INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6” x 9” black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600
Ecophysiology and genetic diversity of hard maples indigenous to eastern North America

by

Rolston St. Hilaire

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

Major: Horticulture
Major Professor: William R. Graves

Iowa State University
Ames, Iowa
1998
Graduate College
Iowa State University

This is to certify that the Doctoral dissertation of
Rolston St. Hilaire

has met the requirements of Iowa State University

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program

Signature was redacted for privacy.

For the Graduate College
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iv</td>
</tr>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Dissertation Organization</td>
<td>1</td>
</tr>
<tr>
<td>Literature Review</td>
<td>2</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>15</td>
</tr>
<tr>
<td>FOLIAR MORPHOLOGY AND ANATOMY OF HARD MAPLES (ACERACEAE) INDOGENOUS NEAR THE 43°N LATITUDE</td>
<td>25</td>
</tr>
<tr>
<td>Abstract</td>
<td>25</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>28</td>
</tr>
<tr>
<td>Results</td>
<td>31</td>
</tr>
<tr>
<td>Discussion</td>
<td>34</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>39</td>
</tr>
<tr>
<td>WATER RELATIONS, GROWTH, AND FOLIAR TRAITS OF DROUGHT-STRESSED HARD MAPLES FROM CENTRAL IOWA, EASTERN IOWA, AND THE EASTERN UNITED STATES</td>
<td>55</td>
</tr>
<tr>
<td>Abstract</td>
<td>55</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>59</td>
</tr>
<tr>
<td>Results</td>
<td>65</td>
</tr>
<tr>
<td>Discussion</td>
<td>67</td>
</tr>
<tr>
<td>Literature Cited</td>
<td>71</td>
</tr>
<tr>
<td>CHLOROPLAST DNA MAY BE USED TO RESOLVE PHYLOGEOGRAPHY OF SUGAR MAPLES AND BLACK MAPLES</td>
<td>83</td>
</tr>
<tr>
<td>Abstract</td>
<td>83</td>
</tr>
<tr>
<td>Introduction</td>
<td>83</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>85</td>
</tr>
<tr>
<td>Results</td>
<td>88</td>
</tr>
<tr>
<td>Discussion</td>
<td>88</td>
</tr>
<tr>
<td>References</td>
<td>90</td>
</tr>
<tr>
<td>GENERAL CONCLUSIONS</td>
<td>97</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>98</td>
</tr>
</tbody>
</table>
ABSTRACT

I examined foliar traits of sugar maple (*Acer saccharum* Marsh.) and black maple (*Acer saccharum* Marsh. ssp. *nigrum* Desm. or *Acer nigrum* Michx. f.) indigenous near 43°N latitude from 70° to 94°W longitude. Laminae from 90° to 94°W had the highest surface area and a slightly higher percentage of their area in the proximal portion of the blade than leaves from further east. Up to 800 trichomes/cm² were on the abaxial surface of laminae from west of 85°W longitude, whereas laminae from further east had fewer than 200 trichomes/cm². Westerly laminae had relatively high stomatal frequency, and stomatal apertures of laminae west of 91°W longitude were particularly narrow. Principal component analysis showed that trees west of 93.1°W longitude in Iowa formed a distinct cluster, while trees from the remaining locations clustered into a second group. In another experiment, I compared water relations, growth, and foliar traits of frequently irrigated and drought-stressed hard maples from central Iowa, eastern Iowa, and the eastern United States. Seedlings irrigated frequently had higher xylem diameter, lamina area, lamina dry mass, root dry mass, shoot : root dry mass ratio, and lamina area : xylem diameter ratio than plants subjected to drought. Although drought reduced predawn leaf water potential, midday water potentials were higher for seedlings subjected to drought (-1.44 MPa) than for frequently irrigated plants (-1.92 MPa). Specific lamina mass and trichome frequency were highest for leaves from central Iowa, for which means were 5.97 mg·cm⁻² and 531 trichomes/cm², respectively. Averaged over regions, drought-stressed plants had 483 abaxial stomates/mm², while plants irrigated more frequently had 596 stomates/mm². Interactions of seedling origin and irrigation treatment affected stem length, shoot and
total dry masses, and stomatal conductance, which was reduced during drought only among plants from central Iowa. Restriction site patterns in chloroplast DNA for plants from central Iowa, eastern Iowa, and the eastern United States were determined. Analysis of the ndhA intron revealed that TaqI gave a polymorphic site that showed chloroplast types were not geographically localized. My results could impact the selection and evaluation of hard maples for use in managed landscapes prone to drought.
GENERAL INTRODUCTION

The genus *Acer* contains the section *Saccharina* Pax., which includes sugar maple (*Acer saccharum* Marsh.) and black maple (*Acer nigrum* Michx. f.). These maples also are called hard maples or rock maples and are economically and aesthetically valuable. Although they are commonly used in urban landscapes where water deficit is a common cause of plant stress, little research has been done to compare hard maples for ecological, morphological, and physiological characteristics related to drought resistance. Many studies about genetic diversity in hard maples have been limited to isozyme analysis. Molecular techniques rarely have been used to study genetic diversity in hard maples even though uncertainty exists about the taxonomic relationship and post-glacial colonization history of the hard maples. The objectives of the research on which I report in this dissertation were: 1. to determine whether foliar traits of black maples and sugar maples that could contribute to drought resistance are related to geographic origin, 2. to examine biomass partitioning, foliar morphology, and water relations in response to deficit irrigation among seedlings of black maple and sugar maple from central Iowa, eastern Iowa, and the eastern United States, and 3. to assess genetic diversity in selected populations of hard maples by using chloroplast DNA variation.

Dissertation Organization

This dissertation consists of three manuscripts. The first manuscript will be submitted to the *American Journal of Botany* and is formatted for that journal. The second manuscript is formatted for submission to the *Journal of the American Society for*
Horticultural Science. The third manuscript will be submitted as a short communication to Molecular Ecology. A literature review and general conclusions of this research are included. Acknowledgments follow the general conclusions.

Literature Review

Taxonomy, origin, distribution, and uses of hard maps

The maple family, Aceraceae Lindl., consists only of trees and shrubs (Rehder, 1940). The samara fruit type distinguishes the family and is used to classify the family into two genera. A samara that is winged all around defines the genus Dipteronia Oliv. Species in the other genus, Acer L., have samaras that are winged only on one side (Rehder, 1940). Dipteronia has two species; both are indigenous only to China. Acer has about 148 species (Olson and Gabriel, 1974) found throughout the northern hemisphere (Olson and Gabriel, 1974; Rehder, 1940). Within Acer, the section Saccharina Pax. contains sugar maple and black maple, which also are called hard maples or rock maples (Rehder, 1940). Because of cross compatibility within this section but not with other sections, and the occurrence of intermediate forms, sugar maple (Acer saccharum Marsh.) has been classified as a single species divided into subspecies based on variations in leaf and bark morphology (Desmarais, 1952; Kriebel and Gabriel, 1969). Kriebel (1957) stated that from physiological and taxonomic perspectives, sugar maple more aptly is considered a single species, Acer saccharum Marsh., but notes that the forms saccharum, nigrum, and floridanum show interrelated physiological responses. Nomenclature of the taxa can vary among authors, so I will retain the classification by Rehder (1940) that considers sugar maples
and black maples as two distinct species, *Acer saccharum* Marsh. and *Acer nigrum* Michx. f., respectively.

Unclear migration routes and mode of speciation may contribute to the confusion in the nomenclature of these species. Sugar maple may have existed as a separate species before the glacial period of the Pleistocene era, and cold migration may have caused speciation (Kriebel and Gabriel, 1969). Although evidence for route of migration is scant, several theories exist. One theory postulates that the initial species migrated to the west and north from the southern Appalachians, and the *saccharum* type was eliminated from the western portion of the range (Kriebel and Gabriel, 1969). These species occur in an extensive geographic region in eastern North America (Kriebel, 1957; Little, 1971), but disjunct populations of sugar maples occur in west-central Oklahoma (Dent and Adams, 1983). Cultivars such as ‘Legacy’, ‘Bonfire’, and ‘Green Mountain’ are used in the ornamental plant industry (Conley et al., 1995; Pair, 1994; Santamour and McArdle, 1982). A southern ecotype, *Acer saccharum* Marsh. *ssp. saccharum*, occurs as a disjunct population in Red Rock Canyon in Caddo County, Oklahoma, and has been called Caddo maple. Sugar maple is valued for its wood and shade (Flint, 1997) and is an important source of syrup and sugar (Kriebel, 1957; Olson and Gabriel, 1974). Black maple is valued for autumn foliage color, but cultivars are scarce. ‘Greencolumn’, which forms upright and columnar trees, is the most widely available cultivar of black maple. The genotype was selected in Boone County, Iowa.
Water stress effects on plant growth and distribution

Water is the most limiting physiochemical factor required for plant growth (Boyer, 1982; Jones, 1983). Landscape trees particularly are susceptible to water stress because of restricted rooting volume and diminished water infiltration into roots that results from compacted soils and impervious pavements (Whitlow and Bassuk, 1988). Plant distribution across a moisture gradient depends on drought avoidance and tolerance mechanisms (Pallardy and Rhoads, 1993; Parker et al., 1982), collectively called mechanisms of drought resistance. Although sugar maples are drought sensitive (Ellsworth and Reich, 1992; Pallardy and Rhoads, 1993), and use radiation, water, and nutrients less efficiently than a closely related species, the Norway maple (Acer platanoides L.) (Kloeppel and Abrams, 1995), certain ecotypes like Caddo maple (Pair, 1994) may be relatively drought resistant. Also, Kriebel (1957) described three ecotypes of sugar maple that can be matched to specific urban conditions and conceded that there are at least two regional groupings of sugar maples when related to drought, drought-resistant trees from central and southern regions, and the more drought-susceptible trees from the northern hardwood region. Although the geographical separation between the two regions is unclear and individual tree responses to drought varied, sugar maples in hot, dry climates had the highest drought resistance, while trees from cool, moist climates had the lowest resistance to drought (Kriebel, 1957).

Because black maple is the predominant hard maple in portions of the northeastern quadrant of the United States where mean annual precipitation is relatively low (United States Environmental Data Service, 1968) and droughts are relatively common, Ware
(1983) and (Desmarais, 1952) speculated that black maple is better adapted to drought than sugar maple. For that reason, black maples have been recommended for planting in areas where the ornamental characteristics of sugar maples are desirable but climatic factors such as drought restrict their usefulness (Kriebel and Gabriel, 1969; Ware, 1983).

Graves (1994) compared the development of seedlings of black maple and sugar maple. He found that deficit irrigation impacted dry matter accumulation, stem length, and lamina area of black maple less than those traits of sugar maple. These results were consistent with prior speculation that black maple is more resistant to drier conditions.

However, Hauer (1995) found that the decline in net CO₂ uptake, stomatal conductance, water use efficiency was less rapid for sugar maple seedlings from Oklahoma (Caddo County), Missouri, and Tennessee than for sugar maple seedlings from Ontario and for black maple seedlings from Iowa when dry preconditioned seedlings were subjected to drought. He also found the increase in ratio of internal to ambient CO₂ concentrations in response to moisture stress was slower sugar maple seedlings from Oklahoma, Missouri, and Tennessee than for sugar maple seedlings from Ontario and for black maple seedlings from Iowa. Hauer (1995) concluded that Caddo maples and sugar maples from Missouri and Tennessee acclimated better to water stress than sugar maples from Ontario or black maples from Iowa. Drought resistance of black maples and sugar maples likely is influenced by numerous factors, many of which are traits of the foliage. But direct comparisons about how foliar characteristics of the taxa are related to geography and precipitation are lacking.
Morphological and anatomical adaptations to water stress

Plant pubescence

Traits of foliar morphology used to differentiate sugar maple and black maple also may affect their drought resistance by influencing water relations. Leaves of black maples have more pubescence on abaxial surfaces than leaves of sugar maples (Dansereau and Desmarais, 1947; Desmarais, 1952). In natural plant communities, pubescence may increase along a gradient of decreasing precipitation (Donselman and Flint, 1982; Ehleringer, 1980; Ehleringer et al., 1976). Because pubescence increases boundary layer resistance to leaf water loss (Johnson, 1975; Schuepp, 1993), increased trichome frequency of leaves of eastern redbud (Cercis canadensis L.) along a gradient of decreasing precipitation was considered an adaptation to water deficit by Donselman and Flint (1982). Evaluations of differences in pubescence on the abaxial leaf surfaces of black maples and sugar maples (Dansereau and Desmarais, 1947; Desmarais, 1952) have not involved quantitative measurements.

Lamina area and dry matter partitioning

Although individual laminae of black maple are thinner (Powers, 1967) and have greater surface area than laminae of sugar maple (Desmarais, 1952; Powers, 1967), the impact of this on water use of whole plants (Abrams, 1986; Donselman and Flint, 1982) could be mitigated by numerous factors, including leaf stance and relatively low total lamina surface area (Graves, 1994). In areas where black maples and sugar maples occur together, black maples often are restricted to the more mesic sites (Kriebel and Gabriel, 1969). But leaf area of trees can vary with environmental conditions, with reduced
lamina area being correlated with decreased moisture availability (Abrams, 1988; Abrams, et al., 1992; Donselman and Flint, 1982). The genetic control over lamina growth can be mitigated by environmental factors (Kriedman, 1986) such as drought, which reduces leaf area (Kriedman, 1986; Roden et al., 1990). A high ratio of root : shoot biomass is a possible mechanism to avoid drought stress because a large proportion of the total plant mass is available for water uptake (Nash and Graves, 1993). The length of sugar maple roots increased in dry soil (Pallardy and Rhoads, 1993). When drought was imposed on seedlings of black maples and sugar maples, drought reduced shoot : root biomass ratio more for black maples than sugar maples (Graves, 1994).

Leaf architecture

Leaves of sugar maple have three to five lobes, are coarsely toothed, and have narrow and deep sinuses (Rehder, 1940). In contrast, leaves of black maples have a relatively entire margin because they lack the basal two lobes (Dansereau and Desmarais, 1947). The reduced depth of their sinuses (Rehder, 1940) might affect convective heat loss (Gottschlish and Smith, 1982) and boundary layer thickness (Baker and Myhre, 1969).

Stomatal traits

Direct comparisons of stomatal traits of black maple and sugar maple have not been reported. The number of stomates per unit area of leaf surface, which also is called the stomatal frequency or density, depends on the genotype (Salisbury, 1927; Willmer and Fricker, 1996), or the environment in which the taxon develops (Abrams, 1986; Abrams et al., 1992; Salisbury, 1927). Abrams (1994) noted that plants from xeric environments
had higher stomatal density than plants from mesic environments. Stomatal density, guard cell length, and leaf thickness of sugar maples were similar among saplings at two urban forests sites that had similar environments in Pennsylvania (Kloeppel and Abrams, 1995). Donselman and Flint (1982) observed that *Cercis canadensis* L. plants in dry environments have reduced water loss because of reductions in stomatal frequency and the size of stomatal apertures.

*Leaf anatomy*

Leaf anatomy affects water use efficiency of plants (Nobel, 1980). Water use efficiency is the ratio of net carbon dioxide absorbed to transpiration rate. Mesophyll surface area represents the available surface for carbon dioxide uptake and could indicate species differences in drought tolerance (Ashton and Berlyn, 1992; Nobel, 1980). Thick leaves can help to reduce transpirational water loss (Tipton and White, 1995). Black maples subjected to drought stress in a greenhouse had a higher specific leaf mass than drought-stressed sugar maples (Graves, 1994), which might reflect differences in leaf thickness or cell size that could influence water economy (Ashton and Berlyn, 1992; Graves, 1994).

*Tissue water relations*

For ornamental trees, the capacity to withstand low water potentials without compromising aesthetic value is an important consideration for selection. Although under landscape conditions gradual decreases in soil moisture content may condition the plant to withstand water stress (Edwards and Dixon, 1995), water deficits commonly affect landscape plants (Whitlow and Bassuk, 1988). Predawn and midday leaf water potentials
indicate leaf water status and could reflect the amount of water available in the root zone or the capacity of the plant to transport water (Nash and Graves, 1993). Reported changes in predawn leaf water potential in response to low water availability in root media are variable (Abrams, 1990), but they may directly relate to net photosynthesis and could be used to evaluate genotypic variation in drought responses (Abrams, 1994). Boyer (1982) showed that the genetic improvement in yield of newly released cultivars of soybeans (*Glycine max* L.) was correlated with average afternoon water potential of the cultivars, and he suggested that accelerated genetic progress would have resulted if plants were selected for drought tolerance. When seedlings of the taxa were subjected to drought, black maples from Iowa had a lower osmotic potential at zero turgor than sugar maples from Oklahoma, Missouri, and Tennessee, but had similar osmotic potential as sugar maple seedlings from Ontario (Hauer, 1995). Osmotic adjustment is a major drought avoidance mechanism (Hinckley et al., 1980; Radin, 1983) because it maintains turgor and restricts desiccation during water stress (Abrams, 1988). Sugar maples (Close et al., 1996; Ellsworth and Reich, 1992), black cherry (*Prunus serotina* Ehrh.) (Abrams et al., 1992), and some species of oak (*Quercus* L. *spp.*) (Parker and Pallardy, 1987; Parker et al., 1982), are among the woody taxa that osmotically adjust their cell contents to maintain turgor. Differences in osmotic potential at zero turgor between sugar maples growing at an urban site and in a forest (Close et al., 1996), and at contrasting sites in southwestern Wisconsin (Ellsworth and Reich, 1992), show that sugar maples have a limited capacity to adjust osmotically (Ellsworth and Reich, 1992).
Introgression between black maple and sugar maple

Ecophysiological and morphological similarities of black maples and sugar maples have led to confusion about the genetic relationship between the two taxa. Unclear genetic links between the two taxa are further strengthened because trees with traits that can be ascribed to either of the two types of taxa occur in the zone of sympatry of black maples and sugar maples. The presence of intermediate characters in hard maples led Desmarais (1952) and Kriebel (1957) to suggest that the two taxa may introgressively hybridize. Evidence for hybridization in black maples and sugar maples is scant. Morphology is used to identify hard maples (Dansereau and Desmarais, 1947; Desmarais, 1952; Rehder, 1940), but individuals that possess morphological characters intermediate between the two taxa may be difficult to identify. Methods that lead to unequivocal identification of the species and individuals within species are needed.

Molecular basis for assessing genetic variation in hard maples

Molecular data are preferred to morphological data to resolve ambiguous cases of introgression (Rieseberg and Wendel, 1993) because environment and stage of development may affect morphological traits (Krahl et al., 1993). Significant advantages of using molecular techniques to investigate genetic diversity include that a large number of characters can be analyzed (Karp et al., 1996), and fine levels of variation at the DNA level are revealed (Krahl et al., 1993). Also, molecular information can be combined with data about morphology, anatomy, physiology, and isozyme variation to help characterize botanical diversity. But, reliance on a single source of molecular data for determining phylogenies may lead to incorrect interpretation of the phylogenetic relationship among
taxa (Wendel et al., 1991). Phylogenies derived from nuclear and organelle information will yield more complete phylogenetic relationships and permit plant evolution processes to be evaluated (Wendel et al., 1991).

Restriction fragment length polymorphism, arbitrary primed DNA, amplified fragment length polymorphism (AFLP), variable number of tandem repeats, sequence-tagged simple sequence repeats (SSRs) and polymerase chain reaction (PCR) sequencing are some molecular techniques that can be used to assess botanical diversity (Karp et al., 1996). Although knowledge of the genetic diversity and phylogeography of forest species is needed for tree improvement programs and germplasm conservation (Petit et al., 1993), molecular assessment of the genetic diversity of hard maples indigenous in a wide geographic region is lacking.

Rogstad et al. (1991) found a distinct set of DNA fragments when plants of pawpaw [Asiminia triloba (L.) Dunal.] were surveyed across widely separated sites within the geographic range. Geographical distribution of white willow (Salix alba L.), crack willow (Salix fragilis L.), and their hybrid, Salix xrubens Schrank, determined by AFLP analysis coincided with the previous distribution that was based on morphology. Also, AFLP analysis distinguished clonal individuals of Salix species and showed that introgression could be occurring between the hybrid Salix xrubens and the parent Salix fragilis (Beismann et al., 1997). For black spruce (Picea mariana Mill. B.S.P) gene diversity estimated by allozyme variation and molecular techniques such as random amplified polymorphic DNA (RAPDs) were congruent (Isabel et al., 1995). Although the significance of isozyme variation to plant adaptation is unclear (Isabel et al., 1995), allozyme variation among
fragmented and continuous populations of sugar maples revealed altered patterns of gene flow and increased genetic diversity in fragmented populations (Young and Merriam, 1994; Young et al., 1993). Le Corre et al. (1997) showed that allozyme variation correlated with chloroplast DNA (cpDNA) variation, but not with geographical distances between 21 populations of sessile oaks \[Quercus petraea\] (Matt.) Liebl. They also showed that allozymes revealed less genetic diversity than RAPDs. Also, genetic distance estimated from RAPDs correlated with geographical separation, but genetic distance estimated from cpDNA did not correlate with geography (Le Corre et al., 1997). These observations led Le Corre et al. (1997) to postulate that allozyme loci have different patterns of variation than RAPDs loci.

Krahl et al. (1993) showed that the use of RAPDs markers enabled the distinction of red maple \(Acer rubrum\) L.) cultivars 'Franksred' and 'October Glory', which appeared to be morphologically identical at an early stage of growth. They found unique banding patterns for nine red maple clones, five silver maple \(Acer saccharinum\) L.) seedlings, and four reported cultivars derived from crosses between red maple and silver maple. RAPDs were concluded to be reliable molecular markers for identification of red maple cultivars (Krahl et al., 1993). Lamboy and Alpha (1998) found grape \(Vitis\) L.) cultivars were difficult to identify when morphological traits were used, but they achieved unequivocal cultivar identity by using SSRs.

**Using chloroplast DNA to assess genetic diversity**

Plants have nuclear, mitochondrial, and chloroplast genomes that can be used as sources for sequences that can be amplified by the PCR. Chloroplasts are double-
membraned, intracellular organelles, and they contain all the enzymatic apparatus for photosynthesis (Esau, 1977). In terrestrial plants such as liverwort (*Marchantia polymorpha* L.), cpDNA contains the genes for transcription, translation, and protein complexes needed for chloroplast photosynthesis (Ohyama et al., 1986). Genes of the chloroplast genome are arranged on a single, circular DNA molecule divided into a large single copy and small single-copy region separated from each other by two inverted repeat regions (Hiratsuka et al., 1989; Ohyama et al., 1986; Shinozaki et al., 1986). The entire cpDNA sequence has been elucidated for tobacco (*Nicotiana tabacum* L.) (Shinozaki et al., 1986), a liverwort (Ohyama et al., 1986) and rice (*Oryza sativa* L.) (Hiratsuka et al., 1989). cpDNA evolves at half the rate of the nuclear genome (Wolfe et al., 1987) and lacks the genetic homogeneity caused by pollen flow (Le Corre et al., 1997). Geographical variation in cpDNA is resistant to change by selective pressures (Le Corre et al., 1997). Among tobacco, rice, and the liverwort, the cpDNA is highly conserved (Hiratsuka et al., 1989. In tobacco the cpDNA genome is made up of 155,844 base pairs (Shinozaki et al., 1986), in rice 134,525 base pairs (Hiratsuka et al., 1989), and in the liverwort 121,024 base pairs. The cpDNA genome is often maternally inherited in angiosperms (Dumolin et al., 1995; Le Corre et al., 1997; Petit et al., 1993) and has allowed for phylogenetic studies and reconstruction of postglacial recolonization routes in white oaks (*Quercus spp.*) (Dumolin-Lapèque et al., 1997), studies of population history in sessile oaks (Le Corre et al., 1997), and assessment of intraspecific genetic variation in North American duckweeds (*Lemma* L. spp. and *Spirodela* Schleid. spp.) (Jordan et al., 1996). Thus we expect cpDNA
variation may be a useful tool to investigate genetic and phylogeographic patterns in hard maples.

The PCR can be used to study sequence variation in the cpDNA because specific areas of the genome can be targeted for analysis. Areas of the chloroplast genome that show a level of sequence variation suitable for the taxonomic level of genetic diversity required may be selected for analysis (Jordan et al., 1996). A set of primers can be developed to select specific areas of the chloroplast genome for amplification. The aim is to place primers for the PCR in conserved regions to allow for the amplification of more variable regions (Taberlet et al., 1991). Non-coding regions of the genome show more variation, so these primers are useful for studying genetic variation in many different taxa (Demesure et al., 1995; Taberlet et al., 1991). Chloroplast fragments amplified by PCR can be digested by restriction endonucleases to detect polymorphisms (Demesure et al., 1995; Dumolin et al., 1995). The ndhA intron, which resides in the small single-copy region of the cpDNA (Hiratsuka et al., 1989; Shinozaki et al., 1986), has recently been used to study cpDNA variation in Ipomopsis L. (Wolf et al., 1997) and cotton (Gossypium L.) spp. L.) (Small et al., 1998). The rpl16 intron (Jordan et al., 1996; Small et al., 1998) and the trnL-trnF spacer region (Small et al., 1998; Taberlet et al., 1991) of the chloroplast genome also may be used to evaluate genetic variation.
Literature Cited

Abrams, M. D. 1986. Physiological plasticity in water relations and leaf structure of
understory versus open grown *Cercis canadensis* L. in northeastern Kansas.

Abrams, M. D. 1988. Genetic variation in leaf morphology and plant and tissue water
relations during drought in *Cercis canadensis* L. For. Sci. 34:200-207.

Abrams, M. D. 1990. Adaptations and responses to drought in *Quercus* species of North
America. Tree Physiol. 7:227-238.

Abrams, M. D. 1994. Genotypic and phenotypic variation as stress adaptations in
temperate tree species: a review of several case studies. Tree Physiol. 14:833-
842.

Abrams, M. D., B. D. Kloeppel, and M. E. Kubiske. 1992. Ecophysiological and
morphological responses to shade and drought in two contrasting ecotypes of
*Prunus serotina*. Tree Physiol. 10:343-355.

Ashton, P. M. S. and G. P. Berlyn. 1992. Leaf adaptations of some *Shorea* species to

Baker, D. N. and D. L. Myhre. 1969. Effects of leaf shape and boundary layer
thickness on photosynthesis in cotton (*Gossypium hirsutum*). Physiol.

on distribution of two *Salix* species and their hybrid along a natural gradient.


FOLIAR MORPHOLOGY AND ANATOMY OF HARD MAPLES
(ACERACEAE) INDIGENOUS NEAR THE 43°N LATITUDE

A paper to be submitted to the American Journal of Botany

Rolston St. Hilaire and William R. Graves

Abstract

We examined foliar traits of sugar maple (Acer saccharum Marsh.) and black maple
(Acer saccharum Marsh. ssp. nigrum Desm. or Acer nigrum Michx. f.) indigenous near
43°N latitude from 94° to 70°W longitude. Laminae from 90° to 94°W tended to have
the highest surface area and a slightly higher percentage of their area in the basipetal
portion of the blade than leaves from further east. Up to 800 trichomes/cm² were present
on the abaxial surface of laminae from west of 85°W longitude, whereas laminae from
further east had fewer than 200 trichomes/cm². Westerly laminae also had relatively high
stomatal frequency, and stomatal apertures of laminae west of 91°W longitude were
particularly narrow. Laminar specific mass and thickness did not vary with longitude and
averaged 5.5 mg/cm² and 90 μm, respectively. By using principal component analysis, we
found that data from trees west of 93.1°W in Iowa formed a small, distinct cluster, while
trees at the remaining locations clustered into a large, second group dominated by sources
from New England. While traits varied predictably and changed incrementally across the
distribution, leaves of hard maples west of 93.1°W longitude in central Iowa are distinct in
ways that may influence leaf water relations.

Key words: Acer nigrum; Acer saccharum; geographic variation; provenance; pubescence.
Sugar maple (*Acer saccharum* Marsh) is an economically important tree species found in Canada and the United States. Although most of its native range in the United States is east of the Mississippi River, the contiguous distribution of sugar maple extends as far west as central Minnesota and eastern Kansas (Kriebel and Gabriel, 1969; Little, 1971). Between these western extremes, sugar maple is restricted to only eastern portions of Iowa. In contrast, the contiguous range of black maple (*Acer saccharum* Marsh. ssp. *nigrum* Desm. (Desmarais, 1952) or *Acer nigrum* Michx. f. (Rehder, 1940)) extends west to central Iowa but is not more westerly in other states than the distribution of sugar maple (Kriebel and Gabriel, 1969). Thus, central Iowa is the most westerly portion of the contiguous range of black maple where sugar maple does not occur. Intermediate phenotypes may result from natural hybridization of the two taxa where they are sympatric from eastern Iowa to Vermont. East of western Pennsylvania and New York, however, black maple is not common and is absent in New Hampshire and Maine, where sugar maple is prevalent (Kriebel and Gabriel, 1969). Because black maple is the predominant hard maple in portions of the northeastern quadrant of the United States where mean annual precipitation is relatively low (United States Environmental Data Service, 1968) and droughts are relatively common, Ware (1983) speculated that black maple may be more resistant to drought than sugar maple.

Traits of foliar morphology used to differentiate sugar maple and black maple also may affect their drought resistance by influencing water relations. Leaves of sugar maple have three to five lobes, are coarsely toothed, and have narrow and deep sinuses (Rehder, 1940). In contrast, leaves of black maple have a relatively entire outline because they lack
the basal two lobes (Dansereau and Desmarais, 1947), and the reduced depth of their sinuses (Rehder, 1940) might affect convective heat loss (Gottschlish and Smith, 1982) and boundary layer thickness (Baker and Myhre, 1969). Although individual laminae of black maple may be thinner (Powers, 1967) and have a greater surface area than laminae of sugar maple (Desmarais, 1952; Powers, 1967), the impact of this on water use of whole plants (Donselman and Flint, 1982; Abrams, 1986) could be mitigated by numerous factors, including leaf stance and relatively low total lamina surface area (Graves, 1994). Leaves of black maple have more pubescence on abaxial surfaces (Dansereau and Desmarais, 1947; Desmarais, 1952) than leaves of sugar maple. Because pubescence increases boundary layer resistance to leaf water loss (Johnson, 1975; Schuepp, 1993), increased trichome frequency of leaves of eastern redbud (Cercis canadensis L.) along a gradient of decreasing precipitation was considered an adaptation to water deficit by Donselman and Flint (1982). Although differences in surface area, lobing, and pubescence are used to distinguish black maple and sugar maple, the consistency of differences between the taxa and across a geographical area in which annual precipitation varies has not been assessed. Foliar traits important for leaf water economy but not previously used to separate sugar maple and black maple also may vary geographically. Direct comparisons of stomatal traits that could cause differences in the water use of sugar maple and black maple have not been reported. Stomatal frequency varies among taxa (Salisbury, 1927; Willmer and Fricker, 1996) and the environment in which a taxon develops (Salisbury, 1927; Abrams, 1986). Donselman and Flint (1982) observed that Cercis canadensis plants in dry environments have reduced water loss because of reductions in stomatal frequency and
the size of stomatal apertures. Black maples subjected to drought stress in a greenhouse had a higher specific leaf mass than drought-stressed sugar maples (Graves, 1994), which might reflect differences in leaf thickness or cell size that could affect water economy (Ashton and Berlyn, 1992; Graves, 1994). How stomatal traits and specific leaf mass of sugar maple and black maple differ in relation to the quantity of precipitation across their natural distributions is not known. The objective of this research was to determine whether foliar traits likely to contribute to leaf water relations of sugar maple and black maple are related to geographic origin. Our approach was to measure morphological and anatomical traits of leaves from trees indigenous near the 43°N latitude and to test for relationships between the traits and longitude of origin. We selected this geographical area so that our sample would include leaves from regions in Iowa where black maple is native and sugar maple is not, regions in New England where only sugar maple occurs, and from a zone of sympatry from eastern Iowa to Vermont (Kriebel and Gabriel, 1969; Little, 1971).

MATERIALS AND METHODS

Plant material—Terminal shoots with at least three nodes were collected from up to 10 hard maple trees at each of 24 (1995) and 36 (1996) sites near 43°N latitude from 94°W longitude in Iowa to 71°W longitude in Maine (Table 1). Trees at each site were chosen to represent the foliar diversity of indigenous hard maples in the area. Samples were collected in July, August and September from growth that formed during the year of collection and that was fully exposed to solar radiation. Shoots were kept between moist paper towels sealed in plastic bags during transport to our laboratory. Each shoot was
designated subjectively as sugar maple or black maple. We considered samples from Dolliver State Park, Pammel Woods, and the YMCA Woodland near 94°W longitude in west-central Iowa (Table 1) as reference black maples based on the distinct cordate bases and shallow sinuses of their leaves, traits ascribed to black maple by Rehder (1940). The pubescence and texture of these leaves also was characteristic of black maple (Ware, 1983). Comparisons with these reference shoots were used to designate each shoot as sugar maple or black maple within one day after they were severed from trees. The basipetal end of each stem then was cut under and held in tap water. Shoots then were enclosed in plastic and kept in a dark cooler at 5°C to rehydrate for 12 h.

**Lamina surface area**—Laminar surface area of one leaf from the third oldest pair of leaves was determined with an area meter (Model 3100, LI-COR, Lincoln, NE). This leaf then was dissected under an Olympus SZ60 microscope so that differences in the partitioning of surface area within the laminae could be assessed. While viewing the abaxial surfaces, cuts were made through the center of the midvein and two pairs of lateral veins to divide leaves into sectors designated as distal, middle, and proximal (Fig. 1). The area of each sector, measured by using the LI-COR 3100 area meter, was expressed as a percentage of the total for each leaf or plotted against longitude.

**Trichome frequency**—Trichomes on the abaxial surface of the leaf used for surface area measurements were counted by viewing three 0.237-cm² areas (Fig. 1) with the Olympus SZ60 microscope. For each leaf, the number of trichomes per 1 cm² was determined as an average of the three areas assessed.
Specific leaf mass—After rehydration of the leaves, a cork borer was used to remove a 0.79-cm² disc of lamina tissue from the leaf opposite the one used for surface area and trichome determinations. Discs were taken from the same region of each leaf, 2 to 3 cm above the petiole attachment (Fig. 1). Mass of each leaf disc was determined after it dried at 67°C for 3 d. Specific leaf mass was calculated by dividing dry mass by disc area.

Microscopy—Two leaf samples were cut transversely perpendicular to the midvein from the leaf used to determine specific mass. From each leaf, a sample 10 mm long and 3 mm wide, taken 2 to 3 cm from the tip of the central lobe (Fig. 1), was preserved in formalin-acetic acid-alcohol (FAA). These samples were dehydrated in a graded series of ethanol, and critical-point dried with liquid carbon dioxide. Dried specimens were mounted on brass discs and sputter-coated with gold-palladium. Trichome morphology was examined with a JEOL (JSM-5800LV) scanning electron microscope.

The second sample from each leaf was 15 mm long and 3 mm wide and was taken adjacent to the area removed to determine specific leaf mass (Fig. 1) and preserved in FAA. A subsection 5 mm long and 3 mm wide removed from the end of each of these samples, was rehydrated into water through 50, 30, and 10% ethanol, pressed between paper towels at 23°C, and painted on the abaxial surface with clear finger nail polish (No. 61, Revlon, NY). The polish was allowed to dry for 15 min. These subsections were placed under an Olympus SZ60 zoom stereo microscope (Olympus Optical Co., Tokyo, Japan) equipped with fiber-optic lighting, and the dry replica was lifted from the leaf sample with Dumont forceps (A. Dumont & Fils, Switzerland). Each clear replica was placed on a glass slide, sealed with a cover slip, and observed with an Olympus BH-2 compound microscope.
fitted with a micrometer and ocular grid. Stomata within one 0.05-mm² grid were counted. The length and width of three guard cells chosen at random within the same grid, and the width of the aperture between these cells, were measured and averaged. The larger subsection remaining from the second sample from each leaf was dehydrated in a graded series of ethanol-tertiary butanol and embedded in Paraplast-xtra (Oxford Labware, St. Louis, MO). Serial transverse sections were cut at 10 μm, stained with safranin-fast green, and observed with an Olympus BH-2 microscope. One randomly selected serial section was used to measure total thickness and thickness of adaxial and abaxial epidermal layers, and mesophyll and palisade parenchyma. Two measurements of each thickness component were taken 5 mm from the midvein and averaged.

**Data analysis**—We tested for linear and quadratic relationships between leaf morphology/anatomy and longitude by using regression. Analysis of variance (ANOVA) with year as the main effect was performed for each dependent variable. Separate regression models for data from 1995 and 1996 were determined if F tests from ANOVA showed a year effect at an α of 0.05. Principal component analysis by using the procedure PrinComp of the Statistical Analysis System (SAS, 1990) was used to determine principal component scores based on foliar morphological and anatomical traits that regression analysis showed were related to longitude.

**RESULTS**

All samples from sites east of Chenango Valley State Park in NY (75.841°W longitude) were subjectively judged to be sugar maples, while all samples from Wentland
Wavels, IA (92.98°W longitude), and further west were deemed black maple (Table 1). Traits of most samples obtained from sites between these longitudinal extremes were consistent with morphological descriptions of sugar maples.

ANOVA showed that the year of collection did not affect the surface area of the selected lamina nor the partitioning of area among distal, middle, and proximal sectors. Laminae from westerly sites had the highest surface area, and the relationship of lamina area and the longitude of origin was best fitted by a quadratic regression function (Fig. 2A). Within laminae across all sites, most surface area was partitioned in the middle and distal sectors. The total area partitioned in the middle and proximal sectors was best represented by a quadratic function (Fig. 3). Specific leaf mass did not vary with longitude and was higher in 1996 (5.8 mg/cm²) than in 1995 (5.2 mg/cm²).

Although ANOVA revealed that the number of trichomes/cm² on the abaxial lamina surface over all longitudes was higher in 1996 than in 1995, the relationship of the number of trichome/cm² of lamina surface (trichome frequency) and longitude was similar both years (Fig. 2B). Fewer than 200 trichomes/cm² were typical on laminae from east of 87°W longitude. Regression functions predicted trichome frequency increased from near zero at 80°W to about 800/cm² at 94°W longitude (Fig. 2B), and leaves at the most westerly sites had a trichome frequency of nearly 1200. Leaves from the most westerly sites had the highest number of stomates/mm² (stomate frequency) on the abaxial leaf surface (Fig. 2C) and lowest stomatal aperture (Fig. 2D). A quadratic regression function best represented changes in stomate frequency and the width of the stomatal pore across the sampled region, which did not differ in 1995 and 1996. Trichomes were uniseriate
and arose from both major and minor veins regardless of the location from which the sample was obtained (Figs. 4, 6, and 7). Sugar maples often were glabrous (Fig. 5). Trichome morphology, including surface texture (Fig. 7), was similar for all samples. Variation in dependent variables related to lamina thickness and the dimensions of pairs of guard cells was not related to year of collection or longitude. Averaged over both years and all collection sites, lamina thickness was 90 μM. Mean adaxial and abaxial epidermis thicknesses were 10 and 7.5 μM, respectively. Thickness of the palisade mesophyll averaged 41.5 μM, and mean thickness of spongy mesophyll was 28.5 μM. Guard cell pair length and width averaged 17.0 μM and 17.5 μM, respectively.

Principal component analysis showed the first two components accounted for 75% of the variation of foliar traits that related to longitude (Table 2). The first principal component had high positive factor loading of trichome frequency, lamina area, and stomatal frequency, and high negative factor loading of guard cell pore width. Lamina area in basipetal lobes was the variable that had the largest contribution to the second principal component. The third principal component explained 9.8% of the total variation (Table 2). A scatter plot depicting the relationship between the first and second principal components illustrated two groups separated at about 0.3 of the first principal component (Fig. 8A). While group A consisted of data from all the states we sampled, data from Iowa, Wisconsin and Michigan tended to be clustered away from data from more easterly locations. Group B consisted of plants from four locations in Iowa, Pine Lake State Park, Pammel Woods, YMCA Woodland, and Dolliver Memorial State Park, at longitudes that ranged from 93.078 to 94.083°W. Data from locations in Iowa showed
the most heterogeneity along the projections of the first principal component (Fig. 8A). A plot of all individual values showed the heterogeneity among the groups (Data not shown).

DISCUSSION

Although variation in foliar traits has been reported previously for sugar maples and black maples (Dansereau and Desmarais, 1947; Desmarais, 1952; Kriebel, 1957), our research broadens the base of knowledge by determining if and how longitude relates to lamina area, pubescence, stomatal frequency, stomatal dimensions, leaf anatomy, and specific leaf mass. Because of their effects on plant water relations, these traits could impact drought tolerance of the taxa. Our analysis of the morphological traits that varied with longitude is consistent with previous research that recognizes a population with characters of both taxa is intermediate between allopatric populations of black maples at the western extreme of the range and of sugar maples occurring at the eastern extreme.

The positive quadratic relationship of lamina area with longitude is consistent with the results of (Desmarais, 1952) who showed that Acer nigrum Michx. f. had larger leaves than Acer floridanum Pax., A. leucoderme Small., A. saccharum Marsh., and A. schneckii Rehd. But the large lamina area of leaves from westerly sites is inconsistent with the results of Donselman and Flint (1982) who observed that the lamina area of Cercis canadensis L. decreased as the natural habitat became drier. Mean annual precipitation at our collection sites decreased with longitude (Table 1), which is consistent with the annual climatic pattern for eastern North America (United States Environmental Data Service, 1968). Leaves between 92 and 94°W have a relatively large portion of the total lamina surface
partitioned in the middle and proximal sectors (Fig. 3). The cordate leaf base of black maple, which is distinct from the acute leaf base of sugar maple (Rehder, 1940), could account for the larger lamina area in the proximal lobes. The significance of differences in the partitioning of lamina area among hard maples is unclear. Baker-Brosh and Peet (1997) have shown that lobing patterns may represent adaptations for rapid initiation of photosynthesis in newly emerging leaves of temperate woody species.

In natural landscapes, the association of hard maples with other species forms distinct vegetation types, such as the *Tilia americana* L. vegetation type in central Iowa (Johnson-Groh, 1985), or the maple-beech (*Acer L.-Fagus L.*) forests of eastern North America (Desmarais, 1952). This relationship with other tree species could create a microclimate that tempers responses to a xeric environment. In forest communities, plants with large and thin lamina maximize surface area per volume of photosynthetic tissue because radiation is limited (Mooney, 1980). Although thin leaves may have a ratio of lamina tissue per unit area, or specific leaf mass, less than that of a thick leaf, this may not be a functional disadvantage for hard maples. Ledig and Korbobo (1983) found that in sugar maples native to different altitudes of the White Mountains of New Hampshire, photosynthesis was correlated negatively to specific leaf mass. No comparative data have been reported for black maples. But in an experiment conducted under environmentally controlled conditions, Graves (1994) found total lamina surface area of black maple seedlings was less than that of sugar maples of the same age. Compared to the sugar maples in that study, lamina area of black maples was less affected by water deficit.
However, specific mass of lamina was greater for black maple than for sugar maple (Graves, 1994).

Carpenter and Smith (1981) compared thickness of sun and shade leaves of southern Appalachian hardwoods and found that the plasticity index of sugar maples was the third highest among 23 species of large forest trees. Plasticity index is the ratio of thickness difference between sun and shade leaves to thickness of sun leaves. Black maple leaves would show a comparatively larger response to fluctuating environmental conditions such as diminishing water supplies if they are more phenotypically plastic than other hard maples. Yet in this study, no clear relationship existed between leaf thickness or specific leaf mass and longitude. Similarly, Kriebel (1957) did not find a relationship for leaf thickness that could explain variation in resistance of hard maples to desiccation. The large lamina area of black maples found in nature (Desmarais, 1952), and the maintenance of total lamina area in greenhouse plants (Graves, 1994), suggests that black maples are more phenotypically plastic because in contrasting conditions, lamina area and possibly photosynthetic potential, are maintained.

Our data on the relationship between trichome frequency and longitude present the first quantitative comparison of pubescence of black maples and sugar maples occurring in a zone of sympatry and in their allopatric extremes. In contrast to leaves from eastern locations, which had little pubescence, plants west of 85°W longitude had up to 1200 trichomes/cm² (Fig. 2B). These data substantiate previous observations of pubescence on black maples and sugar maples by Dansereau and Desmarais (1947), Desmarais (1952) and Graves (1994) who concluded that black maples are more pubescent than sugar
maples but provided no quantification. Pubescence is positively associated with xeric environments (Ehleringer, 1980; Donselman and Flint, 1982), and may be a strategy for reducing the loss of water vapor from leaf blades (Johnson, 1975; Schuepp, 1993). Differing environmental conditions in a single location can affect trichome development (Johnson, 1975), which may explain the difference between years in the relationship of pubescence with longitude.

Stomatal frequency had a positive quadratic relationship with longitude, while stomatal aperture had a negative quadratic relationship with longitude (Figs. 2C and D). Annual precipitation is lowest at the western sites we sampled. Thus plants with the greatest number of stomates per unit area and the smallest stomatal apertures were found at the western extreme of our range, where the least precipitation occurs. Our data are consistent with previous observations that plants in xeric environments have higher stomatal densities and smaller stomatal apertures than plants on mesic sites (Salisbury, 1927; Carpenter and Smith, 1981; Abrams et al., 1992). Stomata provide a major resistance to water loss (Ludlow, 1980; Jones, 1983), and stomatal aperture is directly related to water loss from the plant (Willmer and Fricker, 1996). If stomatal frequency is related to environment and has no adaptational relevance to transpiration or assimilation (Salisbury, 1927), leaves from eastern regions have the potential to lose more water per unit area than plants from western regions because of larger stomatal apertures. In addition, stomatal conductance under conditions of water stress could differ between plants of the two regions and affect water use efficiency and plant performance.
Principal component analysis of plant morphological variables that correlated with longitude illustrated that a small group of maples from Iowa were different from samples from other locations. These maples were found in west-central Iowa, at the Pine Lake State Park, Pammel Woods, YMCA Woodland, and Dolliver Memorial State Park sites. Samples from Pammel Woods, YMCA Woodland, and Dolliver Memorial State Park were our reference population of black maples because they showed reduced and shallow lobes, cordate leaf bases, and the occasional presence of stipules as described by Rehder (1940). Their extreme position and homogeneity on the axis of the first principal component (Fig. 8) shows that this group had high positive scores of lamina area, pubescence, and stomatal frequency, and had high negative scores on stomate aperture. If further research indicates the extent to which these traits influence drought resistance, detection of these aberrant genotypes may be relevant to those seeking hard maple clones for planting in managed landscapes prone to drought (Abrams et al., 1990; Pair, 1994).

A group of plants occupied a central position on first principal component, corresponding to the intermediate characteristics of the traits that varied with longitude. Another group of plants that was dominated by those of the relatively mesic environment of New York and the New England states displayed heterogeneity along the axis of the second principal component, but was clustered along the axis of the first principal component. The absence of central Iowa sources from that group suggests that these phenotypes may have a selective disadvantage in comparatively mesic regions (Dansereau and Desmarais, 1947). Our data provide quantitative support for the observation by
Desmarais (1952) that sugar maples and black maples are two distinct groups united by a large intermediate population.

LITERATURE CITED


DANSEREAU, P. AND Y. DESMARAIS. 1947. Introgression in sugar maples II. 
*The American Midland Naturalist* 37: 146-161.


Table 1. Latitude, longitude, elevation, mean annual temperature, mean annual rainfall for collection sites of hard maples in the Midwestern and eastern United States.

<table>
<thead>
<tr>
<th>Site</th>
<th>Year(s)</th>
<th>Longitude (°W)</th>
<th>Latitude (°N)</th>
<th>Altitude (m)</th>
<th>Precipitation (mm)</th>
<th>Temperature (°C)</th>
<th>Taxon composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradbury Mountain State Park, ME</td>
<td>1996</td>
<td>70.183</td>
<td>43.905</td>
<td>122</td>
<td>1149</td>
<td>7.4</td>
<td>0/10</td>
</tr>
<tr>
<td>Harris farm, ME</td>
<td>1995</td>
<td>71.000</td>
<td>43.500</td>
<td>61</td>
<td>1084</td>
<td>6.7</td>
<td>0/10</td>
</tr>
<tr>
<td>Kingston State Park, NH</td>
<td>1995, 1996</td>
<td>71.059</td>
<td>42.927</td>
<td>40</td>
<td>922</td>
<td>7.3</td>
<td>0/20</td>
</tr>
<tr>
<td>Bear Brook State Park, NH</td>
<td>1995, 1996</td>
<td>71.353</td>
<td>43.108</td>
<td>198</td>
<td>964</td>
<td>7.7</td>
<td>0/20</td>
</tr>
<tr>
<td>Greenfield State Park, NH</td>
<td>1995, 1996</td>
<td>71.833</td>
<td>42.958</td>
<td>244</td>
<td>922</td>
<td>7.3</td>
<td>0/20</td>
</tr>
<tr>
<td>Fox State Park, NH</td>
<td>1995, 1996</td>
<td>71.911</td>
<td>43.142</td>
<td>244</td>
<td>964</td>
<td>7.7</td>
<td>0/20</td>
</tr>
<tr>
<td>Hunter Brook Partnership, VT</td>
<td>1996</td>
<td>72.763</td>
<td>42.944</td>
<td>110</td>
<td>1118</td>
<td>7.5</td>
<td>0/8</td>
</tr>
<tr>
<td>Jamaica State Park, VT</td>
<td>1995</td>
<td>72.775</td>
<td>43.107</td>
<td>73</td>
<td>1016</td>
<td>6.0</td>
<td>0/10</td>
</tr>
<tr>
<td>Green Mountain National Forest, VT</td>
<td>1995, 1996</td>
<td>73.118</td>
<td>44.008</td>
<td>488</td>
<td>838</td>
<td>2.0</td>
<td>0/16</td>
</tr>
</tbody>
</table>
| Park Name                          | Year   | Percent Correct | Errors | Insect Species | DG Weight | Error Rate | Error Rate

| Arlington State Park, VT          | 1995   | 73.204          | 43.032 | 701            | 1016      | 6.0        | 0/10        |
| Saratoga National Historical Park, NY | 1995, 1996 | 73.625          | 43.000 | 61             | 1038      | 8.3        | 0/10        |
| Grafton Lakes State Park, NY      | 1996   | 73.667          | 42.750 | 457            | 919       | 8.6        | 0/10        |
| Pack Forest, NY                   | 1995, 1996 | 73.792          | 43.542 | 242            | 891       | 7.4        | 0/20        |
| Chenango Valley State Park, NY    | 1996   | 75.841          | 42.213 | 274            | 940       | 7.7        | 1/9         |
| Fillmore Glen State Park, NY      | 1996   | 76.396          | 42.694 | 305            | 972       | 7.8        | 0/9         |
| Watkins Glen State Park, NY       | 1996   | 76.901          | 42.369 | 305            | 1092      | 7.2        | 0/10        |
| Allegany State Park, NY           | 1995, 1996 | 78.750          | 42.158 | 549            | 1193      | 8.3        | 0/20        |
| Lakeport State Park, MI           | 1995, 1996 | 82.496          | 43.123 | 183            | 787       | 8.6        | 0/7         |
| Sleepy Hollow State Park, MI      | 1995, 1996 | 84.413          | 42.939 | 244            | 787       | 8.6        | 2/17        |
| Saugatuck Dunes State Park, MI    | 1996   | 86.208          | 42.708 | 202            | 973       | 9.9        | 1/9         |
| Silver Lake State Park, MI        | 1996   | 86.511          | 43.679 | 55             | 749       | 4.2        | 0/10        |
| Kettle Moraine State Forest, WI   | 1995, 1996 | 88.500          | 42.867 | 317            | 789       | 3.7        | 0/18        |
| Village of Maple Bluff, WI        | 1996   | 89.383          | 43.075 | 265            | 783       | 4.4        | 0/10        |
Table 1. continued

<table>
<thead>
<tr>
<th>Location</th>
<th>Year(s)</th>
<th>t-values</th>
<th>g-values</th>
<th>MRs</th>
<th>Densities</th>
<th>Distance</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abraham Woods, WI</td>
<td>1995, 1996</td>
<td>89.483</td>
<td>42.667</td>
<td>253</td>
<td>1016</td>
<td>7.7</td>
<td>0/20</td>
</tr>
<tr>
<td>Yellowstone Lake State Park, WI</td>
<td>1996</td>
<td>89.987</td>
<td>42.759</td>
<td>279</td>
<td>838</td>
<td>7.2</td>
<td>0/3</td>
</tr>
<tr>
<td>Dean Thomas Property, WI</td>
<td>1995</td>
<td>90.420</td>
<td>42.975</td>
<td>275</td>
<td>789</td>
<td>7.4</td>
<td>1/8</td>
</tr>
<tr>
<td>Wildcat Mountain State Park, WI</td>
<td>1996</td>
<td>90.561</td>
<td>43.700</td>
<td>305</td>
<td>845</td>
<td>7.8</td>
<td>0/9</td>
</tr>
<tr>
<td>Wyalusing State Park, WI</td>
<td>1996</td>
<td>91.122</td>
<td>42.992</td>
<td>275</td>
<td>807</td>
<td>7.5</td>
<td>0/10</td>
</tr>
<tr>
<td>Yellow River State Forest, IA</td>
<td>1995, 1996</td>
<td>91.250</td>
<td>43.167</td>
<td>317</td>
<td>807</td>
<td>7.5</td>
<td>20/0</td>
</tr>
<tr>
<td>Wapsipinicon State Park, IA</td>
<td>1996</td>
<td>91.283</td>
<td>42.095</td>
<td>293</td>
<td>673</td>
<td>8.8</td>
<td>0/10</td>
</tr>
<tr>
<td>Palisades State Park, IA</td>
<td>1996</td>
<td>91.506</td>
<td>41.907</td>
<td>244</td>
<td>746</td>
<td>8.9</td>
<td>0/10</td>
</tr>
<tr>
<td>Backbone State Park, IA</td>
<td>1995</td>
<td>91.559</td>
<td>42.616</td>
<td>342</td>
<td>895</td>
<td>7.6</td>
<td>0/10</td>
</tr>
<tr>
<td>Maralie Educational Forest, IA</td>
<td>1995</td>
<td>91.667</td>
<td>43.208</td>
<td>366</td>
<td>813</td>
<td>8.2</td>
<td>1/4</td>
</tr>
<tr>
<td>Volga River Recreation Area, IA</td>
<td>1996</td>
<td>91.722</td>
<td>42.865</td>
<td>305</td>
<td>871</td>
<td>7.9</td>
<td>0/10</td>
</tr>
<tr>
<td>Echo Valley Recreation Area, IA</td>
<td>1996</td>
<td>91.765</td>
<td>42.944</td>
<td>305</td>
<td>871</td>
<td>7.9</td>
<td>0/10</td>
</tr>
<tr>
<td>Wentland Wavels, IA</td>
<td>1995, 1996</td>
<td>92.733</td>
<td>43.117</td>
<td>336</td>
<td>843</td>
<td>8.1</td>
<td>10/0</td>
</tr>
<tr>
<td>Mather's Woods, IA</td>
<td>1996</td>
<td>92.983</td>
<td>43.15</td>
<td>342</td>
<td>807</td>
<td>8.1</td>
<td>10/0</td>
</tr>
</tbody>
</table>
Table 1. continued

<table>
<thead>
<tr>
<th>Location</th>
<th>Year(s)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Sample Size</th>
<th>Diameter</th>
<th>Height</th>
<th>BA</th>
<th>Age</th>
<th>Crown Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine Lake State Park, IA</td>
<td>1995, 1996</td>
<td>93.078</td>
<td>42.371</td>
<td>305</td>
<td>856</td>
<td>8.2</td>
<td>20/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Claybanks Forest, IA</td>
<td>1996</td>
<td>93.083</td>
<td>43.207</td>
<td>348</td>
<td>884</td>
<td>8.1</td>
<td>10/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pammel Woods, IA</td>
<td>1995, 1996</td>
<td>93.525</td>
<td>42.500</td>
<td>275</td>
<td>837</td>
<td>7.9</td>
<td>20/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YMCA Woodland, IA</td>
<td>1995, 1996</td>
<td>93.944</td>
<td>42.140</td>
<td>336</td>
<td>837</td>
<td>8.7</td>
<td>20/0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolliver Memorial State Park, IA</td>
<td>1995, 1996</td>
<td>94.083</td>
<td>42.387</td>
<td>305</td>
<td>862</td>
<td>8.4</td>
<td>20/0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Number of samples out of the total number subjectively judged to be *Acer nigrum*. All others were judged to be *Acer saccharum*.  

46
Table 2. Eigenvalues and eigenvectors of the first three principal components from five foliar traits of hard maples indigenous near the 43°N latitude.

<table>
<thead>
<tr>
<th>Principal component</th>
<th>Eigenvalue</th>
<th>Variance</th>
<th>Percentage</th>
<th>Cumulative percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>3.12</td>
<td>62.3</td>
<td>62.3</td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>0.64</td>
<td>12.8</td>
<td>75.1</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>0.49</td>
<td>9.8</td>
<td>84.9</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>First principal component</th>
<th>Second principal component</th>
<th>Third principal component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamina area</td>
<td>0.462</td>
<td>0.194</td>
<td>0.055</td>
</tr>
<tr>
<td>Lamina area in basipetal sectors</td>
<td>0.400</td>
<td>0.816</td>
<td>0.099</td>
</tr>
<tr>
<td>Stomatal density</td>
<td>0.462</td>
<td>-0.372</td>
<td>0.272</td>
</tr>
<tr>
<td>Trichome frequency</td>
<td>0.467</td>
<td>-0.355</td>
<td>0.408</td>
</tr>
<tr>
<td>Stomatal aperture</td>
<td>-0.441</td>
<td>0.179</td>
<td>0.864</td>
</tr>
</tbody>
</table>
Fig. 1. Schematic representation of the areas on the pair of hard maple leaves from which data were taken. The third oldest pair of leaves from terminal shoots of trees were sampled. Trees were from 60 sites near the 43 °N latitude in eastern North America.

Fig. 2. Foliar traits that varied with longitude for hard maples sampled from 60 sites near the 43 °N latitude in eastern North America. (●) Sites sampled in 1995. (○) Site sampled in 1996. Each point is a mean of up to 10 values. (A) Mean lamina area; Lamina area = 1820 - 442 long + 0.28 long^2, r^2 = 0.46. (B) Trichomes on the abaxial leaf surface; For 1995, no. of trichomes/cm^2 = 17700 - 458 long + 2.9 long^2, r^2 = 0.84; for 1996, no. of trichomes/cm^2 = 18400 - 477 long + 3.1 long^2, r^2 = 0.64. (C) Stomates on the abaxial leaf surface; No. of stomates/cm^2 = 3400 - 80 long + 0.54 long^2, r^2 = 0.52. (D) Stomatal aperture; Stomatal aperture = -10.6 +0.44 long - 0.0028 long^2, r^2 = 0.29.

Fig. 3. Relationship between lamina area partitioned in the distal, middle, and proximal sectors of hard maple leaves collected from 60 sites near the 43 °N latitude in eastern North America. (●) Sites sampled in 1995. (○) Sites sampled in 1996. Each point is a mean of up to 10 values. For the middle sector, area = 600 - 15 long + 0.09 long^2, r^2 = 0.42. For the proximal sector, area = 196 - 4.8 long + 0.03 long^2, r^2 = 0.45. No statistical relationship was found for the area partitioned in the distal sectors and longitude.

Figs. 4-7. SEM images of the abaxial leaf surface of hard maples collected near the 43 °N latitude in eastern North America. 4. Black maple leaf collected from
Dolliver Memorial State Park in Iowa. Bar = 100 μm. 5. A sugar maple leaf collected from Fox State Park in New Hampshire. Bar = 100 μm. 6. Black maple leaf collected from Dolliver Memorial State Park in Iowa. Notice that numerous trichomes were present on the main vein. Bar = 100 μm. 7. Trichome from a black maple leaf from Dolliver Memorial State Park in Iowa. Notice the ridges on the trichome surface. Bar = 5 μm.

Fig. 8. Plot of the relationship between the first and second principal components from the analysis of morphological traits that varied with longitude for hard maple leaves sampled near the near the 43°N latitude. (A) Each point represents the average of samples from each site. Points from four geographical regions have the following symbols: (●) Iowa; (○) New York, New Hampshire, Vermont, and Maine; (■) Michigan; (□) Wisconsin. Notice that most plants in group A were between 70.18° to 92.98 °W longitude. Plants in group B were within 93.08 to 94.08 °W longitude. (B) Individual points of the same data set as in Fig. 8A are shown.
Fig. 1
<table>
<thead>
<tr>
<th>Number of stomates/mm²</th>
<th>Number of abaxial trichomes /cm²</th>
<th>Lamina area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>400</td>
<td>500</td>
<td>600</td>
</tr>
<tr>
<td>700</td>
<td>800</td>
<td>900</td>
</tr>
<tr>
<td>1000</td>
<td>1200</td>
<td>150</td>
</tr>
<tr>
<td>200</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

Stomatal aperture (µm):
- 4.5
- 5.5
- 6.5
- 7.5
- 8.5

Fig. 2

Longitude (°W)
Fig. 3
Figs. 4, 5, 6, and 7
Second principal component

Fig. 8
WATER RELATIONS, GROWTH, AND FOLIAR TRAITS OF
DROUGHT-STRESSED HARD MAPLES FROM CENTRAL IOWA, EASTERN IOWA,
AND THE EASTERN UNITED STATES

A paper to be submitted to the Journal of the American Society for Horticultural Science
Rolston St. Hilaire and William R. Graves

Additional index words. Acer nigrum, Acer saccharum, pubescence, stomates, woody landscape
plants, leaf anatomy

ABSTRACT. We compared growth, dry matter partitioning, leaf anatomy and morphology,
and leaf water relations of frequently irrigated and drought-stressed seedlings of hard
maples from central Iowa, eastern Iowa, and the eastern United States. Seedlings two
months old from both areas of Iowa had shorter stems and higher specific lamina mass,
root : shoot dry mass ratio, root : lamina dry mass ratio than seedlings from the eastern
United States when the drought treatment began in 1996. Frequently irrigated plants had
higher xylem diameter, lamina area, lamina dry mass, root dry mass, shoot dry mass : root
dry mass ratio, and lamina area : xylem diameter ratio than plants subjected to at least
four drought cycles in 1996 and five drought cycles 1997. Although drought reduced
predawn leaf water potential, midday water potentials were higher for seedlings subjected
to drought (-1.44 MPa) than for frequently irrigated plants (-1.92 MPa). Neither region,
irrigation treatment, nor their interaction affected osmotic potential at full turgor, which
averaged -1.5 MPa. The highest specific lamina mass and trichome frequency, 5.97
mg·cm⁻² and 531 trichomes/cm² respectively, were associated with leaves from central
Iowa. Over the three regions, drought-stressed plants averaged 483 stomates/mm² of
abaxial leaf surface, while frequently irrigated plants had 596 stomates/mm². Neither
irrigation nor region influenced dimensions of stomates. The interaction between origin of
seedlings and irrigation frequency affected stem length, shoot dry mass, total dry mass, and
stomatal conductance. Plants subjected to drought from central Iowa retained 28% of
their stem length relative to their corresponding controls, while plants from eastern Iowa
and the eastern United States maintained 24 and 22%, respectively. High specific leaf
mass, high trichome frequency, low biomass accumulation in the shoots relative to the
roots, and decreased stomatal conductance, are among the potential mechanisms of
drought resistance expressed relatively strongly among plants from westerly regions during
the first two years of seedling development.

Landscape trees are susceptible to water stress because of restricted rooting volume
and the diminished water infiltration into roots that results from soil compaction and
impervious pavements (Whitlow and Bassuk, 1988). Sugar maples (*Acer saccharum*
Marsh.) are drought-sensitive landscape trees (Ellsworth and Reich, 1993; Pallardy and
Rhoads, 1993), and use light, water, and nutrients less efficiently than Norway maple
(*Acer platanoides* L.), which is frequently planted in urban landscapes (KloeppeL and
Abrams, 1995). Certain ecotypes of sugar maple and its allied taxa may be drought
tolerant (Kriebel, 1957; Pair, 1994).
Because black maple \([\textit{Acer saccharum} \text{Marsh. ssp. nigrum} \text{Desm. (Desmarais, 1952)}]\) or \([\textit{Acer nigrum} \text{Michx. f. (Rehder, 1940)}]\) is the predominant hard maple in portions of the northeastern quadrant of the United States where mean annual precipitation is relatively low (United States Environmental Data Service, 1968) and droughts are relatively common, Ware (1983) and (Desmarais, 1952) speculated that black maple is better adapted to drought than sugar maple. Black maples have been recommended for planting in areas where the ornamental characteristics of sugar maples are desirable but, water stress limits tree function (Kriebel and Gabriel, 1969; Ware, 1983). Yet, little work has been done to compare the morphological and physiological responses of black maple and sugar maple to water deficit.

Graves (1994) compared the development of seedlings of black maples and sugar maple and found that deficit irrigation impacted dry matter accumulation, stem length, and lamina area of black maple less than deficit irrigation affected these traits of sugar maple. Drought-stressed black maple had a higher specific leaf mass and a lower shoot : root biomass ratio than drought-stressed sugar maples. These results were consistent with prior speculation that black maple is more resistant to drier conditions, but drought tolerance of the taxa likely is influenced by numerous factors, including several foliar traits. Although in nature individual laminae of black maple are thinner (Powers, 1967) and have a larger surface area than those of sugar maple (Desmarais, 1952; Powers, 1967), the impact of this on water use of whole plants could be mitigated by numerous factors, including leaf stance and relatively low total lamina surface area (Graves, 1994). In several plant taxa, drought stress reduces leaf area (Abrams, 1988; Abrams et al., 1992;
Kriedman, 1986; Roden et al., 1990), but foliar traits such as pubescence (Donselman and Flint, 1982; Ehleringer, 1980; Ehleringer et al., 1976; Johnson, 1975; Schuepp, 1993) and thick leaves (Tipton and White, 1995) also could temper the effects of drought.

Stomata have a strong influence on plant water relations (Jones, 1983), but direct comparisons of stomatal traits of black maple and sugar maple have not been reported. Stomatal frequency depends on the species (Salisbury, 1927; Willmer and Fricker, 1997), and the environment in which the taxon develops (Abrams, 1986; Abrams et al., 1992; Salisbury, 1927). Plants from xeric environments have higher stomatal frequency than plants from mesic environments (Abrams, 1994; Donselman and Flint, 1982).

Predawn (Ψpd) and midday (Ψmd) leaf water potentials indicate leaf water status and could reflect the amount of water available in the root zone or the capacity of the plant to transport water. Ψpd has been used to evaluate genotypic variation in drought responses (Abrams, 1994). Osmotic adjustment is a major drought avoidance mechanism (Hinckley et al., 1980; Radin et al., 1983) because it maintains turgor and restricts desiccation during water stress (Abrams, 1988). Sugar maples (Close et al., 1996, Ellsworth and Reich, 1992), black cherry (Prunus serotina Ehrh.) (Abrams et al., 1992), and some oak (Quercus L. spp (Parker and Pallardy, 1987; Parker et al., 1982;) are among the woody species that osmotically adjust their cell contents to maintain turgor. Although sugar maples have the capacity to adjust osmotic potential at zero turgor during drought, that capacity may be limited compared with other taxa (Ellsworth and Reich, 1992).

Our objective was to determine drought effects on water relations, growth, and foliar morphology and anatomy for hard maple plants grown from seeds collected from
trees indigenous to central Iowa, eastern Iowa, and Eastern United States. Seedlings native
to these three regions were chosen for this analysis because they represented regions where
black maples and sugar maples are sympatric and allopatric.

**Materials and Methods**

**Plant Material.** In Sept. 1995, samaras were collected from sugar maple trees in
eastern Iowa and from black maple trees in central Iowa. On 11 Jan. 1996, we received
sugar maple samaras that had been cold-stratified from the Uihlein Sugar Maple Field
Station in Lake Placid, N.Y. Central Iowa sources were two trees from the campus of Iowa
State Univ., one of which was in Pammel Woods, one tree from the YMCA Woodland in
Boone County, and two trees from Dolliver Memorial State Park in Webster County.
Samaras from eastern Iowa were from two trees in Yellow River State Forest in Allamakee
County, one tree from Palisades-Kepler State Park in Linn County, and two trees from
Backbone State Park in Delaware County. Sources from the eastern United States were
one tree each in Underhill and Starksboro in Vt., one tree in Strafford, N.H., and two trees
in Lake Placid, N.Y. Seed sources from central and eastern Iowa were stratified for 19
weeks. We continued the stratification of sources from the eastern United States for nine
weeks. All samaras we received were stratified at 4 °C in opaque plastic bags filled with
damp Sphagnum moss.

On 19 and 20 March 1996, samaras were sown into standard plastic pots (Belden
Plastics, St. Paul, Minn.) filled with 3 coarse-grade perlite : 1 medium-grade vermiculite
(Strong-Lite, Seneca, Ill.) (by volume). Plastic screens were placed over drainage holes at
the bottom of the pots to retain the rooting medium during the experiment. Samaras were
sown singly in a pot if the radicle had emerged, or three to a pot if the radicles were not visible. After sowing, seeds were misted daily with tap water for 16 d. On 27 March, pots that had multiple seedlings were thinned to contain a single seedling. Then, seedlings were irrigated with a solution that contained 3 Peters Excel All Purpose 21N-2.2P-16.6K : 1 Peters Excel Calcium-Magnesium Special 15N-2.2P-12.5K (Grace Sierra, Milpitas, Calif.) by mass. This solution provided nitrogen at 175 mg•L⁻¹ and was used for subsequent irrigations, unless stated otherwise.

MAINTENANCE OF IRRIGATION TREATMENT IN 1996. On 21 May, 20 uniform plants from each seed source were selected. Plants were arranged randomly on a glasshouse bench. A border row of plants was placed around the plants selected for the experiment to minimize edge effects. Plants received supplemental lighting from Slyvania incandescent lamps (GTE Products, St. Mary's, Penn.) to maintain daylength at 16 h. Mean daily midday temperature and photosynthetically active radiation monitored at canopy level were 28 °C and 380 μmol•m⁻²•s⁻¹. Ten plants from each of 20 from each seed source were assigned randomly to water deficit treatment, and the other 10 plants from each seed source were irrigated every other day to serve as controls. On the day the experiment started, one plant from each of irrigation treatment-seed source combination was selected randomly and harvested. Stem length of these plants was measured. Lamina area of leaves with laminae that were ≥ 2.5 cm long was determined with an area meter (LI-3100, LI-COR, Lincoln, Neb.). Shoot dry mass was determined and included petiole, epicotyl, leaves with laminae < 2.5 cm long, and 3 cm of the hypocotyl adjacent to the cotyledons or their scars. Dry mass of the remainder of the hypocotyl was included with root dry mass. A disc
(0.95 cm$^2$) of lamina tissue was taken from between the midvein and the most basipetal first-order lateral vein from a leaf of the third pair of fully expanded leaves. Specific mass of lamina was determined by dividing disc dry mass by disc area after the discs were dried at 67 °C for 3 d.

Six of the remaining nine plants assigned to water-deficit treatments within seed sources served as indicators pots. For each samara source, plants assigned randomly to water-deficit treatments were irrigated to container capacity and allowed to drain when the mass of the indicator pots decreased by 40%; this constituted the end of a drought cycle. Volumetric water content at 6 cm below the surface of the rooting medium was determined at the end the fifth drought cycle with a Theta Probe (type HH1, type MLI sensor; Delta-T Devices, Cambridge, England), and averaged 0.037 m$^3$·m$^{-3}$. At the end of each drought cycle, seedlings were irrigated and allowed to drain for 2 h. A new initial mass of indicator pots of each source then was taken; these updated mass values were used to determine subsequent decreases in pot mass for the next drought cycle. Drought treatments were maintained until 8 Sept., when plant survival was assessed. On 9 Sept., plants were moved to a glasshouse with no supplemental irradiance and kept at 13 °C until 30 Oct. All plants were irrigated with tap water as needed to keep the medium moist. On 1 Nov., plants were moved to dark coolers maintained at 5 °C where they were misted with tap water every 10 d for 16 weeks.

MAINTENANCE OF IRRIGATION TREATMENT IN 1997. On 27 Feb. 1997, plants were transferred to a glasshouse maintained at 21 °C. Dead and diseased plants or those that did not break bud were discarded. On 26 Mar., plants were randomized, and high-pressure
sodium lamps were used in 16-h photoperiods. Plants selected in 1996 for irrigation every other day and for drought treatments were retained in their same treatments in 1997. Except for plants from the Pammel Woods, Lake Placid, and Underhill seed sources, which each had two plants assigned to drought, three plants from each seed source were assigned randomly to drought treatments and also were used as indicator pots. Irrigations were managed as in 1996. Treatments were maintained through five drought cycles.

On the day that each seed-source group ended the fifth drought cycle, irrigation was withheld. On the following morning, ψpd of one leaf from the youngest pair of mature leaves was estimated for each plant within that source by using a pressure chamber (PMS Instruments, Corvalis, Ore.). At midday, stomatal conductance of the opposite leaf was measured with a steady state porometer (LI-1600, LI-COR, Lincoln, Neb.), and immediately afterwards, ψmd of that leaf was determined. Well-watered plants and plants in the drought treatments were measured in alternate order for ψpd and ψmd. The pair of leaves immediately basipetal to the leaves used for ψpd and ψmd from each of two randomly selected plants in each irrigation treatment was removed for development of pressure-volume curves. Petioles of these leaves were cut under deionized water. Leaves were rehydrated overnight by submerging the cut petiole ends in deionized water and enclosing them in opaque plastic bags. The bagged leaves were stored in the dark and kept at 4 °C. A pressure-volume curve for one leaf from each plant was determined by using the pressure chamber and the techniques of Tyree and Hammel (1972). One leaf was retained as an alternate for the leaf used for pressure-volume analysis. On the other leaf of both plants, two discs (each 0.95 cm²) of lamina tissue from either side of the midvein and 2 to 3 cm
above the petiole attachment were taken. One disc was dried at 67 °C for 3 d, and dry mass was divided by disc area to determine specific leaf mass. The other disc was used for microscopy.

MICROSCOPY. The disc selected for microscopy was divided transversely along its axis into three equal sections and preserved in formalin-acetic acid-alcohol (FAA; Berlyn and Mische, 1976). An outer section was chosen randomly and rehydrated into water through 50%, 30%, and 10% ethanol. The section was pressed, and stomate impressions were made by applying a layer of clear fingernail polish (No. 61, Revlon, N.Y.) on the sample. The polish was allowed to dry for 15 min, and the clear matrix was lifted with forceps and placed on a glass slide. A cover slip was placed over the matrix, and the ends of cover slip were sealed with Permont (Fisher Scientific, Fair Lawn, N.J.). Each clear matrix was observed with an Olympus BH-2 compound microscope fitted with an ocular grid and micrometer. Stomata within one 0.05-mm² grid were counted. Three guard cell pairs were chosen at random within the same grid. The length and width of one guard cell in each of the three pairs of cells was determined. Also, the width of the aperture between each of the three guard cell pairs was determined, and an average stomatal aperture was determined. The middle section was dehydrated in a graded series of ethanol-tertiary butanol and embedded in Paraplast-xtra (Oxford Labware, St. Louis, Mo.). Serial sections were cut at 8 μm, stained with safranin-fast green, and observed with the Olympus BH-2 microscope. Total leaf thickness, thickness of the abaxial and adaxial epidermis, and thickness of palisade and spongy parenchyma were measured. Surface area of the leaf from which the discs were taken was measured with the area meter. The space created by disc removal was
blocked with opaque adhesive tape before leaf area was measured. Dry mass of the disc used for microscopy was estimated from leaf specific mass. After lamina area was determined, trichome frequency on that leaf was determined by counting trichomes on the abaxial leaf surface within the field of view (0.237 cm²) of an Olympus SZ60 (Olympus Optical Co., Tokyo, Japan) stereomicroscope fitted with fiber optic lighting. Three areas were counted, and a mean value was determined. One area was along the midvein, 3 cm from the apex of the central lobe, and two locations were immediately above the areas where discs had been removed.

DESTRUCTIVE HARVEST. All seed-source groups had completed five drought cycles on 27 July. On 31 July plants were harvested destructively. Epicotyl length, lamina area, and shoot and root dry mass were determined in the same way as described for the initial harvest. Lamina area and dry mass of leaves used for pressure-volume curves and microscopy were added to total lamina area and dry mass.

DATA ANALYSIS. For the initial harvest, data were analyzed as a completely randomized design with 10 single-plant replications in each of three regions. The experimental unit was a single plant in a pot. Osmotic potential at full turgor was determined by analyzing the linear portion of pressure volume curves with PROC REG of Statistical Analysis System (SAS Institute, Cary, N.C.). All other data were analyzed as a completely randomized design with a factorial combination of two irrigation regimens, five seed sources in each of three regions. Seed source was nested within regions. For each region, the mean values for irrigation were plotted against the mean variable value averaged over irrigation treatments when the interaction of main effects was significant.
Results

INITIAL SEEDLING DEVELOPMENT. Epicotyl length, lamina area, and specific leaf mass of seedlings differed among regions the day the treatment started. Seedlings from seed sources in the eastern United States had the longest epicotyls, which averaged 68 mm (Table 1). Total lamina area of plants from the eastern United States was higher than that for plants in central Iowa and eastern Iowa. Specific lamina mass was the lowest (3.8 mg·cm⁻²) in plants from the eastern United States. Plants from central Iowa and eastern Iowa had similar lamina area (Table 1). Shoot and root dry mass were similar for seedlings from all regions (Table 1). Seedlings from central Iowa and eastern Iowa had higher root : shoot dry mass ratio and root dry mass : lamina dry mass ratio than plants from the eastern United States (Table 1). Lamina dry mass and total seedling dry mass did not vary with region.

SEEDLING SURVIVAL IN 1996. In the second season of drought, 47, 53, and 51 plants were retained for central Iowa, eastern Iowa, and the eastern United States respectively. Among plants from central Iowa, one plant died, and 31 were infected with Fusarium wilt. Fusarium infected 23 plants from eastern Iowa, and 28 plants from the eastern United States. For eastern Iowa and the eastern United States respectively, six and four plants died. Eleven, eight, and seven plants from central Iowa, eastern Iowa, and the eastern United States respectively, did not break bud.

DESTRUCTIVE HARVEST IN 1997. At the end of the experiment in 1997, well-watered plants had higher lamina area, lamina dry mass, xylem diameter, root dry mass, shoot : root dry mass ratio, and lamina area : xylem diameter ratio than plants subjected to
fifth drought cycles (Table 2). Interactions of seedling origin and irrigation treatment affected epicotyl length (plant height) (Fig. 1A), total dry mass (Fig. 1B), shoot dry mass (Fig. 1C), and stomatal conductance (Fig. 1D). Plants subjected to drought from central Iowa retained 28% of their epicotyl length relative to their corresponding controls while plants from eastern Iowa and the eastern United States maintained 24 and 22%, respectively (Fig. 1A). Stomatal conductance was not reduced by drought among plants from eastern Iowa and the eastern United States, but plants from central Iowa showed a 48% decrease in stomatal conductance in response to drought (Fig. 1D).

**FOLIAR TRAITS.** Specific lamina mass and trichome frequency were unaffected by irrigation regimen but varied among the regions of origin (Table 3). Leaves from central Iowa had the highest specific mass, 5.97 mg·cm⁻². Leaves from this region also were the most pubescent and had an average of 531 trichomes/cm² on the abaxial surface. Leaves from the eastern United States were glabrous (Table 3).

Although the interaction of these main effects was not significant, origin of seedlings and irrigation treatment affected the number of stomates on the abaxial leaf surface (Table 4). Plants from central Iowa had more stomates than those from eastern Iowa and the eastern United States (Table 4). Plants that were well-watered had more stomates on the abaxial leaf surface than drought-stressed plants (Table 4). Guard cell pair width and length, and stomatal aperture averaged 16, 15, and 5 μm, and were not affected by region, irrigation treatment, or their interaction. Mean lamina thickness was 91 μm. Plants subjected to drought had an average lamina thickness of 93 μm, while frequently irrigated plants had an average lamina thickness of 89 μm. Individual lamina
showed variation in thickness and internal anatomy, but neither region, irrigation
treatment, nor their interaction affected leaf thickness and that of the abaxial and adaxial
epidermis and spongy and palisade parenchyma.

LEAF WATER RELATIONS. $\psi_{pd}$ was lower for plants subjected to drought (-0.85 MPa)
than for well-irrigated plants (-0.77 MPa) (Table 2). $\psi_{md}$ was lower among plants in the
control treatment (-1.92 MPa) than in plants subjected to drought (-1.44 MPa). Neither
region, irrigation treatment, nor their interaction affected osmotic potential at full turgor,
which averaged

-1.5 MPa.

Discussion

Our results show that drought affected growth and water relations of all plants
regardless of geographic origin. The lack of regional differences in water relations and the
paucity of regional differences among growth parameters and foliar attributes suggests little
difference in drought resistance may exist among plants from eastern and western
locations. However, growth and biomass partitioning of plants harvested before deficit
irrigation treatments started and the differences in specific lamina mass, pubescence, and
stomate frequency, indicate that western populations possess traits often associated with
plants relatively resistant to xeric conditions, at least early in seedling ontogeny.

Plants from xeric environments tend to have smaller and thicker leaves with higher
specific leaf mass (Carpenter and Smith, 1981), and these plants often partition more
biomass below the ground than to the shoot system (Abrams, 1990; Pallardy and Rhoads,
1993; Ware, 1983) compared to plants from mesic sites. These traits are consistent with
the early growth traits of black maples we observed in 1996 (Table 2) and may represent strategies to minimize water losses and simultaneously establish a root system that can explore a relatively large soil volume for moisture. If these traits persist throughout ontogeny in the field, black maples may need less water for survival in the landscape (Graves, 1994) than other taxa of hard maple. However, drought (Khalil and Grace, 1992) or prolonged growth (Pallardy and Rhoads, 1993) may alter the pattern of biomass partitioning over time. Indeed, our data from plants destructively harvested in 1997, show the low lamina area and high root : shoot biomass ratio of black maples relative to sugar maples in eastern Iowa was lost by 1997.

Deficit irrigation reduced growth and biomass accumulation regardless of the source of seedlings. But the percentage reduction in lamina area (76%) was more than that of root dry mass (46%). This consistent with the previous contention that one of the most pronounced effects of drought is reduced leaf area (Kriedman, 1986; Roden et al., 1990), but the effects drought on root growth may be species dependent (Pallardy and Rhoads, 1993) or could be confounded by the changing mechanical properties of the medium in response to an altered moisture content (Hsiao, 1973). Although plants from eastern Iowa and the eastern United States had comparatively higher shoot growth than plants from central Iowa at the start of the experiment, those plants were more severely affected by drought than the black maples. For example, droughted plants from central Iowa retained 28% of their epicotyl length relative to their corresponding controls, while plants from eastern Iowa and the eastern United States maintained 24 and 22%, respectively (Fig. 1A). Frequently irrigated plants from central Iowa had the highest stomatal conductances.
However, when these plants were subjected to deficit irrigation they showed the largest percentage (48%) decrease in conductance compared to plants from eastern Iowa and the eastern United States. Stomatal conductance of plants in eastern Iowa increased slightly in response to drought (Fig. 1D), but the biological significance of this is not known.

$\psi_{pd}$ and $\psi_{md}$ were intended to indicate plant water status at the least stressed and most stressed periods of the photoperiod. We found no regional differences in these dependent variables (Table 2). This result for $\psi_{pd}$ suggests that plants from different regions were able to rehydrate overnight to similar extent. The significantly lower $\psi_{md}$ of controls compared with drought-stressed plants may be due to the comparatively large lamina area of the controls (Table 2). At midday, evapotranspiration from the larger lamina area of well-irrigated plants is likely to be higher because of the increased surface for water loss.

Our data on osmotic potential at full turgor show that no osmotic adjustment occurred during this study. Similarly, limited osmotic adjustment has been found in several tree species (Ellsworth and Reich, 1992; Hinckley et al., 1980), and this result is consistent previous observations that sugar maples have a limited capacity to osmotically adjust cell contents (Ellsworth and Reich, 1992). Although osmotic adjustment was not evaluated, Hauer (1995) found that black maples from Floyd County, Iowa, had a lower osmotic potential at zero turgor than sugar maples from Oklahoma, Missouri, and Tennessee, but had an osmotic potential at zero turgor similar to that of sugar maples from Ontario, Canada. Differences in the capacity to adjust cell contents osmotically is often unrelated to geographic origin (Abrams et al., 1990), but associated with the season of growth
Mechanisms of drought resistance in black and sugar maples may be unrelated to cell solute accumulation.

Although the values for leaf thickness tended to be larger for drought-stressed plants, this difference was not significant. The lack of an effect of drought stress on leaf thickness is consistent with the observation that for sugar maples, no link exists between leaf thickness and resistance to desiccation (Kriebel, 1957). Specific lamina mass was highest (5.97 mg cm$^{-2}$) in plants from central Iowa (Table 4), and was not affected by irrigation. Because no regional differences in leaf thickness and the partitioning of leaf layers were found, the relatively high specific leaf mass of black maples may be due, among other factors, to a dense mass of chloroplasts (Kloeppel and Abrams, 1995).

We know of no other study that quantitatively compares pubescence of black maples and sugar maples grown under similar environmental conditions. Our study substantiates previous observations of the extent of pubescence on black maples and sugar maples by Dansereau and Desmarais (1947), Desmarais (1952), and Graves (1994), who concluded that black maples are more pubescent than sugar maples but provided no quantification. Sugar maple leaves from the eastern United States were glabrous, while black maples from central Iowa had up to 531 trichomes/cm$^2$. Pubescence may be a mechanism for reducing the loss of water vapor from leaf blades (Johnson, 1975; Schuepp, 1993).
Our data are consistent with the observation that plants indigenous to xeric environments have higher stomate frequency than plants form mesic environments (Ehleringer, 1980; Donselman and Flint, 1982). In contrast, water deficit stress may limit cell division (Kriedman, 1986), which may explain why drought-stressed plants had fewer stomates than well-irrigated plants (Table 4). However, the decrease in stomatal conductance between drought-stressed plants and well-irrigated controls was comparatively larger for black maples than for sugar maples. Stomatal sensitivity to drought depends on the species and could be influenced by prior water stress (Hsiao, 1973; Ludlow, 1980).

Reduced stomatal conductance, pubescence, high stomatal density, high specific mass of lamina, and low investment in shoot growth suggest that black maples may use less water in landscapes prone to drought than sugar maples. The lack of regional differences in tissue water relations suggests that mechanisms of drought resistance of hard maples may be related primarily to foliar morphology and biomass partitioning between root and shoot tissues, which appears particularly important for young seedlings.

Literature Cited


Hauer, R. J. 1995. Physiological responses to drought of Acer saccharum ssp. saccharum and Acer saccharum ssp. nigrum seedlings from different geographic seed sources. MS Thesis, Dept. of Forestry, Univ. of Illinois, Urbana-Champaign.


Kloeppe1, B. D. and M. D. Abrams. 1995. Ecophysiological attributes of the native

_Acer saccharum_ and exotic _Acer platanoides_ in urban oak forests in Pennsylvania,

USA. Tree Physiol. 15:739-746.


Pallardy, S. G. and J. L. Rhoads. 1993. Morphological adaptations to drought in


Pair, J. C. 1994. Stress tolerant trees for the southern great plains. J. Arboric. 20:130-

133.

Parker, W. C. and S. G. Pallardy. 1987. Leaf and root osmotic adjustment in

drought-stressed _Quercus alba, Q. macrocarpa_, and _Q. stelleta_ seedlings. Can. J.

For. Res. 18:1-5.

Parker, W. C., S. G. Pallardy, T. M. Hinckley, and R. O. Teskey. 1982. Seasonal

changes in tissue water relations of three woody species of the _Quercus-Carya_


Table 1. Growth and biomass partitioning characteristics and specific lamina mass of black maple and sugar maple seedlings harvested before irrigation treatments were initiated. Values are means of 10 replicates. Least significant difference (LSD) at the 5% level is given.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Central Iowa</th>
<th>Eastern Iowa</th>
<th>Eastern United States</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem length (mm)</td>
<td>42.5</td>
<td>53.1</td>
<td>67.6</td>
<td>13</td>
</tr>
<tr>
<td>Lamina area (cm²)</td>
<td>113.9</td>
<td>118.5</td>
<td>166.5</td>
<td>38</td>
</tr>
<tr>
<td>Specific lamina mass (mg·cm⁻²)</td>
<td>4.8</td>
<td>4.4</td>
<td>3.8</td>
<td>0.45</td>
</tr>
<tr>
<td>Shoot dry mass (g)</td>
<td>0.63</td>
<td>0.59</td>
<td>0.64</td>
<td>0.16</td>
</tr>
<tr>
<td>Root dry mass (g)</td>
<td>0.20</td>
<td>0.17</td>
<td>0.15</td>
<td>0.06</td>
</tr>
<tr>
<td>Root : shoot dry mass ratio</td>
<td>0.30</td>
<td>0.28</td>
<td>0.21</td>
<td>0.05</td>
</tr>
<tr>
<td>Root : lamina dry mass ratio</td>
<td>1.80</td>
<td>1.54</td>
<td>0.95</td>
<td>0.35</td>
</tr>
</tbody>
</table>
Table 2. Mean values for characteristics of black maples and sugar maples that were affected by five cycles of water deficit irrigation. Values are means of 90 replicates for control, and 61 for drought. Least significant difference (LSD) at the 5% level is given. Only the irrigation treatment effect was significant. No regional differences existed, and main effects showed no interactions.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Irrigation treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Lamina area (cm²)</td>
<td>1675</td>
</tr>
<tr>
<td>Lamina dry mass (g)</td>
<td>10.4</td>
</tr>
<tr>
<td>Xylem diameter (mm)</td>
<td>7.1</td>
</tr>
<tr>
<td>Root dry mass (g)</td>
<td>7.1</td>
</tr>
<tr>
<td>Epicotyl dry mass : root dry mass ratio</td>
<td>4.9</td>
</tr>
<tr>
<td>Shoot : root dry mass ratio</td>
<td>7.3</td>
</tr>
<tr>
<td>Lamina area : xylem diameter ratio</td>
<td>23.3</td>
</tr>
<tr>
<td>Predawn leaf water potential (-MPa)</td>
<td>0.77</td>
</tr>
<tr>
<td>Midday leaf water potential (-MPa)</td>
<td>1.92</td>
</tr>
</tbody>
</table>
Table 3. Mean values for characteristics of black maples and sugar maples that reflected regional differences. Values are means of 20 replicates. Least significant difference (LSD) at the 5% level is given. No irrigation treatment differences existed and main effects showed no interactions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Central Iowa</th>
<th>Eastern Iowa</th>
<th>States</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific lamina mass (mg·cm⁻²)</td>
<td>5.97</td>
<td>5.03</td>
<td>5.31</td>
<td>0.34</td>
</tr>
<tr>
<td>Number of trichomes/cm²</td>
<td>531</td>
<td>360</td>
<td>0</td>
<td>314</td>
</tr>
</tbody>
</table>
Table 4. Mean values for characteristics of black maples and sugar maples that reflected regional differences, and were affected by water deficit treatment. Values are means of 20 replicates for each region, 90 replicates for control, and 61 for drought. Least significant difference (LSD) is at the 5% level. Seedling origin and irrigation treatment showed no interactions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Central Iowa</th>
<th>Eastern Iowa</th>
<th>Eastern United States</th>
<th>LSD</th>
<th>Control</th>
<th>Drought</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomate frequency (no./mm²)</td>
<td>628</td>
<td>516</td>
<td>475</td>
<td>112</td>
<td>596</td>
<td>483</td>
<td>92</td>
</tr>
</tbody>
</table>
Fig. 1. Plots of the variables that were affected by the interaction of irrigation treatment and region of seedling origin. For each variable the mean value for plants subjected to drought (●) and frequently irrigated plants (■) was plotted against the mean value of irrigation treatments within regions. Plots are for (A) height, (B) total dry mass, (C) shoot dry mass, and (D) stomatal conductance. Values are means of 90 replicates for well-irrigated plants and 61 replicates for plants that were subjected to drought.
Mean shoot dry mass for control or drought (g)

Mean height for control or drought (mm)

Mean stomatal conductance for control or drought (mmol·m⁻²·s⁻¹)

Mean total dry mass for control or drought (g)

Fig. 1

LSD = 2.3

CIA, EUS

Mean shoot dry mass for each region (g)

Mean height for each region (mm)

Mean stomatal conductance for each region (mmol·m⁻²·s⁻¹)

Mean total dry mass for each region (g)

LSD = 2.7

EUS, CIA

LSD = 5.6

EIA, CIA

LSD = 2.3

CIA, EUS

EIA, CIA

EIA, CIA
CHLOROPLAST DNA MAY BE USED TO RESOLVE PHYLOGEOGRAPHY OF SUGAR MAPLES AND BLACK MAPLES

A short communication to be submitted to Molecular Ecology

Rolston St. Hilaire, William R. Graves, Randall L. Small, and Jonathan F. Wendel

Abstract

We examined restriction site patterns in chloroplast DNA from populations of sugar maples (Acer saccharum Marsh.) and black maples (Acer saccharum Marsh. ssp. nigrum Desm. or Acer nigrum Michx. f.) representing their zones of allopatry and sympatry in eastern North America. Restriction site analysis of the ndhA intron revealed that Hinfl and Sau3AI did not produce polymorphisms. TaqI did not produce taxon-specific markers but gave a single polymorphic site that showed chloroplast types were not geographically localized. This result could impact the selection of hard maples based on morphology for studies that assess physiological differences among the taxa.

Introduction

Sugar maples (Acer saccharum Marsh.) and black maples [(Acer saccharum Marsh. ssp. nigrum Desm. (Desmarais, 1952) or Acer nigrum Michx. f. (Rehder, 1940)) have ecophysiological and morphological similarities that lead to confusion about their taxonomy and the genetic relationship between the two taxa. Desmarais (1952) and Kriebel (1957) suggest that the two taxa introgressively hybridize because trees with morphological traits that can be ascribed to either taxa occur in the zone of sympatry of
black maples and sugar maples. Although knowledge of the genetic diversity and phylogeography of forest species is needed for tree improvement programs and germplasm conservation (Petit et al. 1993), molecular assessment of genetic diversity among black maples and sugar maples across a wide geographic region is lacking.

Chloroplast DNA (cpDNA) has been used widely in population genetic and phylogenetic studies of plants (reviewed in Olmstead & Palmer 1994, and in McCauley 1995). The polymerase chain reaction (PCR) technology has improved our ability to screen individuals for genetic variation. Chloroplast DNA provides a simple system for these analyses because it is haploid, nonrecombinant, and often uniparentally and maternally inherited in angiosperms (Clegg et al. 1994; Olmstead & Palmer 1994). Uses of cpDNA variation among tree species include reconstruction of post-glacial colonization routes in European oaks (*Quercus* spp.) (Dumolin-Lapèque et al. 1997; Petit et al. 1997), population history in sessile oak (*Quercus petraea* (Matt) Liebl.) (Le Corre et al. 1997), phylogeographic studies of the Moroccan argan tree (*Argania spinosa* (L.) (El Mousadik & Petit 1996)), and a phylogeny of conifer species (Tsumura et al. 1995). Thus we expect cpDNA variation may be a useful tool to investigate genetic and phylogeographic patterns in maples.

Sugar maple is a North American tree species valued for its wood, syrup, shade, and ornate foliage. In the United States, sugar maples are contiguously distributed from the eastern United States to as far west as central Minnesota and eastern Kansas (Kriebel & Gabriel 1969; Little 1971). At the western portion of the range, sugar maples occur only in the eastern portions of Iowa. The contiguous range of black maple extends west to
central Iowa but is not more westerly in other states than the distribution of sugar maple (Kriebel & Gabriel 1969). In the United States, sugar maples and black maples are sympatric between eastern Iowa and Vermont.

The purpose of this study was to investigate variation in cp DNA restriction site patterns for black maples and sugar maples in their zones of sympatry and allopatry. In 1995 and 1996, we obtained seeds from three regions, central Iowa, eastern Iowa, and the eastern United States, to assess regional differences in phenotype among seedlings. Tissues from seedlings in this collection were used in this study to characterize genetic diversity.

Materials and Methods

Plant material and DNA extraction

One-year-old seedlings of sugar maples and black maples were grown from seed collected from trees indigenous to central Iowa, eastern Iowa, and the eastern United States (Table 1). Seedlings were maintained in a greenhouse. In October, 1997, three or four of the youngest leaves were taken from each plant, frozen in liquid nitrogen, and stored at -80 °C until needed for DNA extraction. Total genomic DNA was extracted (Doyle & Doyle, 1987), except that the extraction buffer was modified to contain 10% w/v PVP-40 (Eastman Kodak, Rochester, NY, USA). Frozen leaf tissue (1.5 g) was ground in liquid nitrogen and then placed in 10 mL of extraction buffer held at 60 °C. The DNA was cleaned by using a protocol for tissues rich in polysaccharides, polyphenolics, and other PCR-inhibiting compounds (Lamboy & Alpha, 1998). For each sample, we placed 50 μL of DNA suspended in Tris EDTA buffer into a 200-μL, thin-walled PCR tube (Usa Scientific, Ocala, FL, USA) and added 5.0 μL (0.1 volume) of 4.0 M NaCl. The mixture
was agitated and placed on ice for 10 min. The sample was microfuged at 13,000 rpm for 15 min at 21 °C, and 30 μL of the supernatant that contained the DNA was transferred to a new tube. Then, 60 μL (2 volumes) of 100% ethanol at -20 °C were added. The tubes were held at -20 °C for 30 min and then microfuged at 13,000 rpm for 15 min. The DNA pellet was washed with buffer (76% ethanol/0.01 M CH₃COONH₄) at -20 °C for 2 min, and the buffer was removed by pipeting. The pellet then was washed with 70% ethanol and spun at 13,000 rpm for 2 min. The ethanol was removed, and the pellet was air-dried at 21°C. The DNA was dissolved in 25 μL of Tris EDTA buffer. DNA concentration was determined with a spectrometer (Lambda Bio; Perkin-Elmer, Norwalk, CT, USA). DNA (10 μg) was digested with EcoRI for 5 hr, and electrophoresiated on a 1.5% agarose gel to assess DNA purity further. Additional cleaning was done with GeneClean (Bio 101, Vista, CA, USA) if better purity was needed.

**PCR procedures**

Initial screening showed that primers for the ndhA and rpl16 intron, and the trnL-trnF spacer region amplified some of our samples. We focused on amplifying the ndhA intron. The primer-pair sequence for ndhA is ndhA-F (5'-GGW CTT CTRY ATG KCR GGA TAT RGM TC-3') and ndhA-R (5'-CTG YGC TTC MAC TAT ATC AAC TGT AC-3') (Small et al. 1998). Amplifications were performed in a 25-μL volume by using a thermocycler (GeneAmp PCR system 2400; Perkin-Elmer). The reaction mixture, overlaid with mineral oil to prevent evaporation during thermocycling was, 15.78 μL of sterile distilled H₂O; 0.25 μL 1 M Tris at pH 8.3; 2 μL 25 mM MgCl₂; 1.25 μL 1 M KCl; 2.5 μL 0.01% gelatin; 0.125 μL nonionic detergent
(Nonidet P-40; Sigma, St. Louis, MO, USA); 1.0 μL of dNTPs mixture at 10 mM; 0.5 μL of forward primer and 0.5 μL of reverse primer, each at 10 μM; 1.0 μL of template DNA (=60 ng), and 0.1 μL (0.5 units) of Taq polymerase (Promega, Madison, WI, USA). The PCR conditions were 5 min at 94°C followed by 30 cycles, each of 1 min at 94 °C; 90 s at 42 °C; and 2 min at 72 °C.

**Enzyme digest and gel electrophoresis**

PCR amplicons (5 μL) were digested with five units of three four-base recognition restriction endonucleases, *Hinfl*, *Sau3AI*, and *TaqI*. The incubation mixture had a total volume of 10 μL, and for *Hinfl* and *Sau3AI*, was incubated at 37 °C for 2.5 h. *TaqI* mixture was incubated at 65°C for 2.5 h. For digestion with *Hinfl*, 0.5 μL enzyme, 1.0 μL of the buffer (React2; GibcoBRL, Grand Island, NY, USA), and 3.5 μL of sterile double-distilled H₂O was added to the amplicon. The digestion mixture for *Sau3AI* contained 0.63 μL enzyme, 1.0 of the buffer (Buffer B; Promega, Madison, WI, USA), 0.1 μL 100x BSA, and 3.3 μL of sterile double distilled H₂O. For digestion with *TaqI*, 0.5 μL enzyme, 1.0 μL of the buffer (Buffer E; Promega, Madison, WI, USA), and 3.5 μL of sterile double distilled H₂O was added to the amplicon. Fragments from the DNA restriction digests were separated by electrophoresis on 3% agarose gel (NuSieve; FMC BioProducts, Rockland, ME) in Tris Boric acid EDTA buffer, and stained with SYBR Green nucleic acid stain (FMC BioProducts). Gels were epi-illuminated with 253.4 nm UV light and photographed through a No. 15 filter. Photographic images were recorded on black and white polaroid film.
Results

Although we extracted DNA from 107 specimens, only 24 samples yielded amplification products (Table 1). However, the 24 samples represented populations from the zone of allopatry and sympatry of the two species (Table 1).

The restriction enzymes HinfI and Sau3AI yielded no polymorphisms for DNA from all plants. Five and three restriction sites were present with HinfI and Sau3AI, respectively. Restriction digestion with TaqI revealed a polymorphic restriction site. Samples from Iowa State University, the sample from Pammel Woods in central Iowa, and the four samples from Palisades-Kepler State Park in eastern Iowa contained two restriction sites. A single TaqI restriction site was present in all the sugar maples from the eastern United States; that pattern was shared by sugar maples in Backbone State Park in eastern Iowa, and black maples in Dolliver Memorial State Park in west-central Iowa. Thus, digests did not detect taxon-specific markers. Based on the number of digests performed on the ndhA intron, the average divergence between plants of the two restriction types 2.5% (one site difference out of 40 surveyed). We designated the cpDNA type as A when two restriction sites were present and as B when one restriction site was present. The geographic distribution of chloroplast types is shown in Fig. 1.

Discussion

The amount of cpDNA variation expected in a given comparison is dependent on several factors, including sample size of individuals or populations, the number of locus/enzyme combinations, rates of molecular evolution, and recency of speciation. According to Kriebel & Gabriel (1969), the speciation event leading to black maples and sugar maples
probably occurred after the pleistocene glacial retreat. Given the very recent origin of the
taxon, a paucity of taxon-specific markers is expected. We assessed one cpDNA locus (the
ndhA intron) in 24 accessions by restriction digestion with enzymes (Hinfl, Sau3AI, and
TaqI) which revealed a total of 10 restriction sites. Among these we observed a single
polymorphic restriction site, and noted no length mutations (insertions or deletions).

Our data show that cpDNA could be used to address questions of genetic relatedness
among geographically separated populations of black maples and sugar maples. Similarities
in chloroplast DNA between sugar maples in eastern Iowa and black maples in central
Iowa indicate that either the taxa are exchanging genes via introgressive hybridization or
that they share an ancestral cpDNA polymorphism. Indeed, the presence of trees with
morphological characters that were intermediate between the two taxa led Desmarais
(1952) and Kriebel (1957) to suggest that the species may introgressively hybridize. All
cpDNA haplotypes detected in Ipomopsis aggregata and Ipomopsis tenuituba were
geographically localized (Wolf et al. 1997), but genes also may be exchanged between
species that are distantly related and have a limited capacity to hybridize (Whittemore &
Schaal 1991). In sugar maples and black maples, the presence of intermediate phenotypes
indicates that interspecific gene flow occurs. Our results are consistent with this hypothesis.

Further evidence that genes are exchanged is shown by the clustering of samples
from Dolliver Memorial State Park in west-central Iowa with samples from eastern United
States and from Backbone State Park in eastern Iowa (Fig. 1). Morphological observations
indicate that black maples are found in west-central Iowa (Desmarais 1952; Little 1971),
While sugar maples are indigenous further east (Little 1971). Morphological characters
and provenance are used to select populations of black maples (Graves 1994) and sugar maples (Close et al. 1996; Graves 1994) for studies to compare their functional value, but if phenotype and genotype are not congruent, interpreting data on physiological function may be confounded by genotypic heterogeneity.

We have shown that cpDNA of black maples and sugar maples could be used to provide clues about the phylogeography of the taxa. Chloroplast types were not exclusive to a taxon or geographic region. Although many studies of cpDNA variation are based on very small samples (Whittemore & Schaal 1991), sampling of additional black maple and sugar maple accessions and locus/enzyme combinations is warranted to describe further patterns of genetic variation among taxa and geographical regions.

References


Phylogeographic structure of white oaks throughout the European continent.  
*Genetics*, 146, 1475-1487.


*Molecular Ecology*, 6, 283-291.

Table 1. Region of the United States, locations, and the number of accessions of the black maples (*Acer saccharum* Marsh. ssp. *nigrum* Desm. or *Acer nigrum* Michx. f.) and sugar maples (*Acer saccharum* Marsh.) used in this study.

<table>
<thead>
<tr>
<th>Region</th>
<th>Geographical origin</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>No. of accessions</th>
<th>Taxon based on morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Iowa</td>
<td>Pammel Woods</td>
<td>42° 30' 15&quot;</td>
<td>93° 31' 40&quot;</td>
<td>6</td>
<td>black maple</td>
</tr>
<tr>
<td></td>
<td>Iowa State University</td>
<td>42° 30' 34&quot;</td>
<td>93° 31' 30&quot;</td>
<td>10</td>
<td>black maple</td>
</tr>
<tr>
<td></td>
<td>Iowa State University</td>
<td>42° 01' 34&quot;</td>
<td>93° 38' 54&quot;</td>
<td>8</td>
<td>black maple</td>
</tr>
<tr>
<td></td>
<td>Dolliver Memorial State Park</td>
<td>42° 23' 13&quot;</td>
<td>94° 05' 00&quot;</td>
<td>10</td>
<td>black maple</td>
</tr>
<tr>
<td>Eastern Iowa</td>
<td>Palisades-Kepler State Park</td>
<td>41° 49' 00&quot;</td>
<td>91° 30' 22&quot;</td>
<td>8</td>
<td>sugar maple</td>
</tr>
<tr>
<td></td>
<td>Palisades-Kepler State Park</td>
<td>41° 50' 27&quot;</td>
<td>91° 31' 00&quot;</td>
<td>10</td>
<td>sugar maple</td>
</tr>
<tr>
<td></td>
<td>Backbone State Park</td>
<td>42° 36' 00&quot;</td>
<td>91° 33' 00&quot;</td>
<td>6</td>
<td>sugar maple</td>
</tr>
<tr>
<td></td>
<td>Backbone State Park</td>
<td>42° 36' 58&quot;</td>
<td>91° 33' 34&quot;</td>
<td>10</td>
<td>sugar maple</td>
</tr>
<tr>
<td>Eastern United States</td>
<td>Strafford, NH</td>
<td>43° 17' 39&quot;</td>
<td>71° 04' 39&quot;</td>
<td>10</td>
<td>sugar maple</td>
</tr>
<tr>
<td></td>
<td>Underhill, VT</td>
<td>44° 31' 33&quot;</td>
<td>72° 56' 44&quot;</td>
<td>9</td>
<td>sugar maple</td>
</tr>
<tr>
<td></td>
<td>Starksboro, VT</td>
<td>44° 13' 38&quot;</td>
<td>73° 03' 28&quot;</td>
<td>10</td>
<td>sugar maple</td>
</tr>
<tr>
<td></td>
<td>Lake Placid, NY</td>
<td>44° 16' 46&quot;</td>
<td>73° 58' 49&quot;</td>
<td>10</td>
<td>sugar maple</td>
</tr>
</tbody>
</table>
Fig. 1. Map of chloroplast type distribution in hard maples sampled near the 43 °N latitude. Points from the three geographical regions have the following symbols: (●) Black maple, cpDNA type B; (○) Black maple, cpDNA type A; (■) Sugar maple, cpDNA type B; (□) Sugar maple, cpDNA type A.
GENERAL CONCLUSIONS

I conclude that foliar morphology of hard maples varies with geographic origin along the 43 °N latitude in the United States. Because foliar traits that may influence leaf water relations varied predictably along a gradient of decreasing precipitation from 70° to 94 °W longitude, foliar attributes may represent important mechanisms of drought resistance of hard maples. My research showed that a group of hard maples with distinct morphological characteristics such as dense foliar pubescence is indigenous to central Iowa, and this is the first report to quantify the difference in pubescence between black maples and sugar maples. Reduced stomatal conductance during drought, pubescence, high stomatal density, high specific mass of lamina, and low initial investment in shoot growth suggest that black maples may conserve more water in landscapes prone to drought than sugar maples. Because of the lack of differences in biomass partitioning in two-year-old seedlings from different regions, I conclude that some mechanisms of drought resistance present in seedlings of black maple during their first year of development are temporary. Also, the lack of regional differences in tissue water relations suggests that selection of hard maples for use in horticultural landscapes should focus primarily on foliar attributes and biomass partitioning as mechanisms of drought resistance. Selections from central Iowa should be compared directly to selections of sugar maple from further east, which now dominate the commercial market, for performance in managed landscapes. Black maples and sugar maples share chloroplast genes. Genotypes of the taxa were not separated geographically based on restriction site analysis of the ndhA intron of the chloroplast genome.
ACKNOWLEDGMENTS

I thank the Department of Horticulture at Iowa State University for providing financial support for my graduate work. I thank Dr. William R. Graves for his guidance and timely assistance throughout my student life at Iowa State University. To Drs. Donald R. Farrar, Paul N. Hinz, Jeffery K. Iles, and Harry T. Horner, I thank you all for serving on my graduate committee and providing assistance. Also, I thank Drs. Nick Christians, Richard Gladon, Clint Hodges, and Gail Nonnecke for the use of key pieces of equipment. Tony Aiello and Carol Foster assisted me in several ways.

I thank Lewis J. Staats of the Uihlein Sugar Maple Field Station in Lake Placid, New York, for providing sugar maple samaras from New Hampshire, New York, and Vermont. The organizations and people from the states listed below cooperated in this research by sending me leaf material.

Maine

Bradbury Mountain State Park; Gary Best.

Harris Farm; Ben Harris, Jason Harris.

New Hampshire

Kingston State Park; Keith Kanoti, Kyle Lombard.

Bear Brook State Park; Keith Kanoti, Kyle Lombard.

Greenfield State Park; Keith Kanoti, Kyle Lombard.

Fox State Park; Keith Kanoti, Kyle Lombard.

Vermont

Arlington State Park; Nathan Fice, Alex Sands.
Green Mountain National Forest; Chris Casey, Alex Smith, Jeff Williams.

Hunter Brook Partnership; Bill Guenther.

Jamaica State Park; Nathan Fice, Alex Sands.

**New York**

Allegany State Park; Terry Dailey.

Chenango Valley State Park; John Michaslki, Jerry Pedini

Fillmore Glen State Park; Ed freeman, Tom Noble, Jim Tratt.

Grafton Lakes State Park; Karl Brownell, Tom Conklin.

Pack Forest; John Peck.

Saratoga National Historical Park; Beth Bidwell, Wendy Borden, Chris Martin.

Watkins Glen State Park; Cliff Lott, Doug Haight.

**Michigan**

Lake Port State Park; Trent Burch, Gail Burgett.

Sleepy Hollow State Park; Terry Ellenwood, Sue Herbert, Jo Latimore.

Saugatuck Dunes State Park; Stephen Macioszek, Gordon Steenbergen.

Silver Lake State Park; Peter Lundborg.

**Wisconsin**

Kettle Moraine State Forest-Northern Unit; Manny Oradei, John Van Ells.

Village of Maple Bluff; Mike Roth, Mark Weber.

Abraham Woods; Greg Armstrong, Kenneth Zuba.

Dean Thomas Property; Tom Singer, James Widder.

Yellow Stone Lake; Andy Gric, Greg Pittv.
Wildcat Mountain State Park; Karen Teed.

Wyalusing State Park; Jaye Maxfield.

**Iowa**

Backbone State Park; Charle Besler, Terry Gaines, Bob Schaut.

Yellow River State Forest; Bob Honeywell.

Wentland Wavels; Gary Beyer.

Maralie Educational Forest; Gary Beyer.

Sincerest thanks to Ellen Anna and Placid Samuel who taught me that the darkest hour is closest to dawn.

To Marlene, thank you for being with me and assisting in every possible way. Jael Andre was always willing to smile and play even at 2.00 a.m. Then, I learned that fatigue is a state of mind.