candidate for selection programs, as habitat for the northeastern sugar maples is decreasing in suitability.

***Acer leucoderme* Small (Chalk Maple, Whitebark Maple, Kalkahorn)**

Native range, precipitation, temperature, and soils

There is scarce literature on the ecology and distribution of *Acer leucoderme* Small. Most of its range overlaps with that of *A. barbatum*, although it is more of an upland tree. Figure 1.2a shows the native range map of *A. leucoderme*. Chalk maple also tends to be a shade tolerant understory tree that can withstand undesirable soils, effected by leaching, as a result of alluvial soil formations. These sandier soils occur in the Piedmont coastal plain and the lower hills of the Appalachian Mountains of Pennsylvania, where a shrubbier form is more pronounced, and the tree form appears as water availability, drainage capacity, and soil organics increase (pers. obs.). Figure 1.2b show soil orders throughout the native range of *A. leucoderme*. Chalk Maple has the same annual precipitation amounts as its relative *A. barbatum*. Seasonal temperatures for chalk maple are within the same range as those for *A. barbatum* (Figure 1.2a-b). Chalk Maple is adapted to the climates of the southeastern region of the hard maple complex range. Not only does chalk maple share a tolerance for higher temperatures and drier conditions, it displays a capacity for thriving on nutrient-

poor soil (pers. obs.). In urban context, these traits can be useful. Urban settings are often the harshest regarding water availability, heat effects, and soil quality; all of which work against the chances of sugar maple's success among the gray infrastructure (Bassuk et al. 2009, Fahey et al. 2013). It is also a consideration that chalk maple grows to a lesser height, but still has the brilliant fall display of its sugar maple relative. A smaller tree form could reduce maintenance challenges for public park and works departments. Existing publications suggest chalk maple for use as a street tree in harsh conditions (Gilman et al. 1993, Coder 2010).

***Acer grandidentatum* Nuttall (Bigtooth Maple, Canyon Maple)**

Native range, precipitation, temperature, and soils

A high level of plasticity in *A. grandidentatum* results in variable ecology of the species. Elevations vary on a state basis for the species, and Tollefson's write-up for The United States Forest Service Forest Ecology Information System (FEIS) website is worth consulting for a summary of this information, as it gives a detailed and thorough description of *A. grandidentatum* site characteristics and ecology (Tollefson 2006). *Acer grandidentatum* Nutt, along with associated infrataxa, represents the western component of the hard maple complex. Bigtooth maple occupies the mountainous northern part of the range in Idaho, Montana (Singhurst et al. 2013) and Wyoming, reaching into southwestern states of Arizona, New Mexico and Texas, and also into northern Mexico, where it shares part of the range with populations identified as Canyon maple (*Acer grandidentatum* var. *sinuosum*) (Gehlbach et al. 1983). Bigtooth maple grows with scrub or white mountain oak (*Quercus gambelii*) on drier sites, but favors lower elevations with cooler temperatures and higher moisture levels, near mountain streams, canyons, and ravines across most of its

range, which is shown in Figure 1.3a. Bigtooth maple can displace the oaks in Arizona and Utah on mesic sites (Tollefson 2006). Populations that do not compete with oaks are present on xeric and mesic sites. In New Mexico, bigtooth maple is confined to riparian areas, and might be restricted to the same area type in Utah, if not for its presence in the mountain brush zone and with firs (Tollefson 2006).

In Utah, as elevation increases, *A. grandidentatum* favors northern aspects with higher moisture and cooler temperatures. Drier southern slopes are not suited to its moisture demands. In Arizona and New Mexico's higher regions, the species finds shaded areas of accumulated water and stream drainages. Bigtooth maple can occupy hillsides or lower sites. Populations associate with riparian sites in canyons, creeks and floodplains in Texas (Tollefson 2006). Local precipitation requirement for bigtooth maple is 40-50 cm annually. In Utah, precipitation is the primary water resource for small trees beyond stream banks, and for larger trees in general. Stream banks are the main source of water for small trees nearby. In parts of Idaho, within the range of big tooth maple, the water table occurs a significant distance below the surface. Bigtooth maple has adapted to low water potentials and is considered a drought tolerant species (Tollefson 2006). Bigtooth maple can withstand temperatures to -35°C (Sorenson et al. 1984). Warm season temperatures above 38°C define bigtooth maple's upper thermal limitation (Tollefson 2006). Bigtooth maple has a dynamic presence in terms of soil depth, texture, and pH and soil orders throughout the native range, as presented in Figure 1.3b. The species is most successful on soils that are characteristically moist with adequate drainage. Soil types include silt loam, clay loam, sand, gravel, and cobble. Soil acidity/alkalinity is preferred between 6.0 and 7.0 pH. Bigtooth maple is noted for its fall leaf color, and is a sought after feature for improving

nursery stock (Barker et al. 1977). Bigtooth maple has adaptive traits of drought tolerance and the ability to withstand temperatures above 38° C. For programs seeking these traits for sensitive species occurring at higher elevations or latitudes, *A. grandidentatum* could provide the genetic resources necessary for breeding opportunities to confer environmental stress tolerance.

***Acer nigrum* Michx, f. (Black Maple, Rock Maple, Hard Maple)**

Native range, precipitation, temperature, and soils

The native range of Black maple resembles that of *A. saccharum*, but the frequency of black maple increases from east to west (Figure 1.4a). As environmental conditions change to the warmer and drier climate of the prairie lands, sugar maple frequency is reduced, and black maple occurs 193 km to the west of the western most populations of sugar maple. It is the sole hard maple complex species that is found in western Iowa and South Dakota (Slabaugh 1958). Moisture demand varies across the range of black maple and is a limiting factor in terms of species distribution (Hilaire et al. 1999). Normal annual precipitation is 61 to 142 cm. Growing season precipitation is 30 to 51 cm and average annual snowfall is from 15 to 150 cm. Effective precipitation is defined as the long-range benefit of precipitation in promoting plant growth and can be influenced by precipitation intensity, season, temperature, and local flora among other environmental parameters, as much as soil profile type, plant available soil moisture retention, and level of disturbance . The link between precipitation and temperature has been investigated by several researchers using a variety of approaches. In a comparative study, potted *Acer rubrum* seedlings from mesic and xeric sites are used to determine species sensitivity to low soil water availability. Well-watered (control) plants from mesic sites at saturating light levels,

responded with elevated rates of several such as carboxylation, leaf conductance, and electron transport. Dry site seedlings also reacted to low soil moisture, with a reduction in osmotic potential at both thresholds of full leaf turgor and turgor loss point (Bauerle et al. 2003). It is conceivable that hard maples may show similar behavioral response. Average annual minimum temperatures vary between -12 and -34°C throughout the range of black maple. An average frost-free period duration is 120 to 210 days, with a precipitation effectiveness index of 48 to 127.

Black maple soils vary with climate throughout the native range. In cooler areas of higher moisture, soils are described as subject to leaching and eluviation of minerals and organic contents. As the North American plains have developed, the western section of black maple's range has been reduced in its sub humid fertility (Gabriel 1990). These soils are included in the orders Millisols, Inceptisols, Entisols, and Spodosols and shown in Figure 1.4b. Throughout the Great Lakes region of the black maple range, soil structure is the result of glacial retreat. In western Ohio, black maple increases in abundance as the soil type changes from a silty clay loam to a silt loam, suggesting species sensitivity to aeration and drainage. Black maple growing in the central parts of Iowa is on rich topsoil that is well drained over slightly sloping terrain. In Quebec and New York, conditions limit black maples to lower areas of rich soils. Missouri black maples grow in the northcentral woods, slopes, ravines, and valleys (Slabaugh 1958). A study of black maple, originating in glaciated and unglaciated areas of Ohio, discussed the likelihood of existing populations there being the result of the species evading selective pressures resulting from glaciation. The author proposes his opinion that the current population in Ohio is a postglacial hybrid swarm between black and sugar maple (Paddock 1961). The environmental adaptation of

the species to drier and warmer climates make it an option for plant selection and breeding programs (Graves 1994). *Acer nigrum* is genetically compatible with *A. saccharum*, leading some to suggest the two do not require separate species designation (Skepner et al. 1997). The species is also a sap producer and can be tapped for maple sugar production (Wendel et al. 1980). Successful crosses of *A. nigrum* and *A. saccharum* have been used to improve sugar production and yield in breeding programs for the ‘Super Sweet’ maple hybrids (Kriebel 1989, 1990).

***Acer saccharum* Marshall (Sugar Maple, Hard Maple)**

Native range, precipitation, temperature, and soils

Acer saccharum Marsh, sugar maple, shares much of its native range with *A. nigrum*, as previously mentioned. Figure 1.4a and 1.5a show overlap in the native ranges. Sugar maple’s range extends northeast of black maple into the cooler temperatures. Sugar maple does not persist into the plains, due to drought capacity differences. Sugar maple occurs across a range of soil types but prefers moist and well-drained soils (Little 1953, Godman et al. 1990). Sugar maple is sensitive to drought and is limited to climatic zones where climate is cool and moist (Bishop et al. 2015). Annual precipitation amounts vary with geographical region. In the northeastern part of the range, where sugar maple has high commercial value, precipitation averages 1270 mm per year. Sugar maple populations in the southern Appalachian Mountains receive the most local precipitation, measuring 2030 mm per year. During the growing season, precipitation averages are bracketed by 380 mm to the west and 1020 mm to the east. Winter precipitation can be more than 2540 mm in the northern section of sugar maple range (Godman et al. 1990). The upper portion of sugar maple range experiences winter temperatures averaging -18°C , with July temperatures of

approximately 16 °C. Populations of sugar maple are exposed to conditions of aspect and moisture that modify average annual temperatures of 10 °C in January to 27 °C in July. Climatic factors defining the range of sugar maple also define species frequency at higher elevations (Godman et al. 1990).

Sugar maple tolerates many types of soils, including sands and loams, but prefers well-drained loams. The species is rarely found on dry or swampy soil types. Sugar maple is more selective on southern sites and displays more soil tolerance in the area surrounding the Great Lakes (Beal 1894, Ewers et al. 2008). Dominant soil orders for *A. saccharum* are Spodosols, Alfisols, and Mollisols. (Godman et al. 1990). Figure 1.5b shows the dominant soil orders found throughout sugar maple's native range. Sugar maple is well adapted to mid-range pH levels of 5.5 to 7.3 pH, but can tolerate soils registering a pH of 3.7 (Godman et al. 1990) . Sugar maple does not thrive in compacted soils, especially in the urban environment, where compacted soils are common (Bassuk et al. 2009, Mullaney et al. 2015). Sugar maple is found at its lowest elevation (490 m) in the western part of the range and at its highest elevations in the southern Appalachian Mountains (1680 m) but occurs at 760 m above sea level in New York and New England. Mountainous areas of New Hampshire and Vermont support sugar maple populations that run along a Boreal forest transition zone. Presence of sugar maple in the southern part of the native range is closely associated with water resources at transitional elevations of an irregular topography (Godman et al. 1990).

Relevance of Acer saccharum Marsh

Sugar maple is an important hardwood forest species that has a dominant presence in the northeastern United States. It has strong ties to the economy of New England and

cultural ties to New York, West Virginia, Wisconsin, and Vermont, where it serves as the respective state trees (Miller 1994). At one time, the United States supplied 80% of the global demand for maple sugar products. Today, Canada is the primary source for maple sugar around the world, due to the decline of sugar maple health in the U.S. and government subsidies for Canada's sugar bush growers. Climate change models predict a reduction in available suitable habitat for northeastern sugar maple over the next 60 years (Prasad et al. 2007). Environmental parameters of temperature and water availability are expected to become less predictable, while increasing in terms of their intensity and duration, forecasting negative circumstances for sugar maple, which is sensitive to thermal loading and water deprivation in both urban and natural settings (Fahey et al. 2013).

Plants are facing rapidly changing climate scenarios. Their sedentary nature requires adaptive strategies that will allow for persistence and success, in the face of abiotic and biotic stress factors. Species equipped with the necessary adaptive traits are better suited for environmental changes. Species lacking these traits will fall out of the current forest landscapes (Aitken et al. 2008). Sugar maple, *A. saccharum*, is at risk due to its intrinsic sensitivities to environmental conditions, and solutions are needed (Prasad et al. 2007).

***Acer skutchii* Rehder (Mexican Maple, Cloud Forest Maple)**

Native range, precipitation, temperature, and soils

Acer skutchii Rehder is now considered a subspecies of *A. saccharum*. Very little has been found in the literature, so most of the following comes from four different sources. Populations reported in Mexico and Guatemala are the western and southern most of the of the hard maple species complex. Mexican maple is very scattered across six locations

of high elevation across the cloud forest mountains throughout Central America (Vargas-Rodriguez 2005). Population sites are described as significantly separated from one another by distance (Vargas-Rodriguez et al. 2012). Cloud forest climate is characterized by the common occurrence of cloud emersion and its persistence at high mountain elevations. Studies report yearly average precipitation amounts between 927 and 1295 mm for native populations in Mexico (Lara-Gomez et al 2005). Mean annual temperatures for cloud forest maples in Mexico are between 13 and 19 °C. Average annual temperatures vary and tend to increase with increasing altitude throughout sites in Mexico, except in the case of a site where *skutchii* grows on north, northeast, and eastern facing slopes (Lara-Gomez et al. 2005). Cloud forest maple occurs on a variety of substrates including Lithosols, Cambisols, Regisols, and Luvisols (Vargas-Rodriguez 2005). The species has adapted to germination, growth, and development at high altitudes, across geographic regions, despite its disjunct occurrence. The species has shown the ability to overcome alkaline soils and drought conditions, while not compromising an increased rate of growth. All characteristics that may be desirable as future environmental challenges develop (Creech 2016). Figure 1.6 shows the native range limits for *Acer saccharum* subsp *skutchii*.

IDENTIFYING CHARACTERISTICS OF HARD MAPLE COMPLEX SPECIES

Generally, identification of maple species considers bark color and texture, leaf characteristics of color, size and absence/presence of leaf pubescence, fall display, seed orientation and bud characterization (Desmarais 1952, Kriebel 1956, Gabriel 1973, Gabriel 1978). Field identification of sugar maple species includes the physical characteristics of maples, and environmental factors of local precipitation, temperatures, and soils (Kriebel 1956, Hauer 1995, Hilaire et al. 1996, St. Hilaire et al. 1998, St. Hilaire et al. 2001). Sugar

maple species can be placed into three categories, based on niche characteristics, which are summarized in Table 1.1. Northern populations occur north of 35° latitude and east of 100° longitude. Southern populations occur south of 35° latitude and east of 100° longitude. Western populations are found north of 30° latitude and west of the 100° degree longitude line. Sugar maple and Black maple represent the northern group; Southern sugar maple, Chalk maple, and Mexican maple characterize the southern set, and Bigtooth maple is the western representative.

MORPHOLOGICAL IDENTIFIERS OF NORTHERN SUGAR MAPLES

Acer saccharum, is identified as having a tree form approximately 40 m in height with gray, deeply furrowed bark; leaves 3-5 lobed and 10 to 15 cm across, upper side is dark green, the abaxial side pale green and glabrous to barely pubescent (Godman et al. 1990). *Acer nigrum* shares height, leaf size and color with *A. saccharum*. Black maple leaves are often a darker green. Greater prolepsis in shoot growth displayed in *A. nigrum* lends itself to less forking of tree branches than documented in *A. saccharum*. The bark of *A. nigrum* is darker with deeper furrows than *A. saccharum*, and pubescence is usually present on leaves and leaf petioles. Black maple is believed better adapted to conditions of drought than its sugar maple relative and persists beyond the western limits of *A. saccharum* by approximately 120 km (Gabriel 1990).

Northern hard maples readily exchange genetic information, resulting in a hybridization zone where the native ranges overlap (Dansereau et al. 1947, Fleak 1967, Gabriel 1973). Misidentification of specimens in this region can happen easily, but the cooler, moister climates of the northeastern United States favor persistence of *A.*

saccharum, increasing accurate species recognition (Paddock 1961). To the west, *A. nigrum* populations thrive in the drier, warmer environment (Gabriel 1990).

MORPHOLOGICAL IDENTIFIERS OF SOUTHERN SUGAR MAPLES

Southern sugar maple, *A. barbatum*, is recognized as a tree at 15 m high with white-gray bark that is thin and furrows with age. At breast height, trunk diameter is up to 70 cm. Leaves of the southern sugar maple are 3 to 5 lobed, 20 cm in length and 17 cm across, with deep including leaf blade and petiole, with broad sinuses. Abaxial leaf side is light green and pilose, with long soft hairs that can become glabrescent, or hairless over time. The petiole of southern sugar maple can be pubescent or pilose (Dirr 1990). Chalk maple, *A. leucoderme*, grows as a tree or shrubby form. Personal observations report a multi-trunk habit in areas of the native range and single trunk specimens in others. Heights of up to 9 m are mentioned, but personal observations would expand on this description. In the field, discerning between *A. leucoderme* and its southern relative *barbatum* are difficult, when tree forms were near one another. When chalk maple was observed in shrub form, it made differentiation easier. The bark of chalk maple can be grayish brown to a chalky white and smooth or minimally fissured. Leaves are 3 to 5 lobed and smaller than other sugar maple species at 5 to 8 cm across with a wavy margin. Abaxial leaf side is a yellow-green with pubescence that is erect and dense (Dirr 1990).

The cloud forest sugar maple, or Skutch maple, *A. skutchii*, is like *A. barbatum*. Skutch maple displays larger leaves and produces the largest samaras of the Sapindaceae *Acer* genus. The Skutch maple is reported to be a fast-growing species, showing tolerance to alkalinity, drought, and adaptations for growth in the southeast and southwest regions of the U.S. (Creech 2016). Field studies of *A. skutchii* leaves found laminae thickness greater

than any of the other hard maple species. Regional variation of *A. skutchii* plantings report variation on fall color, spring growth color, and cold tolerance.

The western hard maple, Bigtooth maple (*A. grandidentatum*) is recognized in two forms. Specimens can have single or multi-stemmed form when found near water and grow to heights of roughly 15 m. When found on the drier canyon slopes at higher elevations, trees are usually multi-stemmed and grow at shorter heights of about 8 m. The bark is thin and peeling and smooth. Leaves are pubescent with 4 lobes and are 2.5 to 5 cm wide. *Acer grandidentatum* is considered drought tolerant in its native range.

GENETICS OF HARD MAPLE SPECIES

In the mid-1950s, Howard Kriebel established two trial plots at the Ohio Agricultural Research Development Center (OARDC) in Ohio, USA. One plot was designated for selection of trees with increased sap sugar content, which was successful in providing the U.S. maple sugar industry with the ‘Super Sweet’ variety as a sap resource (Kriebel 1955, Kriebel et al. 1969, Kriebel 1990). Kriebel’s other plot was an environmental tolerance trial. Kriebel collected germplasm from 18 different provenance sources across the range of hard maple species. Trees were from seed-germinated greenhouse stock or from seedlings transplanted to Wooster. Kriebel notes physiological and morphological behaviors for a period of two years in his dissertation work at Yale’s Department of Forestry (Kriebel 1956). Kriebel’s work concluded that sugar maples in his common garden plots displayed a high level of geographic variation in drought tolerance and leaf injury potential from high exposure to solar radiation. Kriebel attributes differences in tolerances associated with cold temperatures and frost-hardiness to cultural variation in apical dominance. Regarding hard maple species phenology, Kriebel describes

a clinal variation pattern from north to south, with transition zones of varying size in between, influenced by summer temperatures and day length (leaf coloration), among other factors (Kriebel 1955, Kriebel 1956, Kriebel 1976).

Kriebel expressed an interesting opinion that the clinal pattern he describes applies to southern (but not northern) populations of sugar maple (Kriebel 1956). He found northern and southern trees to be physiologically distinct and northern species were different only when considering juvenile tree form and seasonal cessation of growth. It is unfortunate that this stress trial was overshadowed by the sugar trial, but fortunately, has been recovered 60 years after its 1954 installation, as a direct result of this dissertation effort. Now a part of the Secrest Arboretum, the plot is maintained due to its great scientific value. The value here is in the plot's history and results it yields in the present day. Certain characteristics of the young trees have become less distinct, such as tree form; however, the knowledge of individual acclimation capacity can inform researchers focused on abating the negative impacts rapid climate change. In the coming decades, northeastern climate is expected to reflect the current climate of the southeastern U.S. The Ohio trees can be a wealth of genetic information regarding the tolerances of sugar maples outside of *A. saccharum*. Further value of this germplasm collection is in the characteristics that may develop within progeny of the original trees. Questions addressing genetic compatibility between hard maple species can be considered using new genetic tools and techniques. Species present in the Kriebel trials are *A. barbatum*, *A. nigrum*, and *A. saccharum*. Drought tolerance has been reported in *A. barbatum* and *A. nigrum*. Black maple, *A. nigrum*, is suggested to present increased drought tolerance, due to its ability to withstand drier and warmer climate of western Iowa (Gabriel 1990). Southern sugar maple, *A.*

barbatum, is reported to have capacity for tolerating drought and temperature conditions found in the southwestern region of the United States (Jones 1990). Kriebel mentions the absence of *A. leucoderme*, chalk maple, in his Ohio studies. Chalk maple is said to occupy the same ecological habitat as *A. barbatum*, but thrives on the sandier, nutrient-poor soils of the upland Piedmont region and can be shrub-like in appearance. Basic research investigating environmental limitations of these species does not exist, but the opportunity for novel study focused on them presents itself.

Native ranges of southern hard maple species are discontinuous throughout the southeast, which may suggest they are genetically incompatible within certain habitats, but it is more likely a circumstance of refugia from glacial retreat creating historical barriers to gene flow between populations. Field observations by this author would suggest, after witnessing a mixed stand during a field campaign to North Augusta, South Carolina, along the Savanna River, that a maladaptive theory has merit. Ted Stephens, the local horticulturist of Nearly Native Plant Nursery of Fayetteville, Georgia, explained his belief that *barbatum* is contained in the area along the riverbank and *A. leucoderme* grows higher, where sandy soils occur. The trail throughout the wooded area adjacent to the river is considered a natural divide between the southern species existing there. Stephens indicated that he had not encountered any specimens that would lead him to suspect any hybridizing was occurring within the stand, but without the genetic information, he would not be able to say this with 100% confidence. Successful genetic crosses have been forced between *A. saccharum* and *A. leucoderme* (Slavin 1950, 1954), though the specimen has not been located at this time by the author.

Issues within the Hard Maple Complex

Economic importance of maple sugar has driven research focused on *A. saccharum*. The list of references available directed at sugar maple in popular and scientific publications is extensive and provides rationale for aspects of sugar maple research. Considering the predicted trajectory of *A. saccharum*'s future, and traits defining a poor outlook, research focused on sugar maple relatives would be considered a valuable source of behavioral and genetic information when solutions are necessary. Unfortunately, there is a void in literature relating to physiology, phenology, and genetic information of sugar maple species beyond *A. saccharum*, *A. nigrum*, and *A. grandidentatum*. Thus, the opportunity arises and must be reviewed for moving the field of plant research forward to increase our understanding of sugar maple relatives and their adaptive ability, in the face of rapidly changing climate regimes (Aitken et al. 2008, Woodall et al. 2009, McCarragher et al. 2011, Hart et al. 2014). Climate models support findings that sugar maple's inability to persist and thrive under conditions of predicted future climate will have it displaced as a dominant forest species. The warmer, drier weather patterns expected to become typical across the native range of *A. saccharum* over the next 60 years are not conducive to its success. Solutions are needed or its dominance as a hardwood forest species will end (Shea et al. 2001, Parry 2007, Prasad et al. 2007, Iverson et al. 2008, Rustad et al. 2012). Tools for quantifying behavioral trait response to the environment have been readily available for decades. Investigations of stress tolerance in *A. saccharum* have been informative. Physiology studies reveal *A. saccharum* sensitivity to environmental parameters of temperature (McCarragher et al. 2011), water (Abrams et al. 1990, Ellsworth et al. 1992), light (Lindroth et al. 1993, Delagrange et al. 2006), ozone (Tjoelker et al. 1993) and carbon dioxide (Parsons et al. 2003). All studies show a reduction in processes associated with

plant productivity. Photosynthesis processes such as, and not limited to, stomatal conductance, carbon assimilation, and integrity of water management are negatively impacted when *A. saccharum* seedlings are subjected to one or more of these parameters at increasing levels of concentration and/or duration. Where the research falls short is the lack of the same knowledge in terms of the other hard maple species. Modern methods, such as molecular markers, have been developed and can be applied to basic research required to fill the existing void in hard maple biology.

Research Questions and Objectives

Sugar maple relatives show genetic affinity and variation in adaptive trait response across the native range of the sugar maple species complex in the United States. It is critical to lay the scientific framework necessary to inform the following research questions addressing the future of an economically and culturally significant species:

1. Are there patterns of behavioral response between northern and southern hard maple populations and are these patterns significantly different?
2. Do northern and southern hard maples respond differently to extreme drought and are the difference (if any) significant?
3. Is genotype reliable as an indicator of drought response in hard maple species?

The dissertation addresses these questions with the design of 2 drought studies and a genotype analysis of hard maple populations of different provenance sources from across the continental United States.

To address the first research question, I conduct a study looking at hard maple populations under three different water regimes in a classic greenhouse trial. The northern cohort consists of *Acer saccharum*, *Acer nigrum*, and *Acer grandidentatum* and a southern

cohort of *Acer barbatum* and *Acer leucoderme* of different provenance. Photosynthetic processes associated with leaf gas exchange are quantified and analyzed to determine significant differences in population mean values of carbon assimilation rate (A_{NET}), rate of stomatal conductance (g_{sw}), transpiration rate (E), and water use efficiency (WUE). This is the only study in hard maples addressing physiological response to water deprivation at this level of inclusion that the author is aware of, making it novel.

Chapter 3 approaches question 2 using gas exchange methods to build response curves of stepwise elevations in CO_2 concentrations for southern study populations from Chapter 2 that are subjected to a second round of water treatments. The relationship between carbon assimilation rate and intercellular concentration of dioxide is used as input data to calculate the potentially limiting rates of maximum carboxylation, electron transport, and triose-phosphate utilization on plant photosynthesis. Study parameters are V_{cmax} (maximum rate of carboxylation), J (electron transport rate), and TPU (triose-phosphate utilization); all have been found to be limiting factors to plant metabolism under stress (Wullschleger, 1993).

In Chapter 4 there are three objectives: (1) to examine similarities/differences between hard maple taxa through a genetic lens in a way that has yet to be done, (2) to determine whether genetic variation within hard maples reflects the current state of hard maple taxonomy, and (3) to determine whether response patterns observed in the drought study described in Chapter 2 are reflected by provenance-level genetic differences. I present results of a genotyping analysis conducted on leaf tissue samples collected from field campaigns to Texas, Florida, South Carolina, and New York. Trees subjected to studies in Chapters 2 and 3 are also included. The discussion focuses on whether (or not)

sugar maple genotypes are indicators of species limitations and/or plasticity in terms of water limitation. The drought study trees from Chapters 2 and 3 are used show the correlation between these genetic associations and drought response.

Chapter 5 discusses how modern research tools quickly provide data and thoroughly quantify a suite of behavioral trait responses in trees at the whole plant level. On the topic of behavior limitations of plant species, physiological techniques have produced data, once properly analyzed, useful to plant breeders and those establishing plant selection criteria for reforestation programs, restoration programs, and the landscape industry. Applying the same methods to studies focused on the sugar maple species complex can lay the groundwork necessary to advance our understanding of a highly variable group of taxa. Integration of physiology and genetic profiling can establish a model for selection and breeding programs focused on targeted traits associated with plant performance. The model can be used across natural and urban landscapes, while informing forest management practices, policy development, and breeding programs focused on building resilient and sustainable plant selection palettes. The future of sugar maple research is clear. Investigating environmental tolerances of the lesser-known sugar maples can help find answers to questions facing an important component of the U.S. economy and of the forested landscape of the eastern United States, while easing an otherwise difficult transition facing the region in the foreseeable future.

Table 1.1 Regional categories of hard maple species

Northern	Southern	Western
<i>Acer Saccharum</i> Marshall Sugar maple	<i>Acer barbatum</i> Micheaux (incl. <i>floridanum</i>) Southern Sugar Maple	<i>Acer grandidentatum</i> Nuttall Bigtooth/Canyon Maple
<i>Acer nigrum</i> Micheaux, F. Black Maple	<i>Acer leucoderme</i> Small Chalkbark Maple	
	<i>Acer skutchii</i> Rehder Mexican Sugar Maple	

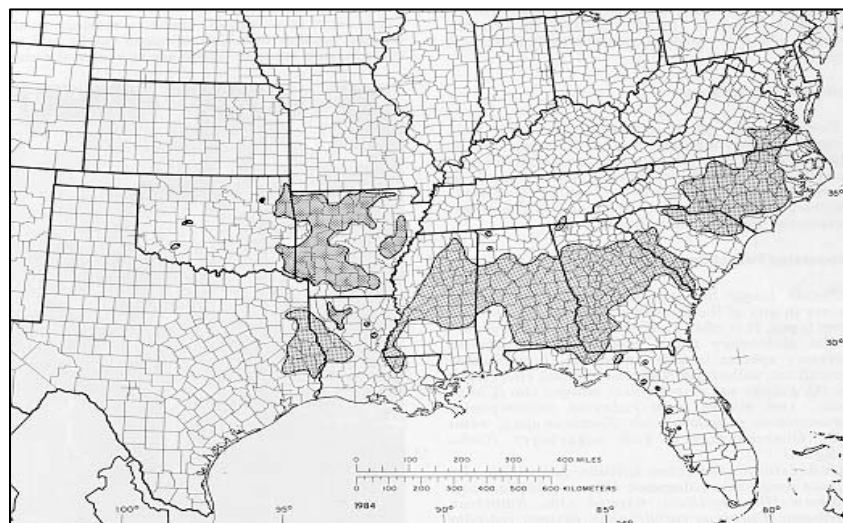


Figure 1.1a. Native range of *Acer barbatum* Michx is shared with *Acer floridanum* in the United States (Little 1953).

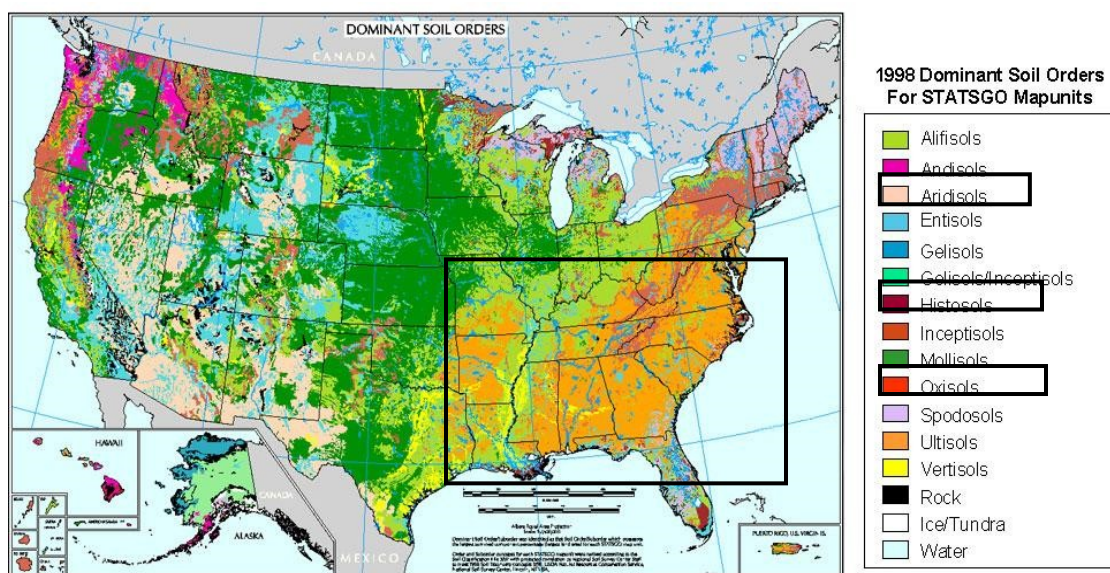


Figure 1.1b. Dominant soil orders for *Acer barbatum* and *Acer floridanum* are Entisols, Inceptisols, and Ultisols (NRCS 2010).

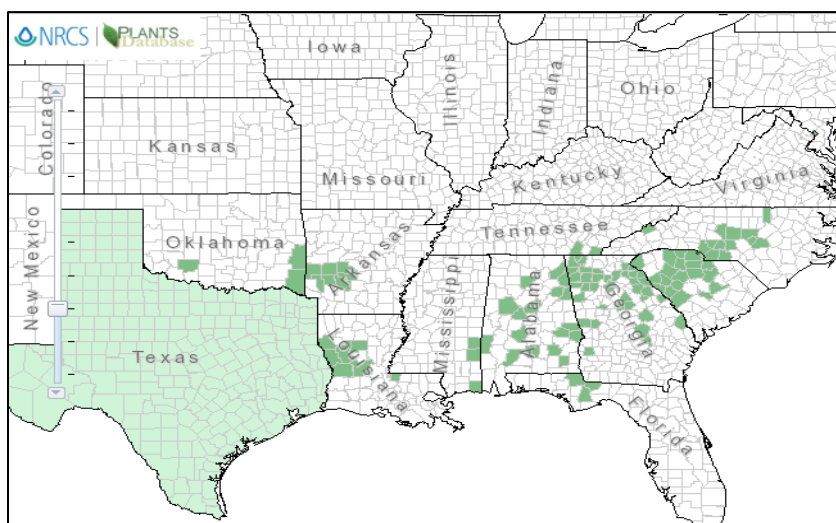


Figure 1.2a. Native range map for *Acer leucoderme* Small, adapted from USDA Plants Database. (1.2b).

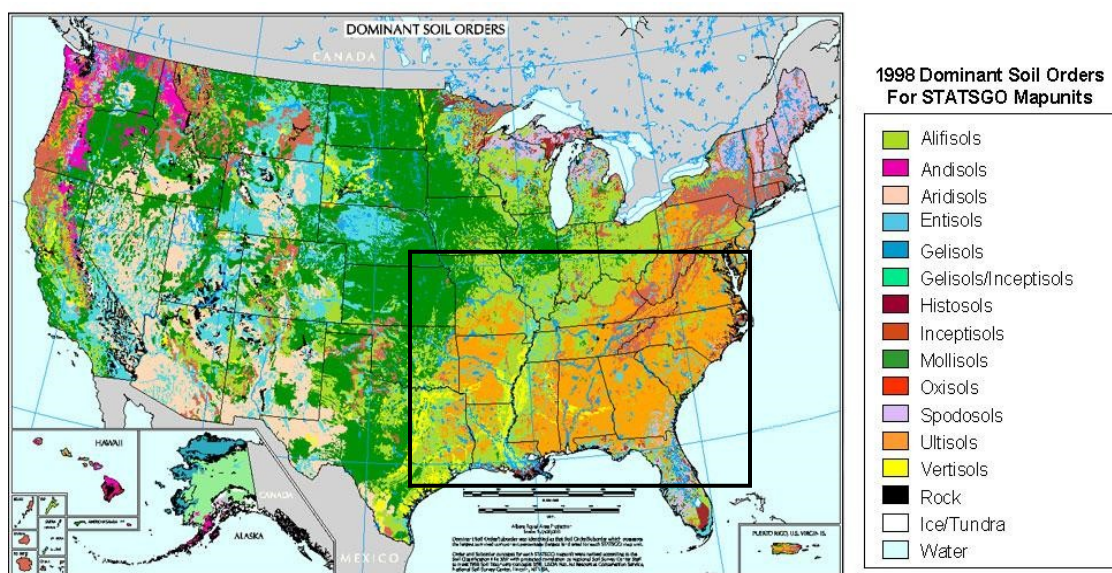


Figure 1.2b. Dominant soil orders found throughout the native of *Acer leucoderme* Small.

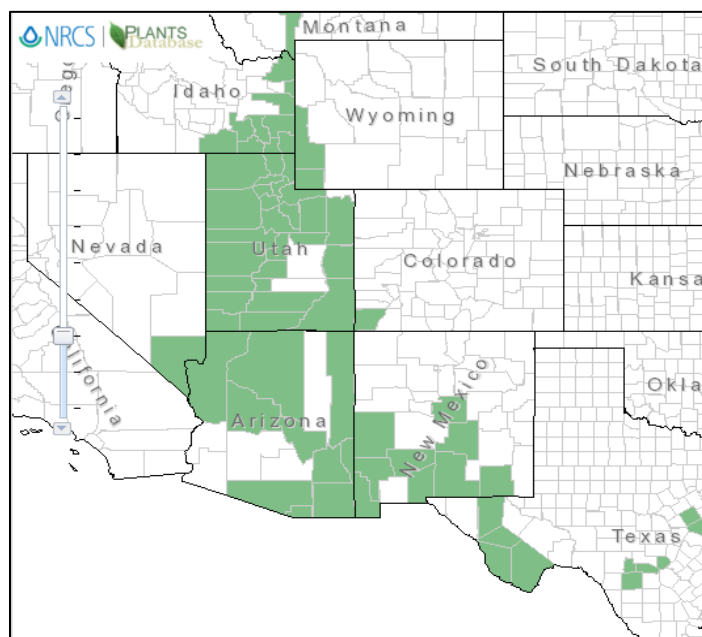
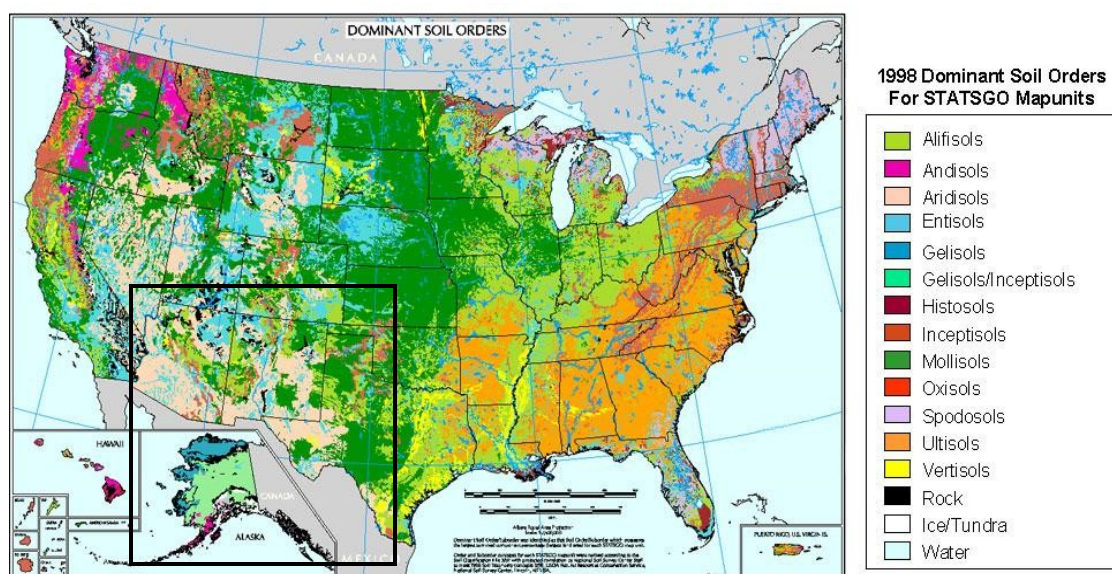


Figure 1.3a. Native range map for *Acer grandidentatum* Nuttall (USDA Plants Database



2017).

Figure 1.3b. *Acer grandidentatum* Nuttall is associated with all dominant soil orders throughout the native range (Tollefson 2006, NRCS 2010)

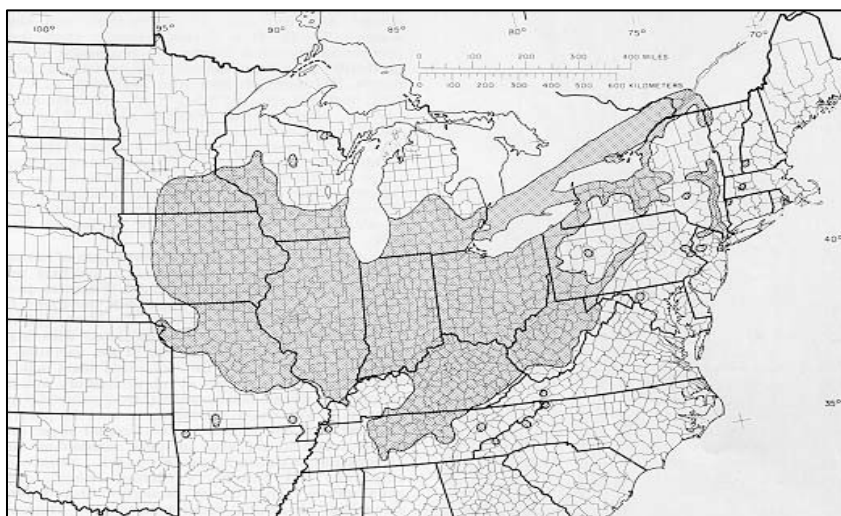


Figure 1.4a. Native range and dominant soil order maps for *Acer nigrum* Michx, f. adapted from Elbert Little (Little 1971).

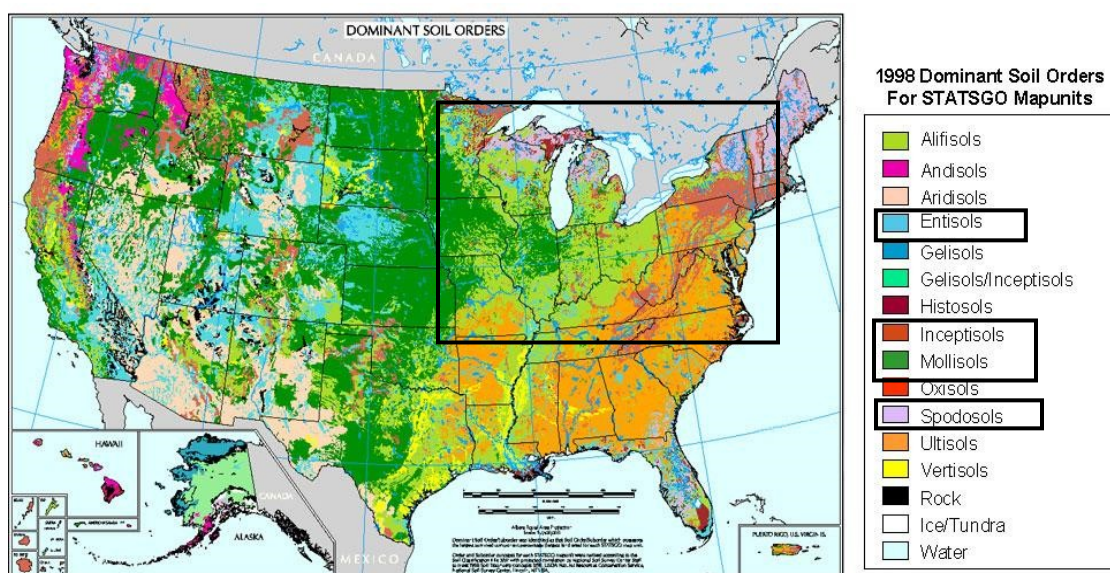


Figure 1.4b. Dominant soil orders for *A. nigrum* are Entisols, Inceptisols, Mollisols, and Spodosols (NRCS 2010).

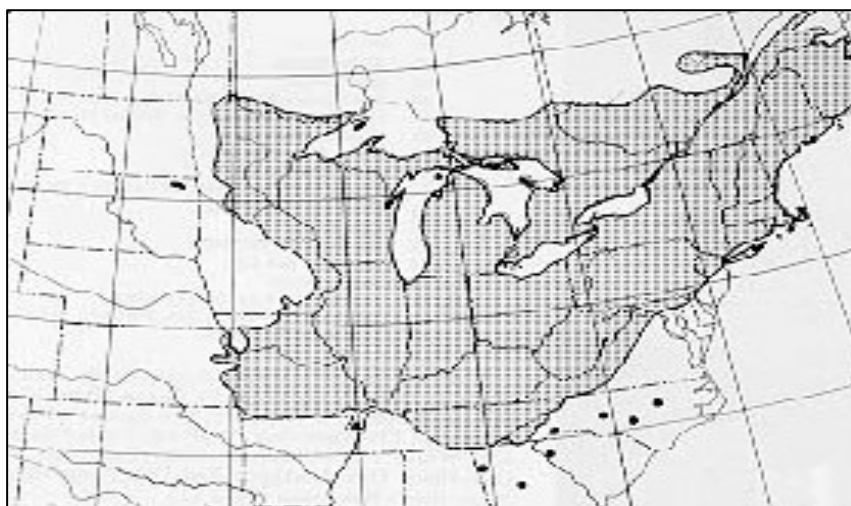


Figure 1.5a. Native range map for *Acer saccharum* adapted from Elbert Little (Little 1971).

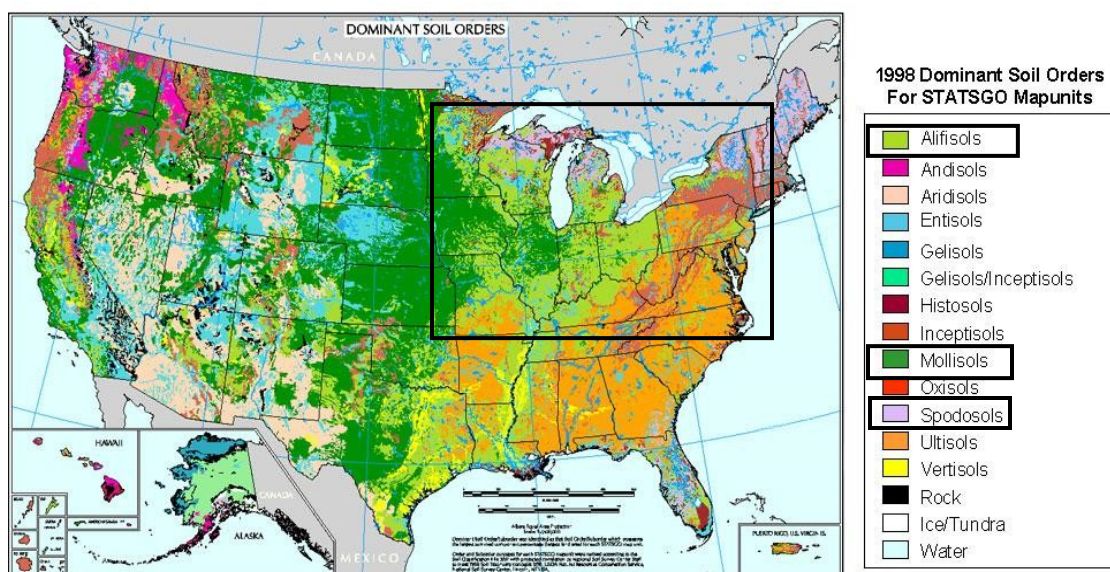


Figure 1.5b. Dominant soil orders for *Acer saccharum* Marshall are Alfisols, Millisols, and Spodosols (NRCS 2010).

Can't really get much out of this one.

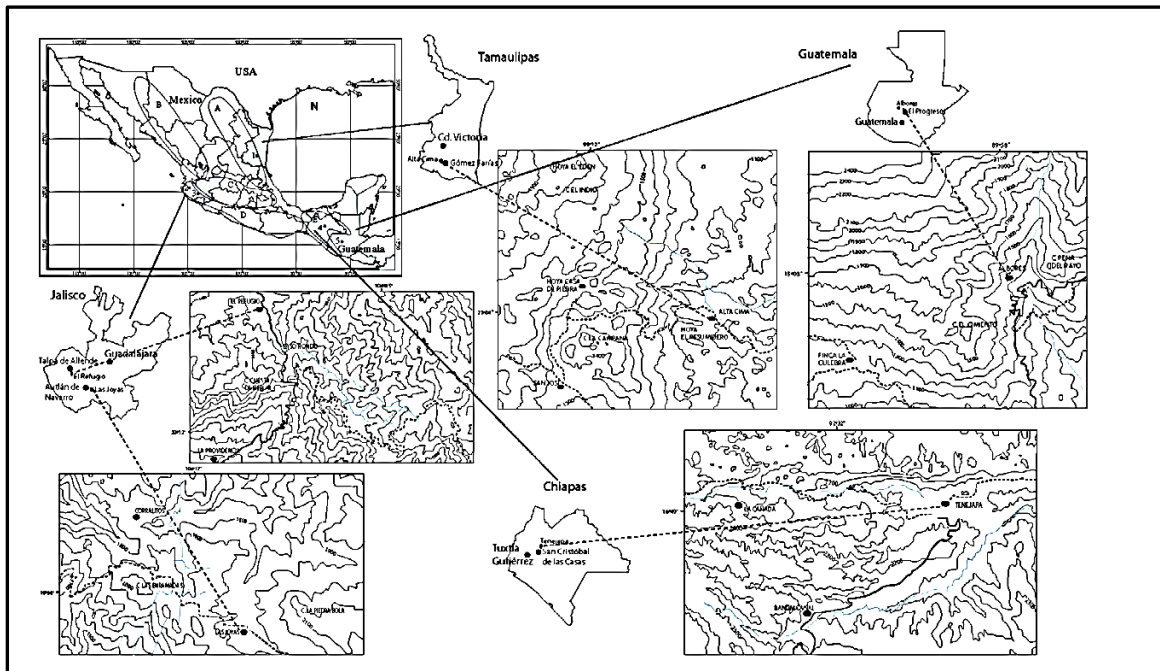


Figure 1.6. Native range of *Acer saccharum* subsp. *skutchii* (Mexican Maple) in Mexico.

Acer saccharum subsp. *skutchii* is associated with Lithosols, Cambisols, Regisols, and Luvisols soil orders throughout its native range (Vargas-Rodriguez 2005).

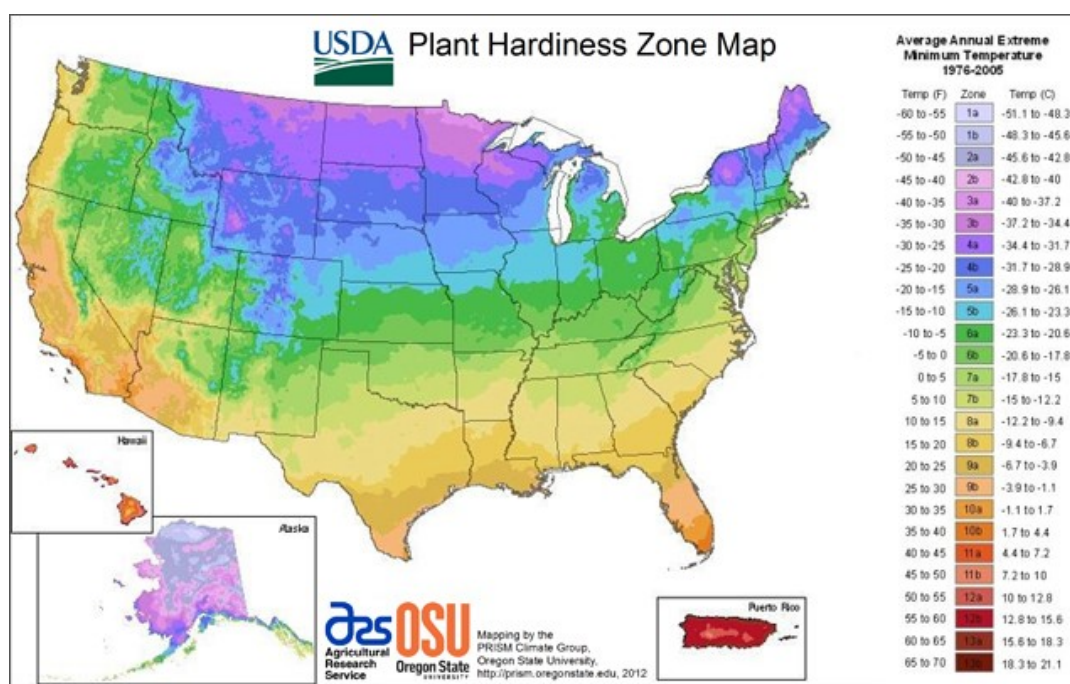


Figure 1.7. USDA Hardiness Zone Map published in 2012.

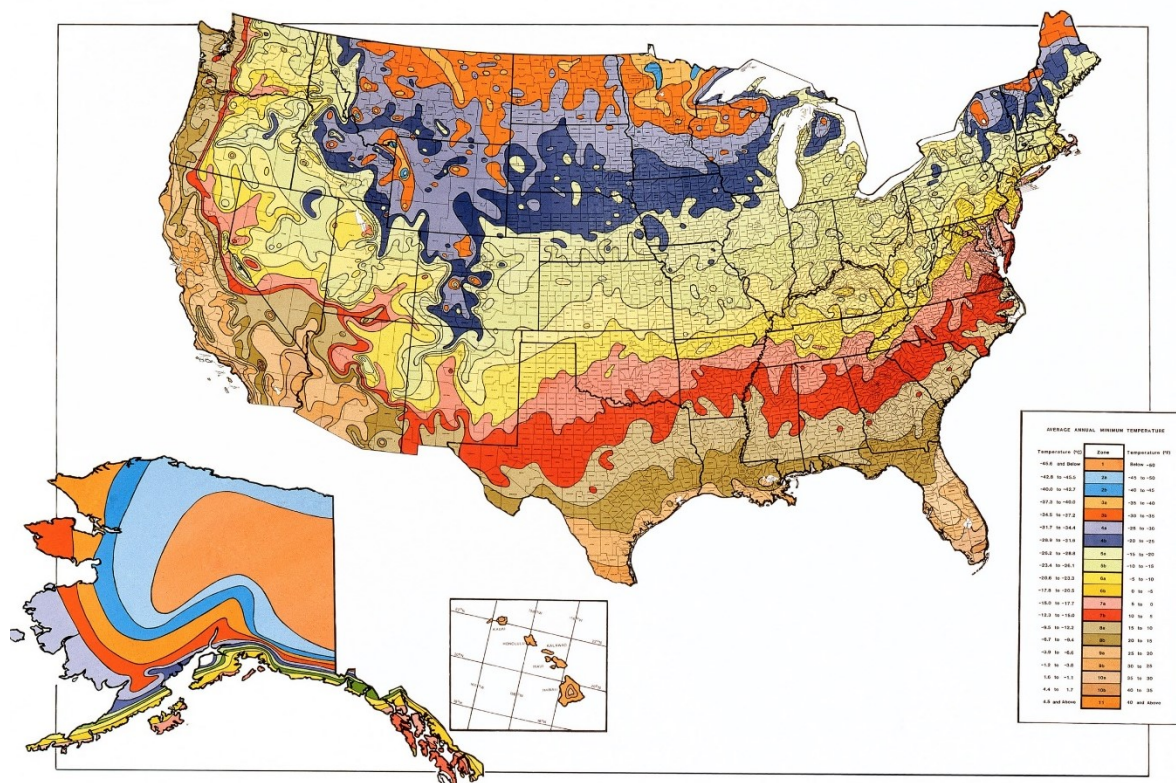


Figure 1.8. USDA Hardiness Zone Map published in 1990.

CHAPTER 2

THE INFLUENCE OF HARD MAPLE PROVENANCE ON STOMATAL CONDUCTANCE, CARBON ASSIMILATION TRANSPIRATION RATE, AND WATER USE EFFICIENCY UNDER THREE DIFFERENT WATER REGIMES

ABSTRACT

This study examines drought responses of nine hard maple populations through gas-exchange methods. A germplasm collection from range extremes is first acclimated to New Jersey climate for several years, then subjected to intermediate and extreme drought treatments in classic greenhouse drought studies. Meaningful population differences were observed for A_{NET} (carbon assimilation rate in $\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), E (transpiration rate in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), g_{sw} (stomatal conductance to water vapor $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and WUE (instantaneous water use efficiency as A_{NET}/E). Population origin (provenance) had the most influence over study means of E , g_{sw} and WUE. Northern hard maple populations (both within and among species) showed delayed physical response to drought treatments, relative to southern hard maples, under the dry-down/rebound treatment. How best to deploy these species as genetic resources is discussed in response to the predicted climatic transition in the northeastern portion of the range for sugar maple, the most commercially valuable member of the group.

INTRODUCTION

Foundational research in sugar maple behavior began in the mid-1950s, initiated by Howard Kriebel, at the Yale School of Forestry. Kriebel built on his doctoral research throughout his career at Ohio State University. Kriebel set up research plots containing 18 provenance sources from throughout the native range of sugar maple at the Secret Arboretum in Wooster, Ohio. After planting the sourced plant material, Kriebel observed the adaptive capacity of the trees to Ohio's climate over three decades (Kriebel 1957, Kriebel and Wang 1962, Kriebel 1963, Kriebel and Gabriel 1969, Kriebel 1976, Kriebel 1981, Kriebel 1989, Kriebel 1990). Kriebel recognized that seedlings originating from hotter/drier climates displayed a higher level of drought tolerance than did those from cooler/wetter climates (Kriebel 1963). Drought resistance manifested as trees from drier regions being better adapted to xeric soils, with trees from humid areas being less adapted to the same conditions. Kriebel (1957, page 51) stated that, "... variation over the entire species may be grouped into three major geographic races or ecotypes, each containing parallel clinal variation which are part of a continental pattern." He describes three ecotypes: 1) Northern hardwood ecotype (*A. saccharum* and *A. nigrum*) with low genetic resistance to drought, sensitive to high insulation, and winter hardy. In the installations in Wooster, Ohio trees of northern provenance showed vulnerability to frosts occurring in late spring; 2) Central ecotype is represented by subspecies *saccharum* and subspecies *nigrum*. The group is characterized by moderately high drought and heat damage, with resistance "increasing in a continuous trend from east to west" (Kriebel 1957, page 148). These trees are not susceptible to winter injury and display a high level of apical dominance; 3) Southern ecotype (subspecies *floridanum* incl. *barbatum*) is described as drought and heat

tolerant with low tolerance to winter injury, with less than desirable tree form resulting from repeated forking of main and lateral shoots. Black maple, *A. nigrum*, is assumed the closest ally of the northern sugar maple (Skepner and Krane 1997). Previous research suggests the taxa do not warrant species-level designation, given the amount of genetic similarity between them (Skepner et al. 1998), but black maple has been described as more drought tolerant than sugar maple (Graves 1994, Hauer 1995) and selections have been made for increased drought tolerance and improved tree form (Kriebel 1957, Heard 1975). Kriebel's initial work was robust, but did not include *A. barbatum* or *A. leucoderme*, two of the hard maple species native to the southeastern U.S. (Kriebel 1957). Popular literature attributes drought tolerance to both species, but *A. leucoderme* occupies an ecological niche very different from that of *A. barbatum* (Dirr 1990). There is a dearth of research on these latter species, providing an opportunity for researchers to help flesh out the full range of hard maple ecology.

Successful genetic crosses are reported between *A. saccharum* and *A. nigrum* (Gabriel 1973, 1990), *A. saccharum* and *A. leucoderme* (Slavin 1950, 1954), and *A. saccharum* and *A. barbatum* (Jones 1990), which have proven useful for improving nursery stock aesthetics, agricultural yield, and timber quality. Still missing from marketing preferences, however, is any development of improving hard maples in overall tolerance to pressures generated by the current trajectory of changing climates. Investigating adaptive traits underlying the persistence of southern hard maples could lay the groundwork for northern species improvement programs that are focused on the development of climatically more resilient species. The immediate question becomes whether significant variation exists in terms of drought response among hard maple

populations. To inform this question I conducted a greenhouse drought screen of select hard maple populations to quantify and analyze several eco-physiological parameters linked to water conservation. The study has two goals: (1) to determine whether differential trait responses to drought are present among northern, western and southern populations of hard maples, and (2) to analyze the biological significance of any differential trait response that may exist. Test population data will offer a current baseline for the northern, more commonly known species, while laying the groundwork for future studies focused on the behavioral drought responses of less studied southern and western species.

MATERIALS AND METHODS

A germplasm collection targeting range extremes of hard maple species was carried out from 2011-2013 (Figure 2.1). One and/or two-year-old seedlings of each provenance / species were purchased, in bare root condition, and shipped to Cook Campus at Rutgers University in New Brunswick, New Jersey (Figure 2.2). Niche characteristics for individual study populations of latitude, longitude, elevation, primary water source, and soil texture characteristics are summarized in Table 2.2. Study populations were assigned a “niche score” to reflect provenance differences. Northern study trees of *Acer saccharum* Marsh seedlings were purchased from Alpha Nurseries, located in Holland, Michigan and Saratoga Tree Nursery in Saratoga Springs, New York. Study seedlings of *Acer nigrum* Michx were purchased from Bailey Nurseries in Newport, Minnesota. Western plant material for *Acer grandidentatum* Nutt was purchased during a field sampling campaign from SFA Gardens in Nacogdoches, Texas. Southern species *Acer leucoderme* Small and *Acer barbatum* Michx, f. populations were bought from Superior Trees Inc., Lee Florida. *Acer leucoderme* of South Carolina provenance was bought from Nearly Native Nurseries

in North Augusta, South Carolina (Figure 2.1). Seedlings were potted in gallon #1 containers, using Fafard potting medium, and were kept for 3 years of acclimatization. Trees were fertilized with (2 teaspoons per container) of Osmocote (15:15:10) slow release fertilizer. Plants were overwintered the first year in a plastic cold frame. Prior to the study trials, plants were placed in an outdoor area and hand irrigated until defoliation had occurred and dormancy had taken hold (Figure 2.2). Trees were shifted into #5 squat containers (A.M. Leonard) at the end of the second year and potted in a customized mix provided by Frye Brothers, INC. (Quarryville, PA). Trees were pruned to a standard height of 61 cm. Selection for drought trials was based on observed caliper size of the main stem and branch number to establish as much biomass uniformity as possible among selected study trees. Prior to the initiation of study treatments, all potted trees were soaked in a plastic pool for 5 minutes to ensure that all planting mix had been thoroughly irrigated and left to drain for 48 hours. Throughout the study, pots were routinely scouted for pests and debris.

Experimental design was a randomized plot, developed for a classic greenhouse screen, to quantify drought responses. Forty-five potted individuals (of unknown parentage) from each study population were randomly selected and assigned ID codes by state and species (Figure 2.4). Three water regimes were distributed among nine plots (3 plots per treatment): Control, DDR (dry down/rebound), and TDD (total dry down). Five randomly selected trees from each of the 9 study populations were placed within each plot ($n = 45$ per plot). Individuals not selected for the study were placed on perimeter benches of the greenhouse to reduce boundary effects (Figures 2.5 and 2.6). As a note for future reference, individual tree specimens were labeled and tracked, since genetic tissue samples

were collected and used in a genotype analysis that is discussed in Chapter 4. Greenhouse conditions were set to simulate environmental conditions of constant summer. Photoperiod was set for day length of 16 hours, simulating day length at 48° latitude for July in the northern mid-west region of the United States. Supplemental lighting was activated when daytime light intensity went below 1000 PAR. Temperatures in the greenhouse space were set for 24 ° C during the day and 18 ° C at night. Relative humidity was regulated at 45%. Due to recognized limitations of labor and timing of gas-exchange measurements, two studies were conducted over a two-year period. Greenhouse conditions were consistent across studies to minimize error in statistical treatments. Least squares model analysis of variance for the two studies did show significant differences in variable response between the single-year studies, but since greenhouse conditions were at the same settings for both trials, study year was left out of the model. Populations were assigned to one of the two studies, based on northern or southern provenance. Two populations of *A. saccharum* from Michigan and New York, *A. nigrum* from Minnesota, and a western population of *A. grandidentatum* from Texas made up one study cohort (March 2014). One population each of Alabama *A. barbatum* and *A. leucoderme*, Florida *A. barbatum* and *A. leucoderme*, and one population of South Carolina *A. leucoderme* comprise the other (August 2014). Study methods are adapted from Bauerle et al. (2003), who examined ecophysiology of *Acer rubrum* L. seedlings from contrasting hydrological habitats, using water regimes similar to those described here, to observe the impact of dry-down and dry-down/rebound on plant processes associated with maintaining water balance. This study uses a modified version of their experimental design and protocol to examine water relations of hard maple study groups (Bauerle et al. 2003). Water treatments are described as follows:

1. *Control treatment (CONT)*: Initial mass data were recorded after pots were subjected to a heavy soak, but prior to the first gas exchange measurements. Pots were weighed every three (3) days to monitor changes in mass. The goal was to avoid water stress entirely for these plots. Control plots were watered as individual study trees reached 80-90% of their initial recorded mass. When study pots approached the targeted percentage of the initial mass, pots were irrigated by filling each pot to the top with water twice, letting the water drain in between each water application. This protocol for watering control plots was carried out for the duration of the study.
2. *Dry down/Rebound treatment (DDR)*: To simulate short-term drought conditions in the soil environment of the pots, time between water applications was extended. Plots receiving intermittent water were irrigated as individual pots approached 65-70% of their initially recorded mass, prior to the first gas exchange measurements. The DDR treatment was applied in two cycles during the study.
3. *Total dry down Treatment (TDD)*: To simulate long-term drought conditions, water was withheld from study individuals until they reached 45-55% of the initially recorded mass, prior to the first gas-exchange measurements.

All plants were then watered during week 5 of the 6-week northern study and week 6 of the 7-week southern study. Boundary plants were placed on the study bench perimeters to reduce potential boundary effects associated with circulation fans. To establish a benchmark for the onset of drought-like soil conditions, pre-dawn water potential was calibrated using a Scholander Pressure Bomb (Scholander et al. 1964, Scholander et al. 1965). Measurements were taken from five (5) CONT, DDR, and TDD trees of each study population. Excised leaves were placed in the leaf pressure chamber for measurement

under predawn conditions, to avoid the interference of plant processes triggered by daylight and measured to the nearest 0.1 bar. Each tree was weighed, and mass and water potential values were calibrated so that the mass of the potted trees could be used to infer the presence of drought conditions (Table 2). Mass data were recorded in kilograms for each potted tree (before being measured), using a Toledo United Balance scale (Mettler-Toledo, LLC, Columbus, OH).

Gas exchange measurements

Snapshot measurements of A_{NET} , g_{sw} , E , and WUE were recorded (Figure 2.7) each week for seven weeks for northern cohort trees and six weeks for southern cohort trees, using the infrared gas analyzer leaf chamber of a LI-COR 6400XL Portable Photosynthesis System (LI-COR, INC., Lincoln, Nebraska). Leaf area measured by the chamber was set at 6 cm². Environmental controls for the leaf chamber held CO₂ reference concentration at 450 ppm, typical of atmospheric levels (Bauerle et al. 2003). Leaf temperature in the chamber was set for 20-25 °C. Relative humidity was monitored by adjusting the desiccant valve, as needed, but held well within the desired range of 45 - 55%. Plant stress responses are dependent on the length and intensity of the stress event (Hinckley et al. 1979). Stomata are a plant's immediate link to conditions of the local environment and stomatal response to environmental modifications can limit or regulate gas exchange activities (Touchette et al. 2007). In terms of drought response, stomatal aperture is a key physical regulator of stomatal conductance to water vapor (g_{sw}), transpiration rate (E), and the flux density uptake of atmospheric carbon dioxide (Farquhar and Sharkey 1982). Snapshot measurements of plant behavioral drought response can be measured and analyzed to determine the variation in trait response, both within and among species. In sensitive

species, mild drought stress triggers the decrease of stomatal aperture on the leaf surface, resulting in the minimization of stomatal gas exchange. Stomatal conductance rates, transpiration rates, and the rate of CO₂ uptake decrease until water is available and the stress impacts are released. Severe or chronic drought events lead to extended periods of stomatal closure and photosynthetic processes associated with stomatal gas exchange are negatively impacted. Under prolonged conditions of stomatal closure, CO₂ concentrations in leaf chloroplasts become reduced enzymatic activity of Rubisco decreases, and O₂ concentrations increase (Farquhar et al. 1980). As the [CO₂]/[O₂] ratio becomes smaller, the assimilation rate of atmospheric CO₂ (A_{NET}) decreases and elevated concentration levels of O₂ enable its interaction with the CO₂ loving Rubisco, catalyzing the energy wasteful process of photorespiration. It has been suggested that characteristics and management of Rubisco are not well-suited for biomass production in current and projected future environments, because of its high energy demand (Carmo-Silva et al. 2015).

Water use efficiency (WUE) describes the relationship between the net rate of carbon assimilation for each unit of water lost via transpiration from plant leaves, and it has been used to determine species capacity to maintain water balance under water stress (Medrano et al. 2015). Each of these parameters are calculated in the LI-6400XT Portable Photosynthesis System manufactured by LI-COR, INC (Lincoln, NE.) and so were used in this study to determine differential trait response to varying water regimes among study populations. These parameters are useful in assessing whether the pathway to stress response is one of biochemical or biomechanical nature. Biosynthetically, plants can regulate chemical pathways to influence mechanical response. In the case of biophysical response to drought stress, adjusting stomatal openings in the leaf surface, via osmotic

adjustment of plant guard cells (Huang 2016). Periods of long-term stress call for a more intricate approach to maintaining plant water balance (Touchette et al. 2007).

Study parameters explanations and equations

Stomatal conductance to water vapor (g_{sw}): Stomatal conductance to water vapor accounts for total conductance to water vapor minus leaf boundary layer conductance to water vapor and is measured in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$. The equation for g_{sw} as measured by the LI-6400XT is:

$$g_{sw} = \left[\frac{1}{q_{tw}} - \frac{k_f}{q_{hw}} \right]^{-1} \quad \text{Equation 2.1}$$

where k_f is the factor based on the stomatal ratio fraction estimate K , which is the relationship

$$k_f = \frac{K^2 + 1}{(K + 1)^2} \quad \text{Equation 2.2}$$

between stomatal densities of abaxial and adaxial leaf sides, g_{tw} is total stomatal conductance to water vapor, and g_{bw} is boundary layer conductance to water vapor. Under drought stress conditions, g_{sw} is expected to decrease as stomata close, conserving plant moisture.

Transpiration rate (E): Surface area of leaf tissue being measured, and transpiration rate are directly linked, since water vaporizes through many stomata on the leaf surface. It is reasonable to expect a decrease in transpiration rate as soil moisture decreases, stomatal aperture becomes reduced, and moisture concentration of the air passing through the leaf chamber becomes reduced. The equation for transpiration rate calculated by the LI-6400XT is:

$$E = \frac{F(Ws - Wr)}{100S(1000 - Ws)} \quad \text{Equation 2.3}$$

where air flow rate is F measured in $\mu\text{mol s}^{-1}$, sample and reference water mole fractions Ws and Wr in $\text{mol H}_2\text{O (mole air)}^{-1}$, and S is measured leaf area.

Net photosynthesis (A_{NET}): Under plant drought stress, stomatal aperture is reduced and the resulting decrease in CO_2 uptake can lead to a deficit in available intercellular concentrations of carbon dioxide for assimilation. The response can be measured by the LI-6400XT open system as

$$A = \frac{F (Cr - Cs) \left(\frac{1000 - Wr}{1000 - Ws} \right)}{100S} \quad \text{Equation 2.4}$$

where F is the air flow rate, measured in $\mu\text{mol m}^{-2}\text{s}^{-1}$, Cr and Cs are sample and reference CO_2 , Wr and Ws are reference and sample fraction of water in $\text{mmol H}_2\text{O (mole air)}^{-1}$, and S is measured leaf area. A is measured in $\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$.

Water use efficiency (WUE): For the purposes of this study, WUE is measured as the ratio of carbon assimilation rate to that of transpiration, or A/E . Previous studies have shown that plants with higher WUE values are better adapted to drought stress (Medrano et al. 2015). Overall WUE values may infer differential trait response to water deficits in study trees at the population level.

For gas exchange measurements, the first mature leaf on current year's growth was selected on each study tree. Measured leaves were marked with flagging tape to enable easy identification and for ongoing study measurements. All 180 individuals were measured and weighed once, between the hours of 8 AM. and solar noon. Temporal records were maintained, as the measurement process took three successive days per cycle, to maintain the measurement series within the targeted measurement hours.

Data analyses

To establish provenance as an indicator of behavioral response to drought for hard maple populations, I analyzed population mean response of stomatal conductance to water vapor in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (g_{sw}), carbon assimilation rate in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (A_{NET}), transpiration rate in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (E), and water use efficiency (WUE) at three treatment levels, using standard least squares analysis of variance in JMP® Pro15 (JMP® 1989-2007). Overall significance of variation of population means for all study variables was evaluated with ANOVA (Table 2.3). Variation in population mean response was found significantly different for all study variables across all study treatments ($P < 0.0001$). Effects tests in the least squares platform calculated the leverage of fixed effects population, treatment, and their interaction to gauge their effects on mean response of study groups. A large population effect on parameter means was significant and indicated a strong link between population origin and behavioral responses to study treatments. Differences in mean behavioral response for study groups were determined by connected letter reports generated from Tukey HSD *post hoc* tests ($P < 0.05$).

RESULTS

Treatment effect

Dry down/rebound (DDR) treatment means (0.18) for WUE were significantly lower than for other treatments. Treatment means for A_{NET} were significantly lower for total dry down trees ($2.64 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$). Treatment means for stomatal conductance rate (g_{sw}) and transpiration rate were not significantly different, according to *post hoc* tests. Treatment mean values are summarized in Table 2.4.

Population Effect

Population mean differences were defined by Tukey HSD tests. Groups assigned different letters had significantly different group means. Patterns of variation observed for g_{sw} , A_{NET} , E and WUE ($P < 0.05$) are described below and presented in Table 2.5. Water use efficiency separated into two significantly different groups, basically northern populations (including Texas *A. grandidentatum*) and southern populations. Significantly different WUE values sorted study populations into the following groups (populations are listed from highest to lowest estimates of WUE):

- Group A: Michigan and New York *A. saccharum*, Minnesota *A. nigrum* and Texas *A. grandidentatum*
- Group B: Florida *A. barbatum*, *A. leucoderme*, Alabama *A. barbatum* and *A. leucoderme*, and South Carolina *A. leucoderme*

Northern populations had higher WUE values than did southern populations, indicating northern hard maples maintain higher rates of carbon assimilation for the amount of water lost through transpiration than do southern hard maples.

Stomatal conductance rate was more variable between study populations and defined by three significantly different groups. Groups in highest to lowest response means were:

- Group A: New York *A. saccharum*
- Group B: Michigan *A. saccharum*, Minnesota *A. nigrum*, and Texas *A. grandidentatum*
- Group C: All five (5) southern populations (no significant difference between groups).

Northern study populations maintained higher rate of stomal conductance than did southern populations, which is also reflected in their observed WUE estimates.

Pairwise tests of carbon assimilation and transpiration rate yielded five (5) significantly different groupings for each variable. Study populations, sorted from highest to lowest, are listed as follows for both carbon assimilation and transpiration rate:

- Group A New York *A. saccharum*
- Group B (from highest to lowest): Michigan *A. saccharum*, Minnesota *A. nigrum*, and Texas *A. grandidentatum*
- Group C was Alabama *A. barbatum*
- Group CD (from highest to lowest): Alabama *A. leucoderme*, Florida *A. barbatum* and *A. leucoderme*
- Group D: South Carolina *A. leucoderme*

Overall results suggest northern study trees function at higher conductance and transpiration rates than do southern study trees. Considering higher WUE calculated for northern populations, it appears that northern trees may trade water lost through transpiration for increasing plant metabolism.

Interaction effect of treatment and population

Table 2.6 summarizes *post hoc* results of effect tests for leverage of fixed effect interaction term Treatment x Population on study variables. The interaction of these effects presented a higher level of variation between treatments and among study populations. Test results were plotted in Figure 2.1 for visualization and clarity of test outcomes. Results showed a large population effect, indicating a strong link between provenance and mean response across all study variables ($P < 0.001$). Treatment effect was present, but not as

strong as the population effects, indicated by a lower F Ratio. Treatment effect was significant for all study variables except for g_{sw} . The interaction term effect was also seen, but with less leverage (←?) (F Ratio) than treatment effect. The interaction term did not significantly affect g_{sw} but had a significant effect on all other study variables.

Percent change in dry down/rebound population means for study variables

The Dry down/Rebound treatment was comprised of two dry down cycles to observe population response to repeat short-term drought events. Average percent changes in A_{NET} , g_{sw} , and E were calculated for populations under the DDR regime from initial (pre-stress) measurements to the end of the first dry down/rebound cycle (DDR1) and from the end of the first cycle to the end of the second (DDR2). Table 2.7 (northern populations) and 2.8 (southern populations) summarize percent changes in population mean response for all study variables under the DDR treatment. Percentages with negative values represent a decrease in rate response and positive values are increases. Water use efficiency was not used for this comparison and will be considered in Chapter 4.

DDR1 percent change in study variables

By the end of the first DDR cycle (DDR1) northern and southern groups showed differences in increased and decreased response rates. The study variable with the largest percent change between northern and southern populations was g_{sw} . Northern groups averaged a 10% increase in g_{sw} . Texas *A. grandidentatum* was the only northern group to show a decrease (6%). Conversely, southern groups reduced stomatal conductance by 38% overall. Alabama *A. barbatum* was the only southern population to increase g_{sw} (42%) by the end of DDR1. Florida *A. barbatum* and *A. leucoderme* showed a large decrease (more than 95%) in g_{sw} by the end of DDR1. The smallest percent change difference between

northern and southern groups was for carbon assimilation rate. Both groups increased A_{NET} by averages of 14% for the northern and 10% for the southern group. Carbon assimilation rate response increased by the end of DDR1 for Michigan and New York *A. saccharum* and Minnesota *A. nigrum*. A small decrease in A_{NET} of 1% was seen in Texas *A. grandidentatum*. Two southern populations reduced A_{NET} : Alabama *A. barbatum* (9%) and South Carolina *A. leucoderme* (34%). Three southern populations increased A_{NET} : Alabama *A. leucoderme* (12%) Florida *A. barbatum* (33%) and *A. leucoderme* (48%). Transpiration rate (E) increased 16 - 44% for northern groups and Texas *A. grandidentatum* by the end of DDR1, while there was a 52-73% decrease in E for all southern populations, except South Carolina *A. leucoderme*, which had an 11% increase in E for this cycle.

DDR2 percent change in study variables

Stomatal conductance rate increased for all groups by the end of DDR2, except for South Carolina *A. leucoderme*. Northern hard maples increased g_{sw} by 24% and southern groups increased g_{sw} by 42% overall. Carbon assimilation rate was held to an average zero percent (0%) change for northern groups, but Texas *A. grandidentatum* did show a reduction in A_{NET} of 23%. New York *A. saccharum* and Minnesota *A. nigrum* showed little to no change in A_{NET} for this cycle. Two southern populations decreased A_{NET} : Florida *A. leucoderme* (14%) South Carolina *A. leucoderme* (40%). Three southern populations increased A_{NET} : Alabama *A. barbatum* (6%) Alabama *A. leucoderme* (68%), and Florida *A. barbatum* (26%). Average percent change for A_{NET} overall was 9% for southern populations. All northern groups reduced transpiration rates by the end of DDR2. New York *A. saccharum* had the largest decrease (73%), while Michigan York *A. saccharum* had the smallest

(21%). Transpiration rates increased for all southern groups, except South Carolina *A. leucoderme*, which decreased this variable by 27%.

These results show that northern trees did not increase carbon assimilation and transpiration rates as much as did southern populations, overall (average of DDR1 and DDR2). Northern trees had a higher increase than did southern trees, in terms of stomatal conductance rate as treatments progressed and drought intensified. These results also support the earlier response to water treatments shown by southern trees, as they reduced conductance rates by the end of DDR1, where northern trees did not exhibit this behavior until the end of DDR2.

DISCUSSION

Hard maple species are reported to persist within distinct ecological zones (Burns et al. 1990, van Gelderen et al. 1994) and variation has been described between members in terms of morphology, physiology, and genetics (Dansereau et al. 1947, Desmarais 1952, Kriebel 1957, Graves 1994, St. Hilaire et al. 1998). Kriebel (1953) describes at least two kinds of drought tolerant sugar maples: (1) populations from central and southern regions of the native range, displaying more drought tolerance, and (2) more drought susceptible trees from the “northern hardwood region.” He suggested that variation in local soil moisture was the reason for this difference, being less recognizable than other features, such as leaf scorching. Variation of intraspecific site requirements for water are exhibited when comparing drought response for study populations that Kriebel describes as “northern hardwood region” drought susceptible trees, represented in this study by New York and Michigan *A. saccharum* study populations. The response patterns of current study populations suggest, however, that under conditions of water stress, plant origin appears to

be a more accurate indicator of response than does nominal ‘ecotype’. New York consistently showed higher levels of productivity than all other study groups for the well-watered treatment and was able to rebound from stress treatments (DDR1, DDR2 and TDD), to pre-stress productive rates. Water use efficiency was highest for the New York *A. saccharum*, but lowest for Michigan *A. saccharum*. As contrasting as these responses are, it is important to consider provenance of seed stock when selection and breeding strategies are proposed.

Elevation and aspect may also influence drought response within species (Griscom et al. 2011, Miller et al. 2013). Mountain populations of *A. grandidentatum* are designated *Acer grandidentatum* Nutt. var. *grandidentatum* (Bigtooth Maple). Trees in this montane environment rely on precipitation events as a primary water source. Canyon Maple (*Acer grandidentatum* Nutt. var. *sinuosum* Rehd.) populations, found at lower elevations, exploit water availability along riverbanks and streams. Since these varieties acquire moisture differently, it is likely that plant water management strategies are also variable (Alder et al. 1996, Fahey et al. 2013). Observations of drought tolerance in *Acer nigrum* are often mentioned in the literature, specifically relative to *Acer saccharum* (Gabriel 1990, Graves 1994). Minnesota *A. nigrum* did not perform as expected, logging lower rates of A_{NET} , E , and g_{sw} across all treatments; these study trees, on average, responded much like *A. saccharum* trees from Michigan under the less intense drought (DDR1/DDR2) and CONT study routines. It is interesting, however, that the *A. nigrum* study group showed less % change to water treatments overall than all other hard maple study groups. WUE values for *A. nigrum* did not exceed those of the other study groups until after the drought release at the end of the TDD treatment. It is possible that a lack of response is itself a manifestation

of stress tolerance or that drought response is not triggered until the stress is intensified, either in intensity or longevity (Touchette et al. 2007).

Southern study populations are not necessarily representative of all southern hard maples, but environmental growing conditions throughout the south are the same for these trees. Southern hard maple species are native to areas where the growth season is characterized as being longer than the northern growing season, with higher amounts of annual precipitation and higher temperatures. The onset of drought treatments showed southern study species logging similar response rates to their northern relatives. As drought treatments increased with intensity and duration, southern species showed earlier fluctuations in response rates, coinciding with advancing drought treatments, suggesting that southern populations are better adapted and have modified systems of growth and development, enabling the ability to thrive under conditions of reduced water availability. Because this study is the first of its kind to analyze this behavior in southern hard maple species, the data can be considered a baseline for further physiological study of southern hard maples. Results from the studies reviewed above have highlighted the role of drought adaptation in plant performance under stress, supporting consideration of provenance when targeting plant selection criteria and genetic resources for breeding programs. The determination of physiological parameters at the provenance and population levels, as a normal part of the species improvement program, are in order.

CONCLUSIONS

Population behavior profiles over time were different, yet presumed typical, for all study populations under the control treatment, which was accepted as a baseline for behavioral differences. Divergence in behavioral responses did increase over the term of the study for the DDR treatment trees. Differential response was most apparent for treatments where water was provided in weeks 4 and 6 of the study, eliciting divergence in rebound and recovery potential, after induced water stress. Out of the four study populations, only the New York *A. saccharum* and Texas *A. grandidentatum* were able to recover to pre-stress conditions of A_{NET} , E , g_{sw} , and WUE. Differences within species in terms of trait response to water stress were also observed. For all populations in this study, divergence in stress responses intensified after week 4 or 5, consistent with the expectation that as drought periods extend, plants are more affected. These data are consistent with findings of other studies that show differential plant response to short or long-term stress events (Touchette et al. 2007, Marchin et al. 2010).

In the past, research focused on sugar maple allies has favored northern and western populations, leaving a void in the literature regarding the southern relatives. Expanding the scope of hard maple research is necessary to better understand the full scale of variation within the hard maple species complex. Southern hard maples adapt to different set of environmental conditions than their northern/western counterparts, but investigations attempting to quantify this adaptive capacity have not been conducted. Study results discussed here have helped establish a current baseline for stomatal drought response in hard maple species, comparing traditionally studied hard maples and those previously overlooked in the literature. Gas-exchange methods establish a baseline for differential

stomatal (mechanical/physical) response to water variability in plants. It has been suggested that characteristics and management of Rubisco are not well-suited for biomass production in current and projected future environments, because of its high energy demand (Carmo-Silva et al. 2015). Investigating non-stomatal/biochemical aspects of hard maple species response under conditions of variable water limitation would further hard maple research and provide more information in terms of rate limiting conditions of drought in sugar maple. Time is not on the side of *A. saccharum*, in terms of the rapidly changing climates predicted for the northeastern United States and adjacent Canada. Provenance selection may provide a transitory solution for a sugar maple industry in jeopardy, while pursuing development of longer-term solutions, buying us some time for a longer-term breeding program solution.

Hard maple research has implications beyond breeding programs and plant selection. A decline in the general health of sugar maple populations has taken its toll on the US maple sugar industry since the 1950s. Sugar maple's ongoing vulnerability to biotic (pathogens and pests) and abiotic (i.e., drought and increasing temperatures) stresses have contributed to ongoing decreases in species health, leading to population decline in the United States and Canada (Lachance et al. 1995, Jarvi 2015). Unfortunately, ongoing climate change is unlikely to improve the industry's prospects. Current models predict a further reduction and/or relocation of suitable habitat for *A. saccharum* over the next 40 years (Prasad et al. 2007). The present forested landscape of the northeastern US is expected to experience significant changes in species composition by the year 2100 (Iverson et al. 2005). The current habitat is fit for the maple-beech-birch forest type but is expected to shift toward the oak-hickory type. Sensitive species, like sugar maple, are

expected to fail, due to a lack of adaptive traits needed to face changing circumstances (Aitken et al. 2008). The discourse surrounding this issue involves human-assisted migration to a more suitable habitat; in the case of northern sugar maple, this would mean into Canada. The existence of climatic limits of sugar maple need to be addressed and understood, even as we explore our future options.

Table 2.1. List of hard maple study populations, their provenance, and suppliers.

Population	Provenance	Supplier
<i>Acer saccharum</i> Marshall (Sugar Maple)	Central Michigan	Alpha Nurseries Holland, Michigan
<i>Acer saccharum</i> Marshall (Sugar Maple)	New York (southern)	Saratoga Tree Nursery Saratoga Springs, New York
<i>Acer nigrum</i> Michaux, f. (Black Maple)	Newport, Minnesota	Bailey Nurseries Newport, Minnesota
<i>Acer grandidentatum</i> Nuttall (Canyon Maple)	Western Texas	SFA Gardens Nacogdoches, Texas
<i>Acer barbatum</i> Michx (Southern Sugar Maple)	Alabama	Superior Trees Lee, Florida
<i>Acer barbatum</i> Michx (Southern Sugar Maple)	Northern Florida	Superior Trees Lee, Florida
<i>Acer leucoderme</i> Small (Chalkbark Maple)	Alabama	Superior Trees Lee, Florida
<i>Acer leucoderme</i> Small (Chalkbark Maple)	Northern Florida	Superior Trees Lee, Florida
<i>Acer leucoderme</i> Small (Chalkbark Maple)	South Carolina	Nearly Native Nursery North Augusta, South Carolina

Table 2.2. Summary of study population provenance location, elevation, water source, soils, and temperature.

Study Population	Provenance	Lat. (°)	Long. (°)	Elevation (m)	Water Source/Mean annual precipitation (mm)	Soil Texture	Heat Zone
<i>Acer nigrum</i>	Washington County, Minnesota/Wisconsin border	45.04 N	92.89 W	309	Annual rainfall 889	upland soils formed in loamy glacial till, well to poorly drained and level to steep	4-5
<i>Acer saccharum</i>	Saratoga County, east coast of New York	43.08 N	73.78 W	93	Annual rainfall 1118	sloping to steep, well-drained, medium textured soils	4-5
<i>Acer saccharum</i>	Allegan/Ottawa County line, west coast of Michigan	42.69 N	86.18 W	202	Annual rainfall 1026	well to poorly drained loamy soils across all existing topography	4
<i>Acer leucoderme</i>	Savannah River Bluffs Heritage Preserve, Georgia/South Carolina central border	33.53 N	81.99 W	200	River/Annual rainfall 1332	Sandy soils of the coastal alluvial plain	8-9
<i>Acer barbatum</i> <i>Acer leucoderme</i>	Wilcox County, south central Alabama	32.01 N	87.34 W	61	Annual rainfall 1237	Coastal plain uplands-shallow to deep, loamy and clayey sediments, claystone, and shale, well to moderately well drained variable drainage can be sandy throughout with some loamy lamellae	9
<i>Acer barbatum</i> <i>Acer leucoderme</i>	Madison County, north central Florida	30.45 N	83.50 W	36	Annual rainfall 1372		10
<i>Acer grandidentatum</i>	Texas Hill Country, central west Texas, Lost Maples Natural Area	29.81 N	99.57 W	549-686	River/Annual rainfall 40-50	Nearly level to gently sloping, deep loamy and clay soils	10-11

Figure 2.1. Little's range maps were used to determine target areas for germplasm collection. Hard maple study populations listed in Table 2.1 were collected from areas indicated by red arrows (Little 1971, USDA 2016).

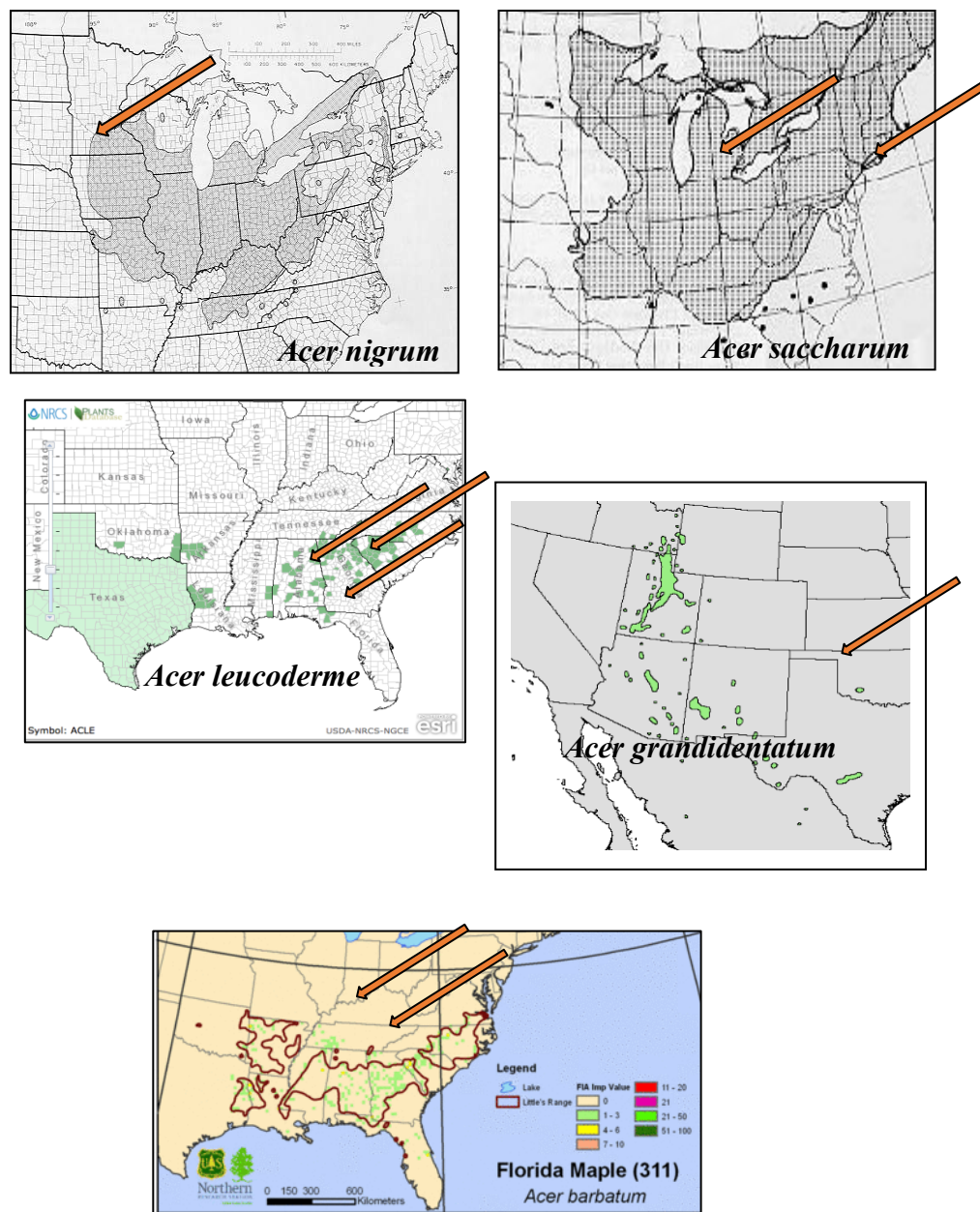


Table 2.3. Whole model analysis of variance (ANOVA) results for population means response of drought study variables: WUE (water use efficiency), A_{NET} (carbon assimilation rate), g_{sw} (rate of stomatal conductance), and E (transpiration rate) and fixed effects of Treatment, Population, and their interaction. The table summarizes Variable, Source (of variation), DF (degrees of freedom), Sum of Squares, Mean Square, F Ratio, and Prob>F.

Variable	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
WUE	Model	26	38.02	1.462	21.2905	<.0001
	Error	2067	141.95	0.069		
	C. Total	2093	179.97			
A_{NET}	Model	26	3512.85	135.109	34.5794	<.0001
	Error	2067	8076.25	3.907		
	C. Total	2093	11589.10			
g_{sw}	Model	26	1.51	0.058	54.5837	<.0001
	Error	2067	2.20	0.001		
	C. Total	2093	3.71			
E	Model	26	1.44E-04	5.52E-06	33.1189	<.0001
	Error	2067	3.44E-04	1.67E-07		
	C. Total	2093	4.88E-04			

Table 2.4. Tukey-Kramer HSD post hoc test shows significance of treatment effect on overall study means for water use efficiency (WUE), carbon assimilation rate in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (A_{NET}), stomatal conductance to water vapor in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (g_{sw}), and transpiration rate in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (E). Populations linked by the same letters do not have significantly different mean response. Significant differences between water treatments are defined by letters. Groups not sharing the same letter are significantly different. Treatment codes are: CONT (control, well-watered), DDR (dry down/rebound), TDD (total dry down).

Parameter	Treatment	Letter	Mean	Std Error
WUE	TDD	A	0.31	3.05E-02
	CONT	A	0.29	2.99E-02
	DDR	B	0.18	2.89E-02
A_{NET} ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	DDR	A	3.90	2.18E-01
	CONT	A	3.88	2.25E-01
	TDD	B	2.64	2.30E-01
g_{sw} ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	CONT	A	0.037	3.72E-03
	DDR	A	0.036	3.60E-03
	TDD	A	0.028	3.79E-03
E ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$)	DDR	A	7.43 E-04	4.51E-05
	CONT	A	7.38E-04	4.65E-05
	TDD	A	5.9E-04	4.74E-05

Table 2.5. Tukey's HSD test shows significance of population effect on overall study parameter means for water treatments: CONT (control), DDR (dry down/rebound) and TDD (total dry down). Groups not sharing a common letter are significantly different for study parameters: stomatal conductance of water vapor in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (g_{sw}), carbon assimilation rate in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (A_{NET}), transpiration rate in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (E). Population codes in order: Ab= Alabama *A. barbatum*, Al = *A. leucoderme*, Fb = Florida *A. barbatum*, Fl = Florida *A. leucoderme*, Mis = Michigan *A. saccharum*, MNn = Minnesota *A. nigrum*, NYs = New York *A. saccharum*, SCl = South Carolina *A. leucoderme*, TXg = Texas *A. grandidentatum*.

Water use efficiency (WUE)				Stomatal Conductance Rate (g_{sw}) $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$			
Pop	Letters	Least Squares Mean	Std Error	Pop	Letters	Least Squares Mean	Std Error
MIIs	A	0.52	0.024	NYs	A	1.18E-01	3.65E-03
NYs	A	0.51	0.029	MIIs	B	8.52E-02	2.98E-03
MNn	A	0.48	0.026	MNn	B	8.45E-02	3.20E-03
TXg	A	0.47	0.027	TXg	B	8.24E-02	3.38E-03
Fb	B	0.33	0.03	Ab	C	3.74E-02	3.72E-03
Fl	B	0.32	0.035	Fl	C	3.47E-02	4.36E-03
Ab	B	0.29	0.03	Fb	C	3.29E-02	3.72E-03
Al	B	0.29	0.033	Al	C	3.03E-02	4.08E-03
SCl	B	0.26	0.04	SCl	C	2.33E-02	4.98E-03
Carbon Assimilation Rate (A_{NET}) $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$				Transpiration Rate (E) $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$			
Pop	Letters	Least Squares Mean	Std Error	Pop	Letters	Least Squares Mean	Std Error
NYs	A	7.68	0.22	NYs	A	1.54E-03	4.56E-05
MIIs	B	5.69	0.18	MNn	B	1.15E-03	4.00E-05
MNn	B	5.31	0.19	MIIs	B	1.12E-03	3.73E-05
TXg	B	5.04	0.2	TXg	B	1.08E-03	4.23E-05
Ab	C	3.88	0.23	Ab	C	7.38E-04	4.07E-05
Fb	CD	3.62	0.23	Fl	CD	6.84E-04	5.45E-05
Fl	CD	3.55	0.26	Fb	CD	6.39E-04	4.65E-05
Al	CD	3.49	0.25	Al	CD	5.86E-04	5.10E-05
SCl	D	2.66	0.3	SCl	D	4.53E-04	6.02E-05

Table 2.6. Test results for fixed effects of Treatment (control, dry down/rebound, total dry down), Population (Ab= Alabama *A. barbatum*, Al = *A. leucoderme*, Fb = Florida *A. barbatum*, Fl = Florida *A. leucoderme*, Mis = Michigan *A. saccharum*, MNn = Minnesota *A. nigrum*, NYs = New York *A. saccharum*, SCl = South Carolina *A. leucoderme*, TXg = Texas *A. grandidentatum*), and their interaction on water use efficiency (WUE), stomatal conductance of water vapor in $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ (g_{sw}), carbon assimilation rate in $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$ (A_{NET}), and transpiration rate in $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ (E), mean response for all study groups.

Variable	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
WUE	Treatment	2	0.83	0.41	6.01	0.003
	Population	8	7.01	0.88	12.76	<.0001
	Treatment*Population	16	3.38	0.21	3.08	<.0001
ANET	Treatment	2	78.89	39.44	10.1	<.0001
	Population	8	1356.50	169.56	43.4	<.0001
	Treatment*Population	16	343.94	21.49	5.5	<.0001
gsw	Treatment	2	0.00	0.00	1.48	0.227
	Population	8	0.69	0.09	80.41	<.0001
	Treatment*Population	16	0.11	0.01	6.7	<.0001
Trans	Treatment	2	1.10E-06	5.30E-07	3.19	0.041
	Population	8	7.12E-05	8.90E-06	53.42	<.0001
	Treatment*Population	16	2.94E-05	1.80E-06	11.03	<.0001

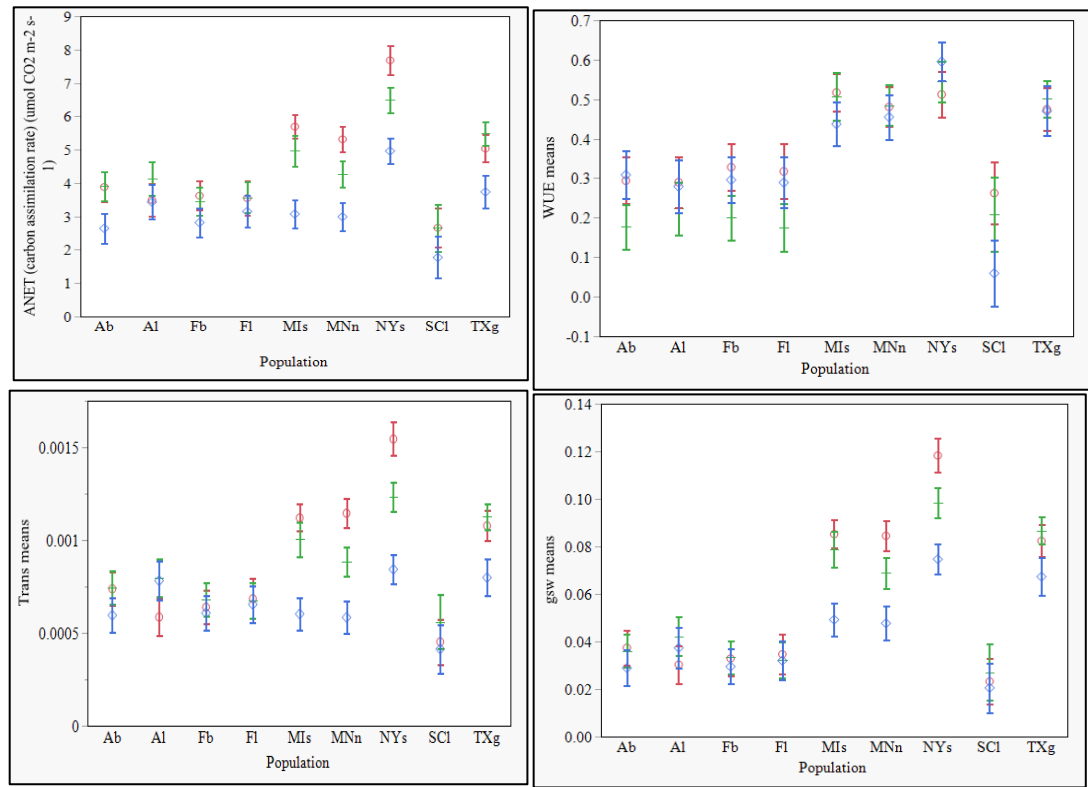


Figure 2.7a-d. Interaction plots of fixed effects of Population and Treatment for mean values and upper/lower confidence values of WUE (a), A_{NET} (b), g_{sw} (c), and Transportation rate (d) response of hard maple study groups. Mean values for study variables are along vertical axes. Population means are overlaid with colors to discern between treatments. Treatment codes are as follows: 1=Control (red), 2= Dry down/Rebound (green), 3= Total dry down (blue). Population codes are as follows: Ab= Alabama *A. barbatum*, Al = *A. leucoderme*, Fb = Florida *A. barbatum*, Fl = Florida *A. leucoderme*, Mis = Michigan *A. saccharum*, MNn = Minnesota *A. nigrum*, NYs = New York *A. saccharum*, SCl = South Carolina *A. leucoderme*, TXg = Texas *A. grandidentatum*.

Table 2.8. Average percent (%) change in northern population mean response for carbon assimilation rate in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (A_{NET}), stomatal conductance of water vapor in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (g_{sw}), transpiration rate in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (E), and water use efficiency (WUE) for all study populations. DDR1 shows percent change from pre-stress Week 1 until the end of the 1st dry down/recovery cycle (Week 4) in the DDR treatment. DDR2 values represent the percent change in mean response from the end of the 1st DDR dry down/recovery cycle until the end of the 2nd DDR dry down/recovery cycle.

Populations	DDR1	DDR2	Average % change
Carbon assimilation rate (A_{NET})			
Michigan <i>A. saccharum</i>	22%	25%	24%
Minnesota <i>A. nigrum</i>	20%	-2%	9%
New York <i>A. saccharum</i>	14%	0%	7%
Texas <i>A. grandidentatum</i>	-1%	-23%	-12%
Averages	14%	0%	7%
Stomatal conductance to water vapor (g_{sw})			
Michigan <i>A. saccharum</i>	11%	25%	18%
Minnesota <i>A. nigrum</i>	18%	28%	23%
New York <i>A. saccharum</i>	17%	21%	19%
Texas <i>A. grandidentatum</i>	-6%	23%	9%
Averages	10%	24%	17%
Transpiration rate (E)			
Michigan <i>A. saccharum</i>	44%	-21%	12%
Minnesota <i>A. nigrum</i>	25%	-27%	-1%
New York <i>A. saccharum</i>	16%	-73%	-29%
Texas <i>A. grandidentatum</i>	17%	-67%	-25%
Averages	26%	-47%	-11%

Table 2.9. Average percent (%) change in southern population mean response for carbon assimilation rate in $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (A_{NET}) stomatal conductance of water vapor in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (g_{sw}), transpiration rate in $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (E), and water use efficiency (WUE) for all study populations. DDR1 shows percent change from pre-stress Week 1 until the end of the 1st dry down/recovery cycle (Week 4) in the DDR treatment. DDR2 values represent the percent change in mean response from the end of the 1st DDR dry down/recovery cycle until the end of the 2nd DDR dry down/recovery cycle.

Populations	DDR1	DDR2	Average % change
Carbon assimilation rate (A_{NET})			
Alabama <i>A. barbatum</i>	-9%	6%	-2%
Alabama <i>A. leucoderme</i>	12%	68%	40%
Florida <i>A. barbatum</i>	33%	26%	30%
Florida <i>A. leucoderme</i>	48%	-14%	17%
South Carolina <i>A. leucoderme</i>	-34%	-40%	-37%
Averages	10%	9%	10%
Stomatal conductance to water vapor (g_{sw})			
Alabama <i>A. barbatum</i>	42%	19%	31%
Alabama <i>A. leucoderme</i>	-19%	151%	66%
Florida <i>A. barbatum</i>	-99%	39%	-30%
Florida <i>A. leucoderme</i>	-98%	19%	-40%
South Carolina <i>A. leucoderme</i>	-18%	-17%	-18%
Averages	-38%	42%	2%
Transpiration rate (E)			
Alabama <i>A. barbatum</i>	31%	3%	17%
Alabama <i>A. leucoderme</i>	-18%	106%	44%
Florida <i>A. barbatum</i>	62%	20%	41%
Florida <i>A. leucoderme</i>	83%	5%	44%
South Carolina <i>A. leucoderme</i>	-14%	-27%	-21%
Averages	29%	21%	25%

Figure 2.3. Photo of hard maple germplasm collection held on landscape fabric on Cook Campus at Rutgers University, New Brunswick, New Jersey.



Figure 2.4. Overwintering potted germplasm collection-first year on campus at Rutgers greenhouse



Figure 2.5. Photograph showing potted study trees with ID labels in the foreground.



Boundary Plants				Boundary Plants			
MNn	MNn	TXg	MNn	MIIs	NYs	MNn	MNn
MNn	NYs	MIIs	NYs	NYs	TXg	MNn	NYs
MNn	NYs	MNn	MNn	MIIs	TXg	NYs	MIIs
TXg	TXg	TXg	MNn	MIIs	NYs	MNn	TXg
NYs	NYs	NYs	TXg	TXg	NYs	MNn	MNn
MIIs	MIIs	MIIs	MNn	NYs	NYs	NYs	TXg
MIIs	TXg	MNn	MIIs	NYs	Mi	NYs	NYs
MIIs	TXg	TXg	TXg	MNn	TXg	MIIs	TXg
NYs	MNn	MIIs	Mi	TXg	NYs	TXg	NYs
NYs	MNn	MIIs	NYs	NYs	MNn	MIIs	NYs
NY	MNn	MNn	MNn	Figure 2.6. Northern plot map for the greenhouse drought study.			
MNn	MNn	MIIs	TXg				
NYs	TXg	MNn	NYs				
TXg	MNn	MIIs	MNn				
MIIs	MNn	MNn	MIIs				
NYs	MNn	MIIs	MIIs	TXg	MNn	NYs	TXg
TXg	MIIs	MIIs	MNn	TXg	TXg	MIIs	NYs
NYs	MNn	TXg	TXg	MNn	MIIs	TXg	TXg
MIIs	TXg	NYs	MIIs	NYs	NYs	MIIs	MNn
TXg	MNn	MIIs	MIIs	NYs	MIIs	TXg	NYs
MIIs	NYs	MIIs	MIIs	TXg	TXg	MIIs	TXg
MIIs	MIIs	NYs	MNn	NYs	MIIs	TXg	MNn
TXg	TXg	MIIs	MIIs	MNn	NYs	MNn	TXg
NYs	NYs	MIIs	TXg	TXg	MIIs	TXg	TXg
MNn	TXg	MIIs	NYs	MNn	MNn	MNn	NYs
Boundary Plants				Boundary Plants			

Boundary Plants				Boundary Plants			
Fb	F1	Ab	F1	F1	Ab	F1	A1
Fb	SC1	SC1	SC1	Fb	A1	Fb	Ab
SC1	Ab	SC1	Ab	A1	F1	Fb	Ab
Fb	Fb	Ab	A1	Ab	SC1	Fb	SC1
Fb	F1	F1	Ab	A1	Fb	Ab	F1
Ab	A1	Ab	A1	Fb	F1	Fb	Ab
F1	F1	Fb	Ab	Fb	SCL	F1	Ab
A1	SC1	Fb	Ab	Fb	SCL	F1	A1
Fb	A1	SC1	Fb	Ab	Fb	Ab	A1
Fb	F1	Ab	F1	Ab	SCL	F1	A1
F1	Fb	Ab	F1	Figure 2.7. Southern plot map for the greenhouse drought study.			
Ab	Fb	Ab	Fb				
Fb	SC1	Ab	F1				
A1	F1	A1	Ab				
SC1	A1	Fb	A1				
Ab	F1	A1	A1	F1	A1	Ab	Ab
F1	Fb	F1	Ab	Fb	SC1	Ab	Ab
Ab	SC1	Fb	Ab	A1	Fb	F1	F1
A1	Fb	A1	Fb	A1	Fb	Fb	Ab
Fb	F1	Ab	SC1	Fb	A1	SC1	F1
Ab	F1	Ab	Fb	Fb	A1	Ab	Fb
F1	A1	Fb	SC1	Fb	SCL	F1	Ab
SC1	Fb	A1	Fb	Fb	A1	F1	A1
A1	F1	F1	Fb	A1	SC1	Ab	F1
Ab	Ab	A1	Ab	Ab	Ab	F1	Fb
Boundary Plants				Boundary Plants			

Figure 2.8. Photo of gas-exchange measurement procedure.



CHAPTER 3

BIOCHEMICAL RESPONSE OF PHOTOSYNTHETIC PROCESSES IN SOUTHERN HARD MAPLE TAXA UNDER WATER TREATMENTS

ABSTRACT

Northern sugar maple, *Acer saccharum* Marsh, is ecologically and economically important in the northeastern United States. Due to drought and temperature vulnerabilities, however, sugar maple is susceptible to potentially negative impacts of projected climate change, and solutions for species improvement are needed. In Chapter 2, I presented a drought study that exposed significant differences in biomechanical response to drought treatments, both within and among populations of sugar maple and related taxa. In this chapter, I have designed a greenhouse experiment to quantify biochemical response parameters of southern hard maple species *Acer barbatum*, *A. leucoderme*, and *A. grandidentatum*, under specific water regimes. I quantified maximum rate of carboxylation by the enzyme Rubisco (V_{cmax}), rate of electron transport (J), and rate of triose phosphate utilization (TPU). Carbon dioxide response curve data were used as inputs for the calculator developed by Sharkey et al. (2007), and calculated values were then subjected to statistical analysis. I found that these southern hard maple taxa, after drought stress, showed no significant differences in their V_{cmax} , J, or TPU values under study conditions. Month (time of measurement) did, however, influence population mean response for study trees, as first month mean differences were significantly different from second and third month mean differences for V_{cmax} , J, and TPU. Results suggest that taxon and provenance, in the context of southern hard maple taxa, do not influence biochemical responses under study conditions of drought stress.

INTRODUCTION

Sugar Maple is moisture sensitive and susceptible to drought-induced damage of biophysical and biochemical processes associated with photosynthesis (Flexas et al. 2002, Dusenge et al. 2019). In Chapter 2, I demonstrated significant differences between biophysical (stomatal) limitations to plant processes associated with photosynthesis, in response to drought for northern, southern, and western hard maple taxa. Throughout the 6-7 weeks of study, northern and western study populations maintained higher rates of stomatal conductance of water vapor, carbon assimilation, and transpiration under control (adequate water provided), mild (short-term) and severe (long-term) drought treatments, than did southern hard maples, all subjected to the same conditions. In general, southern trees exhibited earlier adjustments in stomatal conductance rates than did northern trees, under drought treatments. Significant differences in carbon assimilation rate, conductance rate, and transpiration rate were seen among northern taxa, between northern and southern taxa, but not among southern taxa. The observed lack of biomechanical response differences among southern hard maple taxa raised the question of whether the same patterns would be seen at a biochemical level.

The question is whether there are differential biochemical responses to water treatments among southern hard maple populations. The overall objective of this study was to investigate the impact of drought on biochemical processes associated with photosynthesis in southern hard maple accessions. Given the patterns of behavioral response seen in the previous chapter, the expectation here was that meaningful differences were likely among southern populations, though - in the absence of previous research, the directions and magnitudes of such differences were not predictable. The

research questions for this chapter are the following, based on the results from Chapter 2:

1. Is there differential biochemical trait response, associated with photosynthesis, among *Acer barbatum*, *A. leucoderme* and *A. grandidentatum*, under variable water conditions?
2. Which biochemical study parameters are most sensitive to specific water regimes?
3. How influential is provenance in the biochemical response to drought treatments within each of *A. barbatum*, *A. leucoderme*?

MATERIALS AND METHODS

Plant material - Selections representing two populations of *A. barbatum*, one each from Alabama and Florida, three populations of *A. leucoderme*, one each from Alabama, Florida, and South Carolina, and one population of *A. grandidentatum*, originating from western Texas, were made from an established germplasm collection, developed through purchases from suppliers able to confirm species provenance, and are listed in Table 3.1. At the end of the drought study described in Chapter 2, all individuals were placed in a holding area, outside the Research Greenhouse at Rutgers University (New Brunswick, NJ), from November 2014 until July 2015. Underlying the broad research questions was an objective to push study individuals to and beyond tolerance limits under water stress. I accomplished this prior to the study's onset by instigating a management strategy of forced dormancy and growth seasons, as a byproduct of neglectful management.

The only water provided was from local precipitation, though potted trees were kept clean of debris and weeds during this time. In June of 2015, surviving trees were transferred back into the greenhouse, where they were provided with irrigation for two weeks, to

stabilize trees impacted by previous stress. All populations from Chapter 2 were subjected to preliminary measurements; data from northern trees were not usable, as their measurement values were negative for certain study variables. For all intents and purposes, the northern provenances had succumbed to the drought conditions of the screen. Southern trees showed stable values for the measured variables, and were judged suitable for further investigation and analyses, thus narrowing the focus of the study to *A. barbatum* and *A. leucoderme* populations.

Greenhouse conditions were set to simulate environmental conditions of constant summer. Photoperiod was set for day length of 16 hours, simulating day length at 48° latitude for July in the northern mid-west region of the United States. Supplemental lighting was activated when daytime light intensity fell below 1000 PAR. Temperatures in the greenhouse were set for 24 °C during the day and 18 °C at night. Relative humidity was regulated at 45%. Gas exchange data were used to build CO₂ response curves. The LI6400XT system (LICOR Biosciences, Inc., Lincoln, NE), used in the previous chapter to quantify biophysical data, was also used for this purpose. Criteria for leaves used for measurements was that they were fully expanded on current year's growth and within reach of the measuring equipment. Measurements were conducted no earlier than 7:30 am and no later than 12:30 pm during the first week of the month for both July and August 2015 (Image 3.1). Photosynthetic response curves were established in a stepwise approach by decreasing and then increasing carbon dioxide concentrations in the leaf sample chamber as follows: 400, 300, 200, 100, 50, 400, 600, 700, 800, 900, 1000 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Reference [CO₂] was maintained at 400 ppm. Relative humidity and leaf temperature were not regulated for this study, but photosynthetic photon flux density (PPFD) was held at 1500

$\mu\text{mol m}^{-2}\text{s}^{-1}$.

Water Regimes - Three water regimes were deployed among nine plots (3 plots per treatment): Control, DDR (dry down/rebound), and TDD (total dry down). The Control group was provided adequate water throughout the study's duration, avoiding water stress for this group. Short-term drought conditions (DDR) were simulated by an intermittent watering schedule, and a long-term drought treatment (TDD) was induced by withholding water for the length of the study, after the initial water application at the onset. One randomly selected tree from each of the 6 study populations was placed in each plot ($n = 6$ per plot). Individuals not selected for the study were placed on perimeter benches of the greenhouse to reduce boundary effects (Figure 2). For the third month, measurements were taken the last few days in September into the first few days in October 2015. Water regimes were modified from Bauerle et al. (2003).

Photosynthesis and Relevant Study Parameters - Plant photosynthesis is complex, with a high level of organization (Farquhar and Sharkey, 1982; Wang et al. 2018). There are generally biomechanical processes associated with leaf level gas-exchange and regulation of stomatal aperture, enabled by biochemical functions that occur within leaf chloroplasts, intercellular organelles, and spaces. In concert, these biomechanical and biophysical aspects of plant photosynthesis can determine net photosynthetic rate (A), which can thus be sensitive to biophysical and/or biochemical system modifications (Flexas et al. 2004; Wilson et al 2000). Farquhar et al. (1980) developed the widely accepted model for biochemical rate-limiting factors of C_3 photosynthesis in plants; the calculation scheme developed (based on the model) uses the relationship between net photosynthetic rate and increasing concentrations of intercellular carbon dioxide

concentrations as data values to determine biochemical constraints on photosynthesis under various scenarios (Sharkey et al. 2007). The outputs are a set of five parameters from photosynthetic biochemistry, often used to describe net photosynthetic rate in plants: CO_2 (the maximum rate of carboxylation), J (electron transport rate, based on NADPH requirements), TPU (rate of triose-phosphate utilization), R_d (dark respiration rate), and g_m (mesophyll conductance). Brief explanations of the derivative analytical constructs relevant to this study follow, but for details, see Dusenje et al. (2019).

Maximum Velocity of Carboxylation - V_{cmax} is the maximum (saturated) rate of carboxylation by the enzyme Rubisco, measured in $\mu\text{mol m}^{-2}\text{s}^{-1}$. Photosynthesis is promoted when internal concentration of CO_2 is high. With increased levels of $[\text{CO}_2]$, availability of the Rubisco enzyme also increases inside leaf tissue, curbing the wasteful process of photorespiration. Under drought stress, stomatal aperture is reduced to conserve plant moisture, resulting in decreased CO_2 uptake through leaf stomata, thus reducing net photosynthesis (A). V_{cmax} is also reduced under drought conditions and can be used as a proxy for plant behavior under water stress (Parry et al. 2002). In Chapter 2, southern study populations showed an earlier reduction in carbon assimilation rates under mild and extreme drought treatments than do northern study trees. It can be inferred that the basic mechanistic response exhibited by southern trees results not only in a reduction in (A), but also in V_{cmax} . Northern trees maintained higher rates of (A) for mild and severe drought treatments, also indicating higher V_{cmax} rates, until stomatal closure was triggered.

Electron Transport Rate- The rate of electron transport, J , in $\mu\text{mol m}^{-2}\text{s}^{-1}$, and regulated by RuBP regeneration, effects rates of NADPH and ATP production. The capacity to regenerate RuBP determines the effectiveness and efficiency of electron

transport and can also be used to investigate the impact of drought conditions on plant photosynthetic processes. Photosynthetic limitations, due to compromised electron transport, are expected to occur at higher concentrations of CO₂ than limitations set by the maximum carboxylation rate, V_{cmax} (Flexas et al. 2004). Under conditions of extended drought, RuBP regeneration is reduced, along with the availability of Rubisco activase, negatively impacting electron transport and reducing photosynthetic capacity of plant leaves (Lawlor et al. 2002). An observed reduction in electron transport (J) would indicate plant response to drought stress.

Triose Phosphate Utilization Rate - At even higher internal concentrations of CO₂, a plant's ability to facilitate the use of triose phosphate to produce starches and sugars, becomes limiting, usually measured as triose-phosphate utilization (TPU in $\mu\text{mol m}^{-2}\text{s}^{-1}$). Under conditions of drought stress, plants have shown “reversed-sensitivity” to increasing CO₂, reflected as limited CO₂ assimilation via TPU. Also referred to as an insensitivity to CO₂, this process can be recognized by a consistent reduction in the rate of carbon assimilation, paralleled by increasing CO_{2s} concentration. Behavioral response exhibiting this relationship can be quantified to determine the impact of severe drought on plant photosynthetic processes (Wullschlegel 1993).

Experimental Design and Statistical Analysis - I used a randomized block design with fixed effects of Month (of measurement), Provenance and Treatment. The plot map for study trees is shown in Figure 3.1. Results were analyzed using the least square means method. Least squares estimate targeted parameters by minimizing squared deviations between observed data and expected values. During this study, several individuals did not survive the total dry down treatment (TDD) and holes in the data set were observed. Least

squares protects against missing data when individuals fail and losses occur, resulting in data gaps, and so was determined as the best approach to data analysis for this trial. Parameters showing significant effects were further analyzed using Tukey's HSD *post hoc* tests to compare significantly different means. Pairwise comparison tables are used to visualize effect data. Data analysis was conducted using the JMP software platform (SAS Institute Inc., 1989-2019)

RESULTS

Population means for V_{cmax} were highest in July 2015 for all study groups across all treatments. Population means were lower in August 2015 for all populations, except *A. barbatum* from Alabama. Control provenance averages were highest for Florida *A. leucoderme* ($81.57 \mu\text{mol m}^{-2} \text{s}^{-1}$) and lowest for *A. leucoderme* from Alabama ($39.07 \mu\text{mol m}^{-2} \text{s}^{-1}$), showing intrinsic intraspecific differences in population response (Table 3.5). Population means for the dry down/rebound treatment (DDR) were also highest in July 2015 ($100.15 \mu\text{mol m}^{-2} \text{s}^{-1}$). August 2015 means ($43.83 \mu\text{mol m}^{-2} \text{s}^{-1}$) were higher than Sept/Oct 2015 ($34.58 \mu\text{mol m}^{-2} \text{s}^{-1}$) means. However, all South Carolina *A. leucoderme* and Texas *A. grandidentatum* study trees failed by the last phase of the study, probably influencing study-wide mean values for the Sept/Oct 2015 values. Of the remaining study populations, none of which failed before the last measurements, Florida *A. barbatum* had the highest V_{cmax} ($66.55 \mu\text{mol m}^{-2} \text{s}^{-1}$) and *A. leucoderme* the lowest ($43.24 \mu\text{mol m}^{-2} \text{s}^{-1}$). Under the total dry down treatment, all study populations failed before the Sept/Oct 2015 measurements, resulting in lower provenance averages, overall. Provenance means were highest for July 2015 ($65.21 \mu\text{mol m}^{-2} \text{s}^{-1}$) and lower in August 2015 ($30.65 \mu\text{mol m}^{-2} \text{s}^{-1}$). V_{cmax} was highest for Alabama *A. leucoderme* under the TDD treatment, with the lowest

V_{cmax} exhibited by *A. leucoderme* ($29.31 \mu\text{mol m}^{-2} \text{s}^{-1}$) from South Carolina. Provenance averages and population means for V_{cmax} are provided in Table 3.2.

Provenance averages for electron transport rate (J) were highest for Florida *A. barbatum* ($94.30 \mu\text{mol m}^{-2}\text{s}^{-1}$) and lowest for Alabama *A. leucoderme* ($60.39 \mu\text{mol m}^{-2}\text{s}^{-1}$) under the Control treatment. Population means for July 2015 posted the highest value ($147.71 \mu\text{mol m}^{-2}\text{s}^{-1}$), much higher than that for August 2015 ($45.28 \mu\text{mol m}^{-2}\text{s}^{-1}$) and that for Sept/Oct 2015 ($46.58 \mu\text{mol m}^{-2}\text{s}^{-1}$). For trees subjected to the dry down/rebound (DDR) treatment, Alabama *A. barbatum* trees maintained the highest average electron transport rate ($90.31 \mu\text{mol m}^{-2}\text{s}^{-1}$), while Alabama *A. leucoderme* maintained the lowest ($56.58 \mu\text{mol m}^{-2}\text{s}^{-1}$). Population mean for electron transport rate were higher in July 2015 ($147.71 \mu\text{mol m}^{-2}\text{s}^{-1}$), decreased in August 2015 ($45.28 \mu\text{mol m}^{-2}\text{s}^{-1}$), and increased a small amount in Sept/Oct 2015 ($46.58 \mu\text{mol m}^{-2}\text{s}^{-1}$). Population means displayed the same pattern for V_{cmax} under the DDR treatment. For trees under the total dry down regime, Alabama *A. leucoderme* had the highest rate of electron transport ($99.12 \mu\text{mol m}^{-2}\text{s}^{-1}$) and Florida *A. barbatum* maintained the lowest ($25.24 \mu\text{mol m}^{-2}\text{s}^{-1}$). South Carolina *A. leucoderme* and Texas *A. grandidentatum* trees did not survive for the measurement routines in August 2015 or Sept/Oct 2015. Overall population means for July 2015 ($69.93 \mu\text{mol m}^{-2}\text{s}^{-1}$) were almost double that for August 2015 ($36.72 \mu\text{mol m}^{-2}\text{s}^{-1}$). Provenance averages and population means for J are provided in Table 3.3.

Provenance averages for TPU (triose-phosphate utilization rate $\mu\text{mol m}^{-2}\text{s}^{-1}$) under the Control treatment were highest for Florida *A. barbatum* ($5.50 \mu\text{mol m}^{-2}\text{s}^{-1}$). This was only slightly higher than that of *A. leucoderme* ($5.49 \mu\text{mol m}^{-2}\text{s}^{-1}$) from Florida. The lowest TPU for Control trees was that of *A. leucoderme* from Alabama ($3.99 \mu\text{mol m}^{-2}\text{s}^{-1}$). Control

population mean was three times higher in July 2015 ($9.57 \mu\text{mol m}^{-2}\text{s}^{-1}$) than in August 2015 ($2.46 \mu\text{mol m}^{-2}\text{s}^{-1}$) and Sept/Oct 2015 ($2.94 \mu\text{mol m}^{-2}\text{s}^{-1}$) across all study groups. Average TPU for provenances subjected to the DDR (dry down/recovery) treatment were highest for Alabama *A. barbatum* ($5.68 \mu\text{mol m}^{-2}\text{s}^{-1}$) and lowest for Alabama *A. leucoderme* ($3.10 \mu\text{mol m}^{-2}\text{s}^{-1}$). Population mean was highest in July 2015 ($7.10 \mu\text{mol m}^{-2}\text{s}^{-1}$) and the mean rate for August 2105 ($3.68 \mu\text{mol m}^{-2}\text{s}^{-1}$) was substantially lower. Sept/Oct 2015 values for TPU were not calculated, due to tree failure for South Carolina and Texas provenances. TPU provenance averages and population mean were not calculated for study groups, for the same reason, for all trees under the total dry down treatment. Alabama *A. leucoderme* sustained the highest TPU ($7.87 \mu\text{mol m}^{-2}\text{s}^{-1}$) during July 2015 and overall provenance averages for surviving study groups. In August 2015, highest TPU was held by Alabama *A. barbatum*. Highest TPU population mean was higher in July 2015 ($4.21 \mu\text{mol m}^{-2}\text{s}^{-1}$) than in August 2015 ($2.79 \mu\text{mol m}^{-2}\text{s}^{-1}$) across all study provenances under the TDD treatment. Provenance averages and population means for TPU are provided in Table 3.4.

Provenance, treatment and time (Month) fixed effects were tested for leverage on study populations mean responses. Table 3.5 shows results for the fixed effects of Provenance, Treatment, and (Provenance*Treatment) interaction. Neither Provenance nor Treatment were significant sources of variation between population means. Effect test results for fixed effects of Provenance, Month (time) and their interaction Table 3.6 A strong time (Month) effect indicated the tight genetic link between population means and length and intensity of drought.

DISCUSSION

In my study, population means declined consistently and significantly from July 2015 to August 2015 to Sept/Oct 2015 for V_{cmax} , J, and TPU, mainly due to the fixed effect of time (Month). The leverage of time has also been seen in other studies. Hauer (1995) compared physiological response in *Acer nigrum* seedlings of different provenances and reported that the interaction of time and seed source had a significant impact on population means for leaf gas exchange and intrinsic water use efficiency (Hauer 1995).

Northern hard maples have been the focus of many ecophysiology and genetic studies, due to strong ties to the economy of the northeastern United States and adjacent Canada (Godman et al 1990). Sugar maple vulnerabilities to fluctuations in environmental conditions, particularly drought and increasing temperatures, have driven efforts to improve industry stock through selection and breeding programs, prioritizing the stabilization of sugar sap quality and increasing sugar content of tree sap for the maple sugar industry (Kriebel 1989, 1990; Kriebel and Gabriel 1969). Given the lack of adaptive capacity driving the improvement of sap sugar characteristics, examinations exploring susceptibility to environmental perturbations are necessary, but there has been little (if any) investigation of these same aspects among related southern hard maple taxa.

In this study, one response variable (V_{cmax}) showed significant differences among provenances overall, so there are evidently differences in photosynthetic biochemical response to drought, suggesting that southern hard maple taxa may have something useful to offer. The remaining study parameters, electron transport rate (J) and triose-phosphate utilization (TPU), displayed no significant differences in trait response for the duration of the study, supporting the null hypothesis of no difference in biochemical response between hard maples native to the southern portion of the range. In Chapter 2, significant mean

differences in biomechanical response to water regimes were observed between northern and southern groups. Southern trees displayed earlier reductions of stomatal conductance under short and long-term drought simulations. Biochemical response to treatments was not significantly different among the southern study groups, except in the case of V_{cmax} , which did show different results for July 2015, reflecting the stomatal response pattern of southern study groups in Chapter 2. The variable most sensitive to study treatments was V_{cmax} , but no further changes in mean response emerged between August and Sept/Oct 2015 (Table 3.7). It was expected that study trees, after being pushed to extreme limits of drought, would experience a decoupling event that would trigger drought response, beyond the adjustment of leaf stomatal aperture. However, my results indicate this is not the case and southern hard maples have evolved and persisted through drought events, probably utilizing alternative strategies.

Kriebel's foundational research in sugar maple began in the 1950s, when he executed critical provenance trials exploring environmental limitations of the hard maple complex. The payoff from Kriebel's work is that drought tolerance is highly correlated with hard maple trees from hot, dry climates and that high air temperature has contributed to evolution of drought resistant genotypes of *A. saccharum* (Kriebel 1957). Kriebel concluded that hard maple taxa were better viewed as ecotypes with specific growth forms, branching patterns, foliar traits, and climatic tolerances, exhibiting a clinal distribution pattern based on climate. Kriebel's trials did not include the western (*A. grandidentatum*) or the southern (*A. leucoderme*), by virtue of plant material being unavailable at that time. To my knowledge, a study focused specifically on southern hard maple behavioral response has yet to be conducted and would be profitable. Based on Kriebel's ecotype rubric for

drought tolerant sugar maples, *A. leucoderme* would be absorbed into the southern group, along with *A. floridanum*. Populations from three different states (Alabama, Florida, and South Carolina) of *A. leucoderme* were represented here, and all had survivorship throughout the harshest treatment presented in this study. It is important to realize these results and the potential of *A. leucoderme* as plant selection palettes develop for both natural and urban environments, where drought and temperature conditions can be stressful. Further trials would elucidate the matter further. In terms of maple sugar production, *A. leucoderme* may be potentially useful as a source of genetic information that might improve drought tolerance in modern day sugar maple stock. Selection strategies focused on planting *A. leucoderme* as a substitute for *A. saccharum* may not be wise, due to the shrubby form of *A. leucoderme* would not make it ideal for tapping tree sap and to the author's knowledge attempts to extract tree sap from *A. leucoderme* have not been made.

CONCLUSIONS

To expand our knowledge of stress response differences among southern hard maples, a greenhouse study with varying water regimes was developed, in which carbon dioxide response curves were developed to model biochemical processes linked to photosynthesis. From a biochemical vantage point, results suggest that photosynthetic processes are not significantly different among southern hard maple taxa, as a result of either provenance or treatment, but that for a particular period within a growing season, such processes can be significantly affected by drought conditions. According to the results found here, southern hard maple taxa from Alabama and Florida were able to withstand study extremes and maintain higher rates of biochemical study parameters than were South

Carolina and Texas trees. Introduction of these valuable traits, whether through selection and/or breeding programs may create opportunities to address the issues surrounding northern hard maple decline soon. Desirable traits of southern hard maples may be the key to changing the very uncertain future of the maple sugar industry in the United States. While this study has answered some questions about behavioral response of southern hard maples, it would be valuable to conduct further research that examines multiple populations of southern hard maple taxa (Kriebel 1957). Future studies should increase the number of populations from across the native ranges of southern members of the complex, while increasing the number of individuals within representative populations. Such studies should yield a broader sense of hard maple stress responses and may help to determine whether a genetically influenced link exists between taxa and/or provenances and the array of behavioral stress responses to be expected from southern members of the group.

Table 3.1. Taxonomic designations, provenance (as reported by supplier), and supplier.

Species (Common name)	Provenance	Supplier
<i>Acer grandidentatum</i> Nutt (Canyon Maple)	Western Texas	SFA Gardens Nacogdoches, Texas
<i>Acer barbatum</i> Michx (Southern Sugar Maple)	Alabama	Superior Trees Lee, Florida
<i>Acer barbatum</i> Michx (Southern Sugar Maple)	Northern Florida	Superior Trees Lee, Florida
<i>Acer leucoderme</i> Small (Chalkbark Maple)	Alabama	Superior Trees Lee, Florida
<i>Acer leucoderme</i> Small (Chalkbark Maple)	Northern Florida	Superior Trees Lee, Florida
<i>Acer leucoderme</i> Small (Chalkbark Maple)	South Carolina	Nearly Native Nursery North Augusta, South Carolina

Figure 3.1. Photosynthetic plant processes were quantified using the LI6400XT portable photosynthesis system in the research greenhouse.



Al 2-49	TXg 6-149	Fl 4-167	Al 2-64	TXg 6-160	Fl 4-12	Al 2-53	Fb 3-114	Fl 4
Fb 3-118	SCI 5-77	Ab 1-34	SCI 5-74	Fb 3-102	Ab 1-36	SCI 5-79	Ab 1-17	TXg 6-126
Plot 3			Plot 6			Plot 9		
SCI 5-75	TXG 6-125	Fb 3-101	Fb 3-132	SCI 5-85	Fl 4-16	TXg 6-146	Al 2-61	Ab 1-7
Al 2-66	Ab 1-11	Fl 4-161	Ab 1-44	TXg 6-129	Al 2-54	Fb 3-117	Fl 4-172	SCI 5-80
Plot 2			Plot 5			Plot 8		
Fl 4-173	TXg 6-147	Al 2-60	Fb 3-96	Fl 4-179	Al 2-37	Fl 4-31	SCI 5-70	Al 2-46
SCI 5-73	FB 3-111	Ab 1-3	SCI 5-78	TXg 6-	Ab 1-23	Fb 3-116	Ab 1-28	TXg 6-155
Plot 1			Plot 4			Plot 7		

Figure 3.2. Plot map of study trees for controlled greenhouse drought study in Chapter 3. Control (CONT) plots= Blue, Dry down/Rebound plots (DDR)= Orange, Total Dry Down (TDD) plots= Red. Provenance codes of individual trees are as follows: Ab = Alabama *Acer barbatum*, Al = Alabama *A. leucoderme*, Fb = Florida *A. barbatum*, Fl = Florida *A. leucoderme*, SCI = South Carolina *A. leucoderme*, TXg = Texas *A. grandidentatum*.

Table 3.2. Provenance averages and population means are provided for study parameter maximum rate of carboxylation (V_{cmax} $\mu\text{mol m}^{-2} \text{s}^{-1}$) for trees under Control (CONT), Dry down/Rebound (DDR), and Total Dry Down (TDD) treatments. Measurements were taken in July 2015, August 2015, and Sept/Oct 2015. Provenance codes are as follows: Ab = Alabama *Acer barbatum*, Al = Alabama *A. leucoderme*, Fb = Florida *A. barbatum*, Fl = Florida *A. leucoderme*, SCl = South Carolina *A. leucoderme*, TXg = Texas *A. grandidentatum*.

Control (CONT)							
Provenance	Ab	Al	Fb	Fl	SCl	TXg	Means
15-Jul	136.83	82	111.86	100.72	138.14	117.03	114.43
15-Aug	58.55	6.85	43.68	56.63	20.96	9.69	32.73
Sept/Oct 2015	27.56	28.35	60	87.37	25.11	31.03	43.24
Prov. Aver	74.31	39.07	71.85	81.57	61.41	52.58	63.47
Dry down/Rebound							
Provenance	Ab	Al	Fb	Fl	SCl	TXg	Means
15-Jul	133.28	65.27	68.61	92.79	152.3	88.62	100.15
15-Aug	42.85	42.22	43.68	87.76	7.41	39.03	43.83
Sept/Oct 2015	13.01	22.22	87.37	15.7	---	---	34.58
Prov. Aver	63.05	43.24	66.55	65.42	79.85	63.83	63.66
Total Dry Down							
Provenance	Ab	Al	Fb	Fl	SCl	TXg	Means
15-Jul	52.37	140.28	41.94	49.69	51.22	55.76	65.21
15-Aug	61.65	42.22	28.14	39.32	7.41	5.16	30.65
Sept/Oct 2015	---	---	---	---	---	---	---
Prov. Aver	57.01	91.25	35.04	44.5	29.31	30.46	47.93

Table 3.3. Provenance averages and population means are provided for study parameter J (electron transport rate $\mu\text{mol m}^{-2} \text{s}^{-1}$) for trees under Control (CONT), Dry down/Rebound (DDR), and Total Dry Down (TDD) treatments. Measurements were taken in July 2015, August 2015, and Sept/Oct 2015. Provenance codes are as follows: Ab= Alabama *A. barbatum*, Al= Alabama *A. leucoderme*, Fb = Florida *A. barbatum*, Fl= Florida *A. leucoderme*, SCl= South Carolina *A. leucoderme*, TXg= Texas *A. grandidentatum*.

Control (CONT)							
Provenance	Ab	Al	Fb	Fl	SCl	TXg	Means
Jul-15	73.98	115	162.03	147.31	188.4	199.51	147.71
Aug-15	66.62	12.68	74.24	68.61	34.07	15.46	45.28
Sep/Oct 15	53.75	53.49	46.63	62.64	38.75	24.23	46.58
Prov. Aver	64.79	60.39	94.3	92.85	87.07	79.73	79.86
Dry Down- Rebound (DDR)							
Provenance	Ab	Al	Fb	Fl	SCl	TXg	Means
Jul-15	193.77	71.67	67.77	124.5	158.44	95.2	118.56
Aug-15	53.88	60.7	74.24	124.7	16.12	57.55	64.53
Sep/Oct 15	23.3	37.37	62.64	13.18	---	---	---
Prov. Aver	90.31	56.58	68.22	87.46	87.28	76.37	77.7
Total Dry Down (TDD)							
Provenance	Ab	Al	Fb	Fl	SCl	TXg	Means
Jul-15	59.68	137.54	48.73	53.79	56.59	63.26	69.93
Aug-15	68.56	60.7	1.75	64.67	16.12	8.53	36.72
Sep/Oct 15	-----	---	---	---	---	---	---
Prov. Aver	64.12	99.12	25.24	59.23	36.36	35.89	44.99

Table 3.4. Provenance averages and population means are provided for study parameter TPU (Triose-phosphate Utilization $\mu\text{mol m}^{-2} \text{s}^{-1}$) for trees under Control (CONT), Dry down/Rebound (DDR), and Total Dry Down (TDD) treatments. Measurements were taken in July 2015, August 2015, and Sept/Oct 2015. Provenance codes are as follows: Ab= Alabama *A. barbatum*, Al= Alabama *A. leucoderme*, Fb = Florida *A. barbatum*, Fl= Florida *A. leucoderme*, SCl= South Carolina *A. leucoderme*, TXg= Texas, *A. grandidentatum*.

Control (CONT)							
Provenance→	Ab	Al	Fb	Fl	SCl	TXg	Means
Jul-2015	4.96	7.5	10.41	9.55	11.68	13.29	9.57
Aug-2015	4.03	0.88	3.05	3.78	1.95	1.06	2.46
Sept/Oct 2015	3.64	3.59	3.05	3.13	2.5	1.7	2.94
Prov. Aver →	4.21	3.99	5.5	5.49	5.38	5.35	4.99
Dry Down- Rebound (DDR)							
Provenance→	Ab	Al	Fb	Fl	SCl	TXg	Means
Jul-2015	12.33	3.62	4.22	7.33	7.99	5.01	7.1
Aug-2015	3.33	3.26	3.05	7.58	1.17	3.66	3.68
Sept/Oct 2015	1.39	2.69	3.13	0.68	---	---	---
Prov. Aver →	5.68	3.19	3.47	5.19	4.58	4.34	4.41
Total Dry Down (TDD)							
Provenance→	Ab	Al	Fb	Fl	SCl	TXg	Means
Jul-2015	3.51	7.87	2.87	3.34	3.46	3.85	4.21
Aug-2015	3.99	3.26	1.75	3.77	1.17	0.54	2.79
Sept/Oct 2015	---	---	---	---	---	---	---
Prov. Aver →	3.75	5.57	2.31	3.56	2.31	2.19	5.81

Table 3.5. Summary for fixed effects of Provenance, Treatment and their interaction (Provenance*Treatment). Table lists effects in the model (Source), number of parameters associated with each effect (DF), Sum of Squares , Mean Square (Sum of Squares for the effect divided by its DF), *F* Ratio (Mean Square of Source divided by the Mean Square for Error), and Tukey *P*-value.

Source	DF	Sum of Squares	Mean Square	F Ratio	Tukey <i>P</i> -value
$V_{\text{cmax}} \mu\text{mol m}^{-2} \text{s}^{-1}$					
Provenance	5	1164.09	232.82	0.11	0.99
Treatment	2	2155.46	1077.73	0.50	0.61
Provenance*Treatment	10	9457.63	945.76	0.44	0.92
Error	28	60614.43	2164.8		
$J \mu\text{mol m}^{-2} \text{s}^{-1}$					
Provenance	5	1511.80	302.36	0.09	0.99
Treatment	2	5796.57	2898.29	0.84	0.44
Provenance*Treatment	10	11552.46	1155.25	0.33	0.96
Error	28	97000.07	3464.29		
$\text{TPU} \mu\text{mol m}^{-2} \text{s}^{-1}$					
Provenance	5	5.16	1.033	0.08	0.99
Treatment	2	21.03	10.51	0.77	0.47
Provenance*Treatment	10	32.99	3.3	0.24	0.99
Error	28	382.49	13.66		

Table 3.6 Summary for fixed effects of Provenance, Month (time) and their interaction (Provenance*Month). Table lists effects in the model (Source), number of parameters associated with each effect (DF), Sum of Squares, Mean Square (Sum of Squares for the effect divided by its DF), *F* Ratio (Mean Square of Source divided by the Mean Square for Error), and Tukey *P*-value.

Source	DF	Sum of Squares	Mean Square	<i>F</i> Ratio	Tukey <i>P</i> -value
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)					
Provenance	5	2037.96	407.59	0.44	0.82
Month	2	34908.63	17454.32	18.94	<.0001
Provenance*Month	10	11166.39	1116.64	1.21	0.33
Error	28	25804.11	921.58		
J $\mu\text{mol m}^{-2} \text{s}^{-1}$					
Provenance	5	1498.41	299.68	0.15	0.98
Month	2	47805.50	23902.75	11.97	0.00
Provenance*Month	10	10073.00	1007.30	0.50	0.87
Error	28	55896.2	1996.29		
TPU $\mu\text{mol m}^{-2} \text{s}^{-1}$					
Provenance	5	4.54	0.91	0.11	0.99
Month	2	182.16	91.08	11.50	0.00
Provenance*Month	10	30.45	3.04	0.38	0.94
Error	28	221.66	7.92		

Table 3.7. Tukey HSD test results for maximum velocity of carboxylation rate, V_{cmax} (maximum carboxylation rate), J (electron transport rate), and TPU (triose-phosphate utilization rate). Actual monthly averages for V_{cmax} , J, and TPU are listed diagonally. Month mean square differences (column – row), lower triangle, are significantly different for all three measures between July 2015 and later months, but August and Sept/Oct 2015 do not exhibit significant mean square differences for these measures. Tukey P -Value for each pairwise comparison is shown in upper triangle cells ($\alpha = 0.05$).

V_{cmax} - maximum velocity of carboxylation ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				
Month	July 2015	August 2015	Sep/Oct 2015	Tukey P -Value
July 2015	93.26	< 0.0001*	0.0003*	July 2015
August 2015	-57.53	35.73	0.98	August 2015
Sep/Oct 2015	-55.44	2.08	39.77	Sep/Oct 2015
Diff (+/-)	July 2015	August 2015	Sep/Oct 2015	Month
J- electron transport rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				
Month	July 2015	August 2015	Sep/Oct 2015	Tukey P -Value
July 2015	112.07	< 0.0006*	0.0013*	July 2015
August 2015	-63.22	48.84	0.88	August 2015
Sep/Oct 2015	-72.15	-8.93	41.59	Sep/Oct 2015
Diff (+/-)	July 2015	August 2015	Sep/Oct 2015	Month
TPU- triose phosphate utilization ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				
Month	July 2015	August 2015	Sep/Oct 2015	Tukey P -Value
July 2015	6.82	0.0006*	0.0021*	July 2015
August 2015	- 3.97	2.85	0.94	August 2015
Sep/Oct 2015	- 4.35	- 0.37	2.55	Sep/Oct 2015
Diff (+/-)	July 2015	August 2015	Sep/Oct 2015	Month

CHAPTER 4

EXPLORING GENETIC AFFINITIES OF HARD MAPLE TAXA

ABSTRACT

Sugar maple has been identified as a vulnerable species in terms of rapid climate change, and sustainable solutions are needed to support its role as sole resource for production of maple sugar in the maple sugar industry. Sugar Maple (*A. saccharum*) is the well-known member of the highly variable hard maple species complex. Its congeneric allies occupy distinct ecological niches throughout the United States, Canada, Guatemala, and Mexico. Classification and taxonomic treatment of hard maple taxa have been a source of contention among botanists and taxonomists for at least a century, but current studies using modern techniques do not exist in the literature. In Chapter 2 I describe a drought study where gas-exchange measurements were used to calculate water use efficiency (WUE) for nine (9) hard maple provenance sources, representing five (5) taxa. To determine the relationship between WUE and genotype, I here describe a study using scored molecular data from 20 microsatellite markers across 278 individual samples to calculate fractions of differentiation between and among eight (8) provenance sources, representing six (6) hard maple taxa. I conducted a principal coordinate analysis (PCoA) to visualize the ordination of geographical and genotype data. Based on the PCoA results, the current species designation of hard maple taxa is reasonable.

INTRODUCTION

Taxonomic treatment of the hard maple species complex has been a matter of controversy for close to a century. Identifying characters for these taxa are highly variable, and individuals can be misidentified or described as new taxa when unnecessary (Winston 1999). Given the subjective aspect of botanical practice, hard maples have been designated as species (Rehder 1940), subspecies (Desmarais 1952), and varieties (Sargent et al. 1891), notwithstanding issues of misspelling and misidentification (Mackenzie 1926). It is typical to find studies using tree leaves as a proxy for taxonomic identification of individuals, but the leaves of hard maples exhibit high levels of plasticity, and novel collection techniques were introduced, to allow for the breadth of observed variation, even within single specimens (Anderson 1941). Early variation studies consisted of extensive analyses of leaf biometrics, using such characters as lobe number and angles, size, color and pubescence of leaves, and patterns of leaf venation (Anderson 1938, Desmarais 1952). Later research included characters of tree form, branching pattern, bark texture and color (Kriebel 1957, Kriebel et al. 1969), as well as phenological and reproductive behavior (Gabriel 1966, Gabriel 1967, Gabriel 1968, Gabriel et al. 1984, Gabriel 1990) across the native range of northern sugar and its allies. There are no such studies beyond (Desmarais 1952) that address these attributes in any southern hard maples, to the best of my knowledge.

Introgression and hybridization studies have been reported between northern hard maples (*A. saccharum* and *A. nigrum*), where their ranges are sympatric, though the scale of hybridization is contested in the literature (Dansereau et al. 1947, Paddock 1961, Gabriel 1973). Such genetic exchange events have compromised the accuracy of identification and taxonomic treatment of hard maple field specimens. One study, conducted over 20 years

ago, analyzed chloroplast DNA of sugar and black maple, to determine taxonomic resolution between them. The authors concluded genetic profiles of the two to be so similar that species designation was not warranted (Skepnor et al. 1997). The study's conclusions contradicted those of Paddock (1967), who claimed the two display themselves distinctly in nature, so much so, that treating the two as individual species should be an easy practice. In terms of southern hard maples, interbreeding is suspected between *A. saccharum* and *A. barbatum* in certain areas of *A. barbatum*'s native range, where intermediates have been reported, (Jones 1990) and forced successful crosses of *A. saccharum* and *A. leucoderme* are reported (Slavin 1950, 1954).

Personal communications with native plant specialists in South Carolina (Ted Stephens) and Texas (Dr. David Creech) revealed the presumption that *A. barbatum* and *A. leucoderme* are genetically incompatible is common. The notion is not convincing, as I observed potential intermediates during a field campaign to South Carolina, where stands were cohabitating along the Savannah river. *Acer barbatum* occupied the area between the riverbank and *A. leucoderme*, which persisted in sandier soils of higher ground. Red maple (*A. rubrum* L.) was also present at the site and it is possible that observations of intermediate specimens were red maple hybrids, either with *A. barbatum* or *A. leucoderme*, due to the adaptive capacity of red maple (Townsend et al. 1979, Townsend et al. 1998).

Claims of hard maple genetic relationships are found in popular literature, but are not supported by empiric data (Dirr 1990, van Gelderen et al. 1994). All these observations suggest something going on within southern hard maples, but researchers are without an even fundamental understanding as to what that might be. The need and the opportunity for novel research looking at genetic relationships among hard maples are timely. In

previous chapters, I have described and quantified perturbations in plant photosynthetic processes for hard maple populations subjected to varying water regimes, displayed by biomechanical (stomatal) and biochemical (photosynthetic) responses. These studies brought to light patterns of behavioral response among hard maple taxa, as well as the importance of provenance, when considering traits for possible breeding and selection programs. While these results are valuable, they do not address the underlying question of whether these differential patterns of response are reflected at the genetic level. Genetic markers for the complex have recently been developed and are available to move a study of this sort forward (Graganic et al. 2013).

Objectives- Considering the highly plastic nature of hard maple morphology, modern molecular studies may be both necessary and opportune, for deeper and more productive exploration of hard maple systematics. Here, we apply modern genetic assay methods to identify and quantify genetic differences and/or similarities within the hard maple group. To determine and quantify the significance of genetic divergence between hard maple study groups, I will describe new studies based on microsatellite data, aimed at determining: (1) Fundamental similarities of genetic profiles across multiple hard maple taxa, using available microsatellite data, (2) determine whether response patterns observed in the previous drought study described in Chapter 2 are reflected by provenance-level genetic differences; and (3) assess whether patterns of response align with geographic/environmental gradients associated with water conservation of hard maple study groups.

MATERIALS AND METHODS

To investigate the provenance level genetic differences within the hard maple complex, tissue samples of southern hard maples from field studies (*Acer barbatum*, *Acer*

leucoderme, *Acer skutchii*) and drought study populations of hard maple species from Chapter 2 and Chapter 3 (*Acer saccharum*, *Acer nigrum*, *Acer leucoderme*, *Acer grandidentatum*) were collected and prepared for DNA profiling (Table 4.1). Graignic et al. (2013) described polymorphic nuclear microsatellite markers specific to sugar maple. Markers were developed using SSR-enriched next generation pyrosequencing and cross-species transfer. The authors concluded that 20 markers were useful for future population genetics studies specific to sugar maple (Table 4.2). These markers were used to genotype the 278 hard maple individuals from populations listed in Table 4.1. DNA extraction and scoring were done by the Rutgers DNA Genotyping Laboratory at Rutgers University (New Brunswick, NJ). Extraction methods followed that of Honig et al. (2018), using a Sigma GenElute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO) and protocols suggested by the manufacturer. DNA samples were genotyped using an Applied Biosystems 3500xl capillary electrophoresis genetic analyzer (Thermo Fisher Scientific). Genemapper 5.0 (Applied Biosystems, Foster City, CA) was used to score to capillary electrophoresis data.

Statistical Analysis- To determine the significance of genetic variation across study populations, raw data output from Genemapper were statistically analyzed with AMOVA (GenAlEx, Peakall and Smouse, 2006, 2012). AMOVA is useful for this data set, because it categorizes the separation of genetic variation within and among populations and species, as well as providing estimates of commonly used analogues of *F*-statistics. The AMOVA platform can utilize data from codominant markers for population structure analysis and accommodate statistical testing, via random permutation. We ignored genetic variation within individuals, beginning with a (278 x 278) matrix of pairwise genetic distances

among the $N = 278$ individuals assayed. In AMOVA, this is accomplished by working under the genotypic *Codom-Genotypic* distance option. Under this preference, Φ_{PT} is the estimate of population genetic differentiation provided by GenAlEx, as an analogue of F_{PT} (Peakall et al. 2006). I calculated the sums of squares and extracted variances for the 20-locus genetic data and partitioned the variance of individuals within the same population, among populations within each taxon, and among taxa. This partition reflects a multiply nested linear model of the form

$$\gamma_{ijk} = \mu + \tau_i + \pi_{j(i)} + \varepsilon_{k(ij)} \quad [\text{Equation 4.1}]$$

where μ is the grand mean, τ_i is the mean offset of the i^{th} taxon from the grand mean, $\pi_{j(i)}$ is the mean offset of the j^{th} provenance from the i^{th} taxon, and $\varepsilon_{k(ij)}$ is the offset of the k^{th} individual from j^{th} provenance mean. That leads to a variance partition of the form

$$V_{GT} = V_{WP} + V_{AP} + V_{AT} \quad [\text{Equation 4.2}]$$

with V_{GT} = total genetic variance, V_{WP} = variance within populations, V_{AP} = variance among provenances (within) taxa, V_{AT} = variance among the six taxa.

Formal partitioning of the total genetic variation within the collection is presented in Figure 4.1, with 74 % of the total contributed by variation among individuals within the same population (V_{WP} / V_{GT}), 9% contributed by variation among populations within the same taxon (V_{AP} / V_{GT}), and the remaining 17% contributed by divergence among the taxa themselves (V_{AT} / V_{GT}). The population to population divergence within the currently recognized taxa (here, within *A. saccharum* and within *A. leucoderme*) is somewhat less than that among the six taxa themselves, suggesting that divergence among hard maple taxa is greater than that among provenances, within taxa.

I computed measures for the divergence among all eight (8) provenances, without regard to taxonomic grouping (\emptyset_{PT}), divergence among provenances with a single species (\emptyset_{PS}) and divergence among the (6) species themselves (\emptyset_{ST}), defined (respectively) as in Peakall et al. (2006):

$$\emptyset_{PT} = \frac{V_{AP} + V_{AS}}{V_{WP} + V_{AP} + V_{AS}} \quad [\text{Equation 4.3}]$$

$$\emptyset_{PS} = \frac{V_{AP}}{V_{WP} + V_{AP}} \quad [\text{Equation 4.4}]$$

$$\emptyset_{ST} = \frac{V_{AS}}{V_{WP} + V_{AP} + V_{AS}} \quad [\text{Equation 4.5}]$$

These \emptyset -statistics can be related (inter se), and are subject to the usual constraint that:

$$(1 - \emptyset_{PT}) = (1 - \emptyset_{PS}) \cdot (1 - \emptyset_{ST}) \quad [\text{Equation 4.6}]$$

Results of this doubly nested AMOVA partition are presented in Table 4.3. Statistical constructs defining the fractions of variation estimated in AMOVA are listed in Table 4.4. The estimated \emptyset -statistics analogs suggest modest genetic diversification among populations within a single taxon (0.11). To elucidate these inter-population differences further, I computed all $(8 \cdot 7)/2 = 28$ pairwise measures of genetic divergence among the eight populations (\emptyset_{PT}) and tested each by permuting relationships between the two populations in question. Interestingly, in each case, permutational testing for all pairs, both those within and those among species, exhibited ($P < 0.001$). These differences are both non-trivial and statistically compelling. Pairwise inter-population divergence was evaluated by calculating a (8×8) matrix of paired-population comparisons, using \emptyset_{PT} values, revealing the population x population pattern of divergence ($P < 0.001$). Pairwise results are listed in Table 4.5. The highest pairwise \emptyset_{PT} values across all study groups (0.45) was between *A. grandidentatum* and *A. barbatum* from Texas, followed by Texas *A.*

grandidentatum and *A. skutchii* of Mexico (0.38) and Texas *A. grandidentatum* and South Carolina *A. leucoderme* (0.31). Lowest divergence ($\Phi_{PT} = 0.07$) was that between Michigan and New York *A. saccharum*, followed by South Carolina and Texas *A. leucoderme* (0.10), Michigan *A. saccharum* and Minnesota *A. nigrum* (0.11), and South Carolina *A. leucoderme* and New York *A. saccharum* (0.16).

Several of these study groups originate in Texas. Of the Texas populations, divergence was highest between *A. barbatum* and *A. grandidentatum* (0.45) and lowest between *A. barbatum* and *A. leucoderme* (0.27). Michigan and New York *A. saccharum* showed the least amount of divergence (0.07). South Carolina and Texas *A. leucoderme* had the second lowest divergence (0.10). To visualize the inferences shown in Table 4.5, I conducted a Principal Coordinates Analysis (PCoA) in GenAlEx. The eight (8) taxa were plotted using the first two (2) principal coordinate axes. The cumulative sum of the first two principal coordinate axes accounted for 53.35% of the total variance among populations, as shown in Table 4.5. Pairwise results shown in Table 4.5 were used to create the chart in Figure 4.2. The figure not only shows population differences between taxa, it shows divergence of provenances within them. For example, in Table 4.5, Texas *A. barbatum* and Texas *A. grandidentatum* have the highest pairwise difference (0.45). In Figure 4.2, these two are the farthest apart. On the other hand, Michigan *A. saccharum* and New York *A. saccharum* have the lowest Φ_{PT} value between them and have the closest proximity to each other. Results here legitimize species designation of hard maple taxa.

Comparison of Water Use Efficiency to Genotyping Results

In Chapter 2, I describe a drought study and quantified significant behavioral differences in biomechanical responses associated with leaf gas-exchange between

northern and southern hard maple taxa. Southern taxa displayed earlier reduction in carbon assimilation rate (A_{NET}), transpiration rate, (E), and rate of stomatal conductance (g_m) than did northern trees, in response to persistent water deprivation. Differences in stomatal response between northern provenances were more significant than were those between southern provenances. Patterns of response were seen for duration of the study. Water use efficiency was calculated for all study groups as the relationship between the rate of atmospheric carbon dioxide uptake and the rate of transpiration in tree leaves. To determine the magnitude of variation for WUE between groups, differences in population (provenance) means were analyzed using Tukey-HSD pairwise analysis and assigned letters (a-d) to delineate existing variation. Pairwise groupings for hard maple study groups in Chapter 2 are shown in Figure 3 below. Provenances from Chapter 2 could be interpreted as two (2) groups, northern and southern, each with its own intermediate group. In this instance, northern populations, New York *A. saccharum* (group a) and Minnesota *A. nigrum* (group b) are significantly different from one another, with intermediates of Michigan *A. saccharum* and Texas *A. grandidentatum* (both group ab). Water use efficiency between southern groups shows Alabama *A. leucoderme* (group c) significantly different from South Carolina *A. leucoderme* (group d), while Alabama *A. barbatum*, Florida *A. barbatum* and Florida *A. leucoderme* are intermediates (group cd).

It is clear from Figure 4.3 (WUE-water use efficiency) and results in Figure 4.5 (genotyping) that both behavioral groupings and genetic groupings exist within hard maples. The within taxon genetic variation demonstrated in this chapter is not matched by the population water use differences analyzed in Chapter 2. For example, Michigan *A. saccharum* and Texas *A. grandidentatum* comprise one group (ab) in terms of water use

efficiency (WUE). Genetic pairwise groupings from this chapter sorted these populations much differently. The populations had a Φ_{ij} value of 0.33, which was the fourth highest differentiation value among all genotypes examined here. Paired provenances within taxa were calculated to have low levels of genetic divergence; ($\Phi = 0.07$) between *A. saccharum* from Michigan and New York; ($\Phi = 0.10$) between *A. leucoderme* from South Carolina and Texas; these were the lowest pairwise values of Φ -values in the study. In the drought study, however, populations of *A. saccharum* did not differ significantly for WUE. *Acer leucoderme* from Alabama (group c) and South Carolina (group d) had significantly different population means for WUE, but their Florida conspecific (group cd) in between (Figure 4.3).

DISCUSSION

I initiated this study for several reasons: (1) to examine similarities/differences within and among hard maple taxa through a genetic lens, (2) to determine whether genetic variation within hard maples reflects the current state of hard maple taxonomy, and (3) to determine whether response patterns observed in the drought study described in Chapter 2 are reflected by provenance-level genetic profiles. I accomplished the first objective by statistically analyzing scored genotype data for 20 microsatellite markers on 278 hard maple individuals from eight (8) provenance sources, within six (6) hard maple taxa. The results indicate substantial genetic variation, both within and among hard maple populations and taxa. Most of the variation is found within single populations (76%), (9%) among populations within currently defined species, and (17%) among the species. Population x population pairwise comparisons showed that all pairs of the populations sampled were convincingly different gene pools ($P < 0.001$). It is important to recognize

that these study populations, as different as they are, represent subsets of individuals from specific portions of collective hard maple range, but they almost surely do not encompass the full range of variation within this section of the genus *Acer*. Self-conducted field campaigns to observe hard maple specimens in their natural habitats provided personal observations of phenotypic variation this group can present under field conditions, and future studies would benefit from an expanded data set with more (and varying) provenance sources. The second objective of this study was to determine whether response patterns observed in the previous drought study (Chapter 2) are reflected by provenance-level genetic differences. I compared the pairwise analysis for water use efficiency (WUE) for study provenances in Chapter 2 with the pairwise analysis results from this study from Table 4.5. The pairwise studies were inconsistent for study populations across these two analyses. Provenances of *A. saccharum* from Michigan and New York, respectively, and *A. leucoderme* from South Carolina and Texas, respectively, had significantly different mean values for WUE in Chapter 2. In the pairwise analysis in this study, the same provenances within taxa were estimated at the lowest levels of differentiation across all study groups. These physiological differences suggest that provenance is more indicative of behavioral traits (such as WUE) than to SSR genotypes. It is realistic to expect differences in stress response from members of the complex, both above and below the nominal species level (Duchesne et al. 2014). This particular *A. nigrum* group may not be representative of *A. nigrum* as a whole, and there may well be ecological divergence within this (or any other) hard maple taxon.

The SSR markers used here are not chosen to reflect species fitness. They were selected for analyzing the ancestral commonality of hard maple populations, *sensu lato*. It

is likely that markers selected for drought tolerance would better inform the question of drought response and more accurately infer water use efficiency in these populations. Adaptive traits have been analyzed through QTL (quantitative trait loci) analysis and subsequent marker assisted analyses (Brendel et al. 2002, Rönnerberg-Wästljung et al. 2005, Regnard et al. 2007, Viger et al. 2013, Wheeler et al. 2015). The QTL approach performs phenotypic analysis across a considerable number of individuals from a particular population, separating out for a variety of genetic markers. The data set is then genotyped, in part or in its entirety, and statistically analyzed to identify the loci regulating the trait (Asins 2002). Drought tolerant loci have been reported for *Eucalyptus* (Mora et al. 2017), *Salix* (Rönnerberg-Wästljung et al. 2005), *Populus* (Viger et al. 2013), and *Pinus* (Brendel et al. 2002). It would be beneficial to conduct such an assay of hard maple taxa and further our understanding of their inherent adaptive capacities, especially among the southern cohort.

CONCLUSIONS

Genetic divergence was estimated between all study groups at higher than negligible values. That is contrasted with lower levels of divergence calculated for pairwise provenance differences within species. South Carolina and Texas *A. leucoderme* (on the one hand) and *A. saccharum* from Michigan and New York (on the other) had the lowest overall Φ_{PT} values. Comparisons of behavioral response to water regimes in Chapter 2 with the ancestral lineage results calculated in this chapter do not support shared ancestral lineage as a reliable indicator of behavioral differences that may exist among hard maple populations in the field. These results support the importance of considering geographic provenance when selections for desirable traits are made. Further genetic testing using QTL

approaches will better inform questions of plant stress behavior within and among hard maple populations.

Table 4.1. Table listing of hard maple species, their sources, latitude and longitude of sources, and number of individuals submitted for genotyping analysis.

Species	Nearest town or landmark	Lat (°)	Long (°)	# of samples
<i>Acer barbatum</i> Michx	Field campaign Arcadia, Texas	31.8° N	94.3° W	52
<i>Acer skutchii</i> Murray	Field campaign Nacogdoches, Texas	31.6° N	94.6° W	50
<i>Acer saccharum</i> Marsh	Greenhouse study Michigan	42.8° N	86.1° W	20
<i>Acer saccharum</i> Marsh	Greenhouse study New York	43.1° N	73.8° W	29
<i>Acer leucoderme</i> Small	Field campaign Little Cow Creek, Texas	30.9° N	93.7° W	51
<i>Acer leucoderme</i> Small	Greenhouse Study South Carolina	33.5° N	82.0° W	44
<i>Acer grandidentatum</i> Nutt	Greenhouse study Texas	29.8° N	99.6° W	11
<i>Acer nigrum</i> Michx f.	Greenhouse study Minnesota	44.9° N	93.0° W	21

Table 4.2. Molecular markers (microsatellites) used for genotype analysis were developed by Gaignic et al (2013).

Microsatellite markers	
Sm11	SM27
Sm60	SM29
Sm26	SM34
Sm47	SM36
Am096	SM37
Am116	SM42
Aop943	SM51
SM14	SM53
SM21A	SM55
SM22	SM57

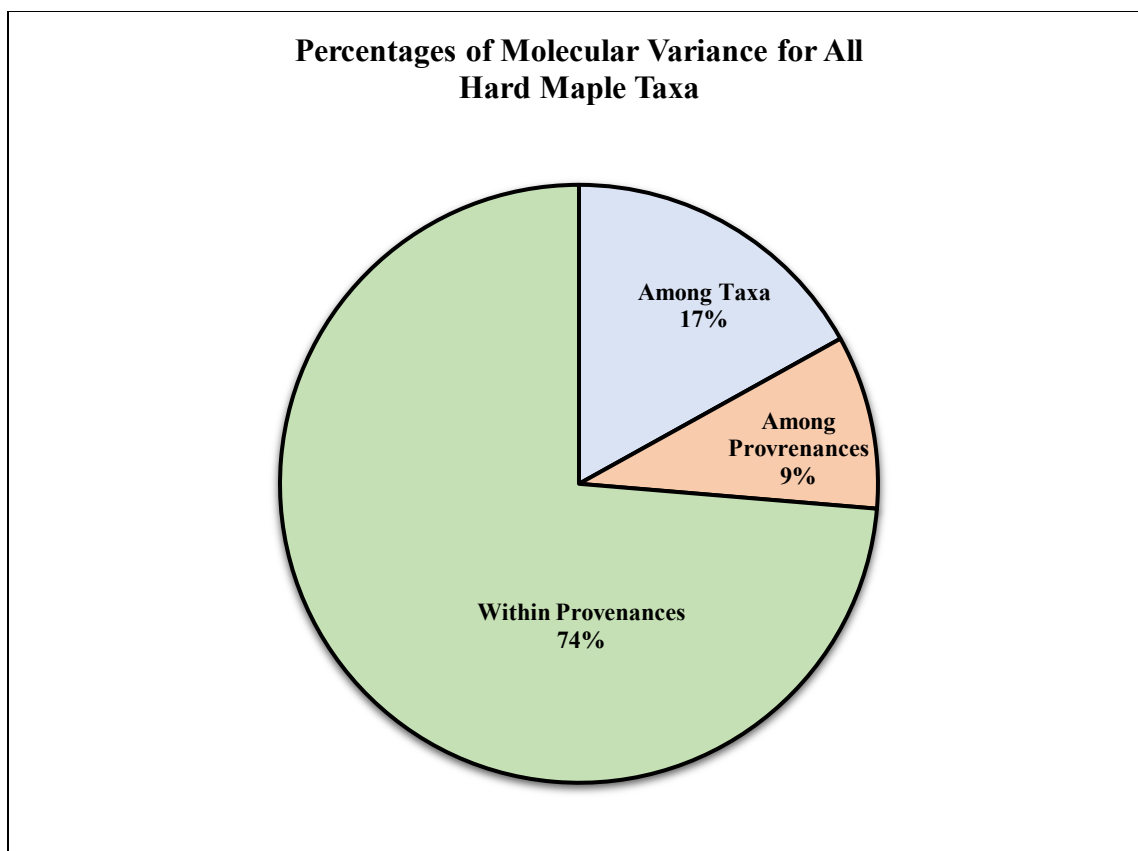


Figure 4.1. Pie diagram, showing relative variances within hard maple study groups. Relative variance within provenances (74%) is shaded in green; relative variance among taxa (17%) is shaded in blue; relative variance among provenances (9%) is shaded in orange.

Table 4.3. AMOVA summary table for the eight hard maple study groups, one provenance each of list them out, two provenances each of *A. saccharum* and of *A. leucoderme*. The AMOVA output lists Source (of variation), df (degrees of freedom), sums of squares (SS), mean square (MS), estimated variance components (Est. Var), and the percentage (%) of total variation contributed by each Source.

Source	df	SS	MS	Est. Var.	%
Among Regions	5	1032.63	206.53	3.13	17%
Among Pops/Regions	2	150.44	75.22	1.74	9%
Within Populations	270	3678.48	13.62	13.62	74%
Total	277	4861.55	---	18.49	100%

Table 4.4. Statistical constructs statistics) estimated by AMOVA. \emptyset_{PS} is the fraction of within-species variation among provenances, \emptyset_{ST} is the fraction of total variance represented by interspecific divergence, and \emptyset_{PT} is the fraction of total variance among the eight populations. (P -values) listed below variance estimates are computed by permuting individual populations.

Constructs	\emptyset_{PS}	\emptyset_{ST}	\emptyset_{PT}
Estimates	0.11	0.17	0.26
P (rand \geq data)	< 0.001	< 0.001	< 0.001

Table 4.5. AMOVA Pairwise population Φ_{ij} values show divergence among and within hard maple taxa. Population ID codes are as follows: TXAb = Texas *A. barbatum*, MXAsk = Mexico *A. skutchii*, MIAs = Michigan *A. saccharum*, NYAs = New York *A. saccharum*, TXAl = Texas *A. leucoderme*, SCAI = South Carolina *A. leucoderme*, TXAg = Texas *A. grandidentatum*, MNAn – Minnesota *A. nigrum*.

Pop ⁿ s	TXAb	MXAsk	NYAs	MIAs	MNAn	TXAl	SCAI	TXAg
TXAb	---							
MXAsk	0.28	---						
NYAs	0.31	0.29	---					
MIAs	0.32	0.28	0.07	---				
MNAn	0.18	0.23	0.14	0.11	---			
TXAl	0.27	0.24	0.18	0.18	0.14	---		
SCAI	0.26	0.21	0.16	0.18	0.15	0.10	---	
TXAg	0.45	0.38	0.36	0.33	0.32	0.29	0.31	---
Pop ⁿ s	TXAb	MXAsk	NYAs	MIAs	MNAn	TXAl	SCAI	TXAg

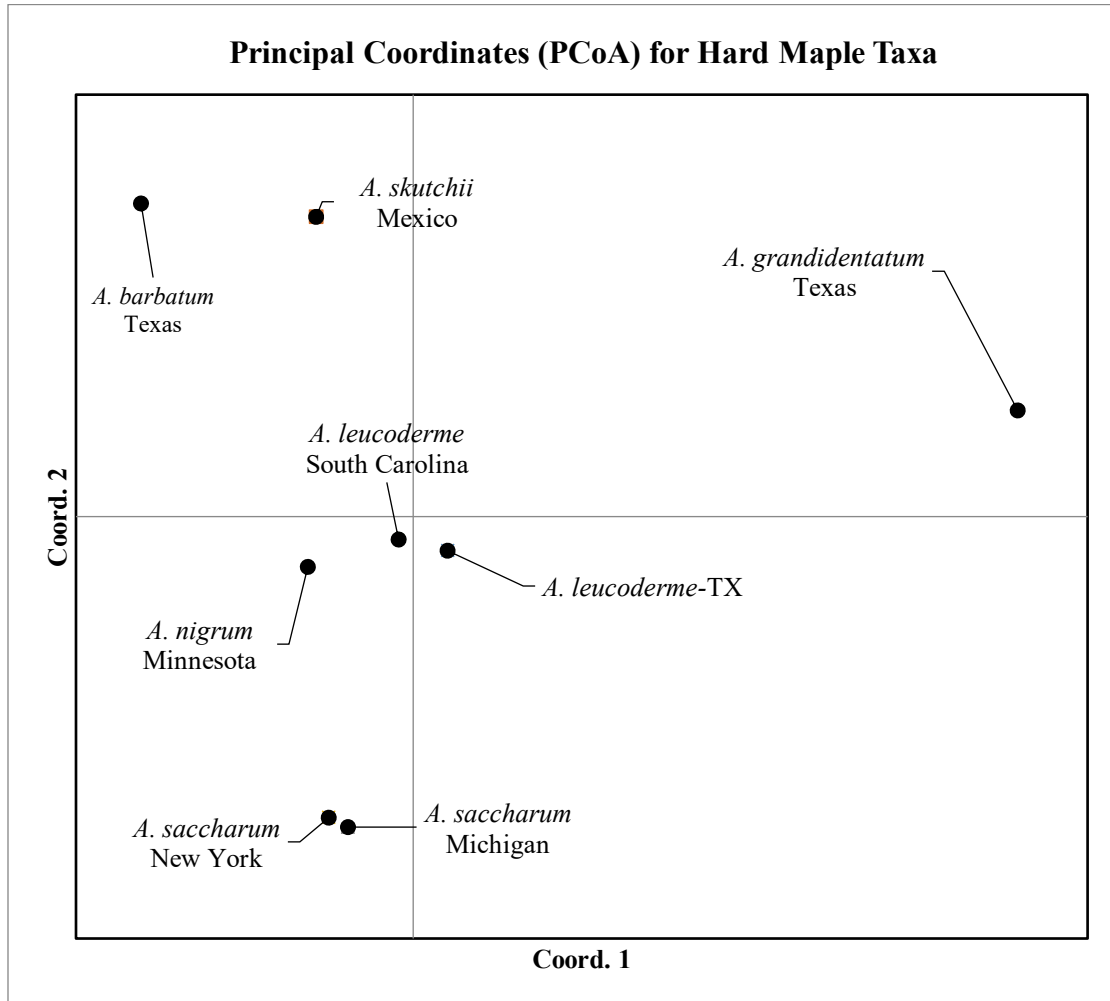


Figure 4.2. Principal Coordinate Analysis results for AMOVA Φ_{ij} pairwise comparisons from Table 4.5. Differentiation levels are determined by distance between populations (provenances). Ordination points are represented by black dots.

Table 4.6 . Principal Coordinates Analysis percentage of variation is explained by the first three (3) axes. Partitioning of variation across individual axes is shown in the first row. The second row shows the cumulative variation across axes. The first two axes account for 53.35% of the total variation.

Axis	1	2	3
%	27.54	25.82	17.11
Cumulative %	27.54	53.35	70.46

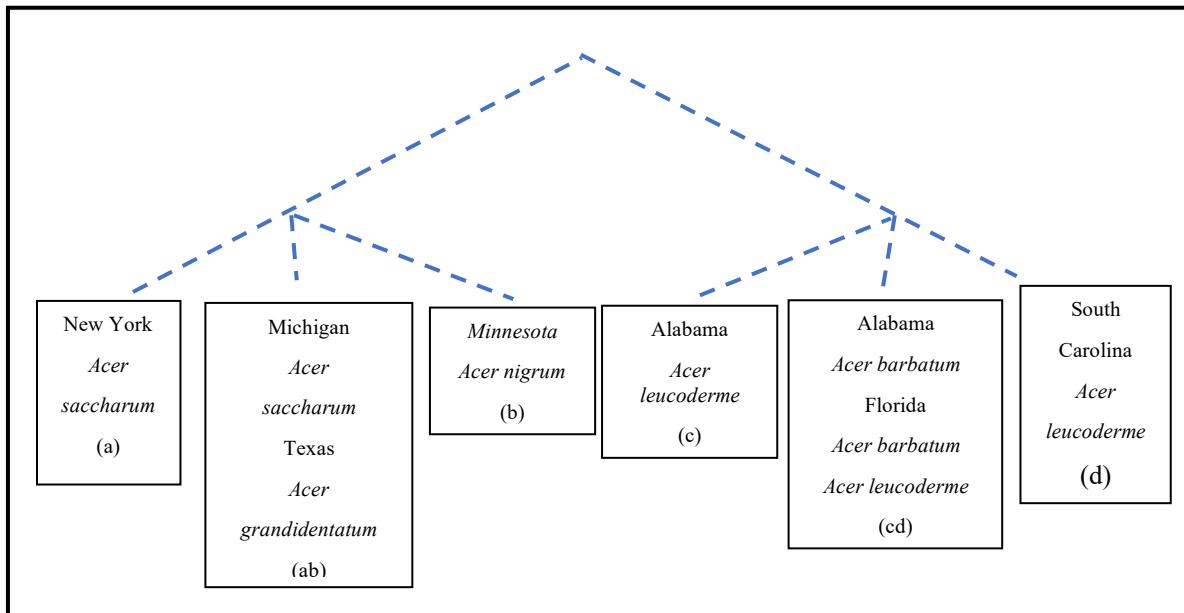


Figure 4.7. Tukey HSD pairwise groupings from Chapter 2 hard maple study populations for WUE (water use efficiency). From left to right, groups (a-d) are organized from highest population mean to lowest for water use efficiency.

CHAPTER 5

Implications of Hard Maple Research and Future Foci

ABSTRACT

This chapter discusses the implications that research described in previous chapters can have for unresolved issues within the hard maple group. I discuss how revitalizing studies focused on genetic variability between sugar maple and its allies is crucial to fill the void in scientific literature, relative to their genetic relationships and adaptive capacities. Once this void is remedied, questions of genetic variability, classification and taxonomic treatment, and environmental limitations of hard maples can be addressed. Research presented in this thesis can also play a role in developing selection criteria that prioritize resilience in plant performance over time. As it is susceptible to negative impacts of rapid climate change, sugar maple is at risk, and solutions are needed to provide strategies aimed at a sustainable future, in terms of sugar production, but also in terms of conservation, reforestation, and forest management practices. I conclude with a discussion of how, building off of this research, integration of tree physiology and forest genetics can be used as a model for hard maple studies in the future.

THE IMPLICATIONS OF HARD MAPLE RESEARCH

Investigations laying fundamental groundwork in hard maple research started with Beal's 1894 comparison of black maple (*A. nigrum*) and sugar maple (*A. saccharum*) in Central Michigan. Anderson and Hubrick's (1938) hybridization study of maples in Illinois, Missouri, Michigan, and Massachusetts inspired the development of new collection techniques to provide researchers with an approach that would account for the large amount of variation observed within sugar maple and its allies (Anderson 1941). Dansereau furthered these studies with Lafond in 1941 and Desmarais in 1947. Desmarais conducted a 13-year study of leaf variation among 523 trees from 14 different locations, collected between 1935-1948. The results were published in 1952 and is one of the most inclusive in terms of hard maple representation. Desmarais reports that only one leaf sample represents *A. leucoderme*, reflecting the state of hard maple research throughout the literature.

Howard Kriebel's work began with his graduate work at Yale in 1954, which he expanded upon for the next 33 years. He reported on many details of hard maple variation, such as tree form, forking tendencies, leaf-out, drought and heat tolerance, and phenology, and survivability (Kriebel 1956, Kriebel et al. 1962, Kriebel et al. 1969, Kriebel 1976). Like Desmarais, however, Kriebel reports that his work did not include samples of *A. leucoderme*. Kriebel's later work narrowed to focus on improvement of sugar maple stock for the maple sugar industry (Kriebel 1955, 1963, Kriebel 1989, 1990). Paddock reported hybrid swarm events involving *A. saccharum* and *A. nigrum* in Ohio in 1961. Long term planting trials were established in West Virginia (Wendel et al. 1975, Wendel et al. 1980). The study addressed provenance performance of 15 sugar maple trees and reported no

significant differences in provenance survival, overall height, dbh over the 10-year study period. The authors also reported that correlations between tree forking and latitude, longitude, and elevation were not strong.

Gabriel's work spanned the 1960s and 1970s, with investigations of comparative sweetness, phenology, reproduction (Gabriel et al. 1961, Gabriel 1966, Gabriel 1967, Gabriel 1972, 1973, Gabriel 1975, Gabriel 1978, Gabriel 1990). Gehlbach et al. (1983) examined the relationship between *A. saccharum* and *A. grandidentatum* and reported observations of intermediates between the two. During the 1980s, studies reporting on sugar maple allies were fewer than in previous decades. In 1995, Hauer's master's thesis out of University of Urbana-Champaign showed differential drought response between sugar and black maple, measured as water use efficiency (WUE), was affected by treatment duration. Between 1994 and 2001, Graves and St. Hilaire expanded on St. Hilaire's doctoral thesis, publishing on the differential physiological response to water treatments for *A. nigrum* and *A. saccharum*, as well as leaf morphological variances at specific latitudes, suggesting that genetic profiles reveal gene flow and the sharing of ancestral DNA (Graves 1994, Graves 1994, Graves et al. 2000, St Hilaire et al. 2001, St. Hilaire et al. 2001).

Sugar maple has been a staple of American cultural identity and has strong ties to the economy of states like Vermont, New Hampshire, Massachusetts and New York (Shea et al. 2001, Parry 2007). These conditions have been the driving force behind research in sugar maple *sensu stricto*, as demands of the maple sugar industry and forestry community needs have evolved over time. Data are available for many aspects of plant biology, including botany, genetics, phenology, and physiology. In the context of plant breeding

and selection, genetic plasticity within species has been a useful resource for understanding plant relationships and developing plant improvement.

Southern hard maple species have simply been overlooked by researchers; empirical data are not available because they do not exist. Revitalizing previous hard maple research and developing future studies less selective for sugar maple and more inclusive in terms of hard maple taxa in general, would provide opportunity for study and help fill the existing void in hard maple expertise.

Breeding and selection programs

Tree improvement programs exist in *A. saccharum* Marsh aimed at increasing sap quality and sugar content. The first sugar and black maple breeding program for “Super-Sweet” crosses was established by Kriebel at Ohio State University in the 1950s at the Secrest Arboretum in Wooster, Ohio. Cornell University’s Uihlein Experiment Station in Lake Placid, NY is second example of a cooperative extension program focused on maple sugar production and marketable ideas for maple sugar producers in the United States. *Acer nigrum* ‘Green Column’ and ‘Temple’s Upright’ are examples of varietal selection aimed at aesthetically desirable tree form (Dirr 1990, van Gelderen et al. 1994). Currently, no other breeding programs in sugar maple are known. Studies have done well to inform questions of sap quality and increased sap yield (Kriebel 1955, Gabriel 1975, Kriebel 1989, 1990, Wild et al. 2015), characteristics of wood (Bragg 1999), and environmental limitations of *A. saccharum* Marsh (Ellsworth et al. 1992, Close et al. 1996, Collins 2015, Collins et al. 2017). The question becomes whether enough research exists to prepare a sustainable future for this important component of the forest landscape in the northeastern United States. Unfortunately, the answer is no.

Though this is discouraging at face value, it provides opportunity for studies that can provide decision-makers with informed conclusions, allowing for the mitigation of the negative results rapid climate change is expected to have on sugar maple populations. It should also be obvious that methods in plant research have increased in accuracy, speed and sophistication. Limited hard maple data are conflated with outdated methods and analyses for group study. Applying modern techniques would not only buttress the quality of past results, it would also bring hard maple research in line with modern research for other valuable forest species.

Sugar Maple decline - Observations of sugar maple decline support the need for ongoing hard maple research. A reduction in northern sugar maple populations have been reported throughout the northeastern portion of the range in the United States and Canada (Westing 1966, Mader et al. 1969, Vogelmann et al. 1988, Horsley et al. 2000, Gavin et al. 2008, Long et al. 2009, Bishop et al. 2015, Oswald et al. 2018). Sugar maple populations in the northeast region of the United States have been identified as vulnerable to negative impacts of rapid climate change that are being reported on a global scale (Menzel et al. 2006, Frumhoff et al. 2007, Reyer et al. 2013, Pauli et al. 2014, Karmalkar et al. 2017). The question, of course, is whether the plants can keep up, by evolving genetically, with the pace of climate change (Aitken et al. 2008). In our Northeastern forest context, the question is whether there is enough genetic plasticity within the existing species for them to keep up with the modeled rates of regional climate change. As concerns for sugar maple populations increase, forest type changes are expected to occur by the year 2100 (Prasad et al. 2007).

Figure 5.1 shows a summary of forest type changes predicted by the Climate

Change Tree Atlas of the United States Forest Service. The figure summarizes forest type changes as outputs of three general circulation models (GCMs), under varying scenarios with atmospheric carbon levels that are high (Hi) or (Lo). The three models are averaged (GCMAvg Hi and GCMAvg Lo) under high and low carbon, as well. In terms of sugar maple, these prognostications are not good. The Hadley Hi scenario, where carbon is high, shows almost all the maple/Beech/Birch forest type being displaced or replaced by the Oak/Hickory type. Under low conditions of carbon, the change is not as dynamic, but there is still a reduction in the maple forest cohort. It is important to realize these modifications in forest structure are predicted to happen over the next 80 years, which indicates a certain urgency regarding solutions for conservation. Historically, reforestation has been sourced by local seed to ensure success of local populations. Climate change challenges this practice for sensitive species as modifications to local climates ensue (Aitken 2013). It may be the best adapted genotypes for novel climates are of different provenance than local specimens, thus preadapted to new environmental conditions and can avoid maladaptation events (Eckert et al. 2008). Environmental factors, such as soil type and condition, elevated levels of atmospheric CO₂, precipitation, species, and temperature play a crucial role in how plants respond to climate change and must be considered when addressing climate change models (Iverson 2008, Iverson 2011, Matthews 2011). These same environmental influences are strongly associated with ensuing selective pressures that influence populations to shift geographically and evolve genetically. Once a habitat becomes unsuitable, whether due to land use changes, a lack of needed resources, or because environmental conditions are no longer within tolerable ranges for development and successful reproduction, only those species able to adapt will survive.

Species plasticity is imperative to ease the transition of ill adapted species lacking suitable traits in the face of climate change, plant selection criteria should prioritize resilience and/or plasticity over aesthetics, yield, growth rate, etc., in order to lay the groundwork for resilient forested landscapes, agricultural crops, and urban plant selection palettes for urban tree planting options. It would be beneficial for existing tree improvement programs to expand selection criteria beyond current environmental parameters to locate individuals that persist along range extremes. In terms of sugar maple, this would mean looking at genotypes from southern areas of sugar maple range or southeastern portions of hard maple ranges for *A. leucoderme*. Genotype analysis in the previous chapter calculated lower population divergence between *A. saccharum* and *A. leucoderme* than between all other hard maple groups. *Acer leucoderme* has adapted to a native climate with drier, and warmer conditions than has its northern relative, making it a viable target for adaptive genetic traits desired in *A. saccharum*. Successful crosses between *A. leucoderme* and *A. saccharum* were reported in the 1950s, but the location of modern day specimens is unknown (Slavin 1950, 1954). Consideration of *A. leucoderme* as a practical choice for local climates.

The common garden approach has been most informative for questions related to genetic plasticity in tree behavioral response to abiotic and biotic stressors. Common garden studies have been conducted in *Abies* (Johnson et al. 1964), *Pinus* (Critchfield 1957, Smouse et al. 1973), *Eucalyptus* (Boden 1958), *Cedar* (Habeck 1958) and, as previously described, *Acer* (Kriebel 1956). Kriebel's work reported on data linked to environmental limitations of several hard maple species. Even though the project was sidelined, it has been recovered and holds empirical value in terms long term adaptive traits

held by provenance sources collected there. Under constraints of time and space, greenhouse studies, like ones I described in Chapters 2 and 3, can be considered controlled common garden trials for juvenile trees (Sagnard et al. 2002, Chambel et al. 2007). Environmental conditions in greenhouse research can be modified to simulate any number of climate scenarios and can be held consistently for brief or extended periods of time to present short- or long-term drought events. By subjecting hard maple populations of different provenance sources, it was possible to better understand the adaptive variation in drought response among and within those populations and how the variation is dispersed across hard maple range. The results can be useful to establish a context for guiding opinions of what material to move and where to move it, in order to avoid maladaptation and for building models to determine species ability to face the impacts of rapidly changing climates (Fady et al. 2016).

Plant selection under changing climates requires preparing not for current climates, but projections of future climates. My studies showed that southern hard maples can be a genetic option for street tree plantings, due to a smaller habit and form and for reforestation efforts focused on resilient species. *A. leucoderme* is adapted to a southern climate similar to climate modeled for the northeastern United States in the future. Revisiting reports of a successful forced genetic cross between the two, conferring traits would seem feasible (Slavin 1950, 1954). Continuing common garden trials in hard maple species would increase our understanding of species response to novel climates, especially in southern hard maple populations. Trial plots representing provenances from range extremes, would provide insight on environmental limitations of hard maples. Provenance sources thriving along climatic transition zones will inform behavioral plasticity of hard maple species. In

terms of the number of provenance sources, the more, the better. The “mass collection” technique was inspired by Anderson after his attempt at ascertaining hard maple hybridization events reported in the field (Anderson 1941). The plastic nature of hard maples has led to misidentification in the field and assayed genotype does not always reflect plant performance, as shown in Chapter 4. Reference genomes for tree species have been slow to develop. Common garden studies and landscape genomic analysis of anonymous markers can be integrated for local and nonlocal observations of variation in population phenotype and used to calculate tree adaptive potential. Adaptive diversity of tree species is valuable information for strategies working towards restoration and conservation on a global scale. Spatial distribution of species adaptive traits can be informed using molecular-based analyses. It should be mentioned that the genetic markers used here (SSRs) are useful because they are not selected as indicators of species fitness. They infer ancestral lineages and therefore genetic similarities or differences between study samples. Markers specific to species fitness would provide genetic profiles of hard maples.

Classification and taxonomic treatment of sugar maple and its allies - The dearth in hard maple scientific literature includes classification of hard maples. Taxonomic treatment of hard maples has been scrutinized by botanists and horticulturalists for more than a century. Hard maple taxa have been described at several levels of taxonomy, species and below (Sargent et al. 1891, Rehder 1940, Desmarais 1952, Kriebel et al. 1969). Inconsistencies are the result of two main factors. The first and most profound is the highly plastic phenotypes hard maples present in the field, which called for new collection techniques. The second is the subjective nature of field observations themselves (Anderson 1938, Anderson 1941, Winston 1999). Genetic studies using plant DNA have been

optimized for speed and accuracy over the last 20 years, but genetic markers specific to sugar maple were only reported recently (Graignic et al. 2013). I am not currently aware of any study where these markers were used to analyze population structure outside of *A. saccharum*, making my genotype study novel. I find this particularly interesting, given the economic and cultural relevance sugar maple itself. It is unclear to me why this group was never investigated beyond the 1980s with much interest. While results revealed differentiation among all study groups to be, at the very least, non-trivial, adaptively neutral markers used for genotyping will explain the evolutionary history or inform the level of speciation among these accessions. Molecular techniques have been applied in studies attempting to differentiate between genetics of black and sugar maple. The first was published in 1998 and used cpDNA to analyze genetic profiles of *A. nigrum* and *A. saccharum*, concluding the two were so similar that they need not be considered different species; cpDNA profiles suggest shared ancestral genes or gene flow between them (St Hilaire et al. 2001). Harrington et al. (2005) conducted a phylogenetic analysis of Aceraceae, and inspired the reassignment of *Acer* into Sapindaceae (Harrington et al. 2005). Molecular markers were developed by Graignic et al. (2013), targeted for use across population genetic studies of sugar maple. One study using these markers has been published, yet is limited to populations of *A. saccharum* Marsh on the northern fringe of the range limit (Graignic et al. 2014). Until the genotypic analysis I conducted in Chapter 4, no genetic studies of southern hard maple species had been done. We now have the groundwork to initiate research studies focused on resolution of taxonomic structure, considering the magnitude of variation within the hard maple species complex. In Chapter 4, one of my objectives was to determine whether hard maple nSSR genotype was a reliable

indicator of water use efficiency among species. Principle Coordinates Analysis revealed clustering patterns among study populations based on genotype data. When compared to pairwise data from Chapter 2 results, however, water use efficiency groupings were not consistent with PCoA results. *Thus, neutral genetic markers are not telling the adaptive story.* Integration of genotype data with robust phenological, histological, and eco-physiological fieldwork would provide accurate and thorough interpretation of genetic analyses that are well founded. Phylogenetic studies in hard maple species would provide insight as to the evolutionary development of hard maple divergence, represented in the distinct gene pools reflected by their genotypic profiles. A clear picture of hard maple ancestral relationships and patterns of diversion will provide data useful in comparative genomics of the sugar maple allies. Questions of genetic compatibility of hard maple species and directionality of genetic exchange can be addressed with the appropriate phylogenetic model.

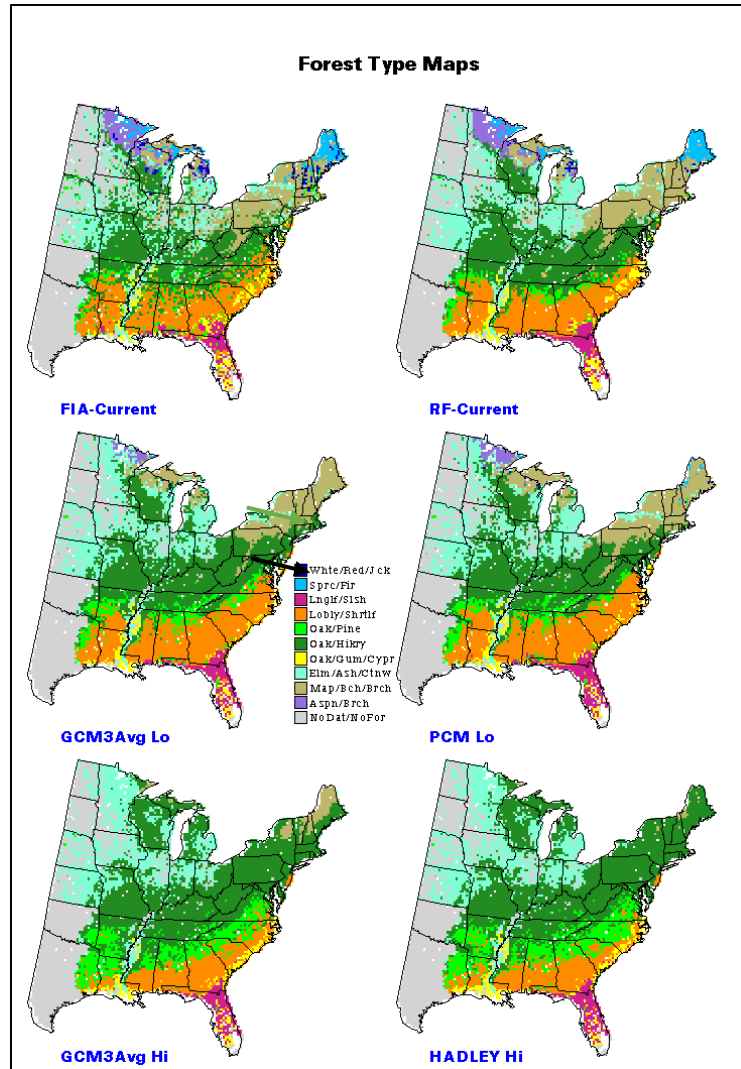
Forest management of hard maples - Advances in forest policy depend on the concern for future societal needs, cooperative efforts across sectors, and particularly on evidence-based data to inform policy makers (Fady et al. 2016). Novel approaches may be required to construct successful policies that consider both societal and environmental demands, while acknowledging the certainty that each of them will fluctuate, often at different rates, over time. Empirical data can inform and influence policy change and development. Several broad questions have been identified, specific to planning for short- and long-term sustainable forest management practices. The first question is whether existing genetic resources in forestry will be appropriate for unpredictable climate change in the future. Desirable traits for forest management will likely be associated with wood

quality and yield and/or tree survivability and overall tree health. Species ill equipped for the transition to novel conditions will not persist, let alone thrive. Coping mechanisms can be delivered to vulnerable species by way of genetic variation. Methods that lead to determination of species adaptive capacities today are needed to verify adaptive potential they may have in the future (Fady et al. 2016). The revitalization of hard maple studies is crucial to providing decision makers with the information to make careful and sustainable policy choices considering future maple forests in United States. It can also inform charge efforts in assisted migration and reforestation planning.

Future Foci

Hard maple research has prioritized sugar maple as a model system, omitting the southern relatives, thus creating a gap in our understanding of hard maple biology and differences within and among the suite of hard maple taxa. It is impossible to overstate the void in the current literature base, relevant to the southern group. Clearly, laying the groundwork for future hard maple investigation is crucial to develop a relevant literature base acknowledging hard maple relationships that is available to students, practitioners, researchers, and, not least of all decision-makers concerned with education and industry. Hard maple species are low-hanging fruit in the context of plant biology research. Opportunity to balance and evolve our understanding of these taxa lies in applications of basic research for plant morphology, physiology, ecology, genetics, and phylogenetics. Reflecting on my results, the direction of hard maple research becomes clear. The integration of tree physiology and forest genomics could be powerful tool to reveal currently unknown genetic relationships among and behavioral differences among hard maple species. The combination of these aspects of plant science would provide more

informed options for selection and breeding programs prioritizing plant resilience in the face of rapid climate change.



(From: https://www.nrs.fs.fed.us/atlas/tree/ew_fortypesn_6pp.png) Figure 5.2. Modeled forest type changes under climate change models of high and low carbon scenarios. Current FIA (forest inventory and analysis) is modeled through the random forest regression model (RF-Current). The most severe scenario is Hadley Hi. The mildest is PCM (parallel climate model) Lo. Averages of three general circulation models (GCMs) are shown as GCM3Avg Lo and GCM3Avg Hi. Green arrow points to legend color for Oak/Hickory forest type, which is modeled to displace Maple/Beech/Birch forest type, indicated in the legend by the black arrow.

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