

**Horticultural & ecophysiological evaluations of leatherwoods (*Dirca* spp.)**

by

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## ABSTRACT

Horticulturists have overlooked *Dirca* spp. despite the many ornamental characteristics of these shade-tolerant but difficult-to-propagate shrubs. The genus is characterized by slow growth, shade tolerance, flowers in late winter or spring, and mature growth approximately 1 to 2 meters tall. The first objective of the work undertaken for this thesis was to evaluate three species of *Dirca* in a common garden setting in central Iowa. As part of an on-going trial, I analyzed data collected from 2007-2010. *Dirca mexicana* had the greatest survival rate with 88.9% alive as of 2010, despite its southerly origins. Within *Dirca palustris*, plants from Thunder Bay survived better than plants from Florida but exhibited poor vigor and achieved low health ratings. *Dirca mexicana* and *D. palustris* from Florida were rated the healthiest, and their stems extended the most annually. *Dirca mexicana* was suggested as the best species for inclusion in landscapes as cold as USDA Hardiness Zones 4 and 5, whereas selections from different provenances, particularly those from Florida, evince a selection gradient for commercially viable traits. *Dirca occidentalis* will require further evaluation for hardier, more climate-suitable genotypes if it's to ever be promoted a landscape plant outside of its endemic distribution. The second objective was to document the edaphic responses of the same three species of *Dirca* under field conditions. Three treatments were imposed on an outdoor trial site; two acidic treatments and one based on the native soil pH of the site. To obtain the two treatments, we modified the soil (native pH 7.65) with 1-M sulfuric acid ( $H_2SO_4$ ) to a pH between 4.5-5.0 and 6.0-6.5 using 200 mL/L medium and 70 mL/L medium, respectively. Stems of *Dirca palustris* extended the most in acidic root zones, but mean health ratings were highest in the slightly acidic and control

treatments, suggesting a range of optimal growth and performance. *Dirca occidentalis* performed best for all response variables under slightly acidic conditions. Stems of *Dirca mexicana* extended the most in slightly basic root zones, and the species was rated the healthiest. Overall, the data suggest that the three species of *Dirca* perform well in a range of soils from pH 6.5 to 7.5, offering initial insights into the proper use of these shrubs in managed landscapes.

## CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

### Thesis Organization

The research undertaken as the subject of this thesis studied the horticulturally underappreciated genus *Dirca*, three to four species of shrubs with an unusual phylogeographic distribution. Three manuscripts are presented in this thesis. Chapter 2 is a short paper submitted to The Royal Horticultural Society's journal *The Plantsman* and included as brief horticultural and botanical introduction to the genus. Chapter 3 is a manuscript prepared for *HortScience* and reports results from the first known trial of *Dirca mexicana*, *Dirca occidentalis*, and *Dirca palustris*, conducted in a common garden setting in central Iowa from 2007-2010. Chapter 4 is a manuscript prepared for the *Journal of Environmental Horticulture* and reports the results of a pH experiment undertaken to assess the edaphic tolerances of *D. mexicana*, *D. occidentalis*, and *D. palustris* under field conditions. Appendices are included to document two attempts to propagate *Dirca* asexually, a study conducted to assess the tolerance of two provenances of *D. palustris* to irradiance, an experiment conducted to evaluate the pH responses of three species of *Dirca* planted in soilless media in a greenhouse, and the first attempted interspecific crosses in the genus.

### Introduction

The effort to introduce and promote new plants has driven ornamental horticulture for decades (Creech, 1966). To meet the demand for new products, horticulturists must continue to explore plant biodiversity and utilize new germplasm. This exploration reveals important interfaces between horticulture and the fields of restoration ecology and conservation biology (Norton and Norton, 2008). Additionally, the ornamental horticulture industry in Iowa is valued

at over \$300 million dollars (Haynes et al., 2007), and total U.S. economic output related to woody ornamental plants is over \$21 billion dollars (Hall et al., 2006), underscoring the need to support a thriving regional and national industry with new plants.

American horticulturists have become increasingly aware of native trees, shrubs, vines, and perennials since the late 1960s, an insight widely supported from a perusal of modern gardening literature (e.g. Christopher, 2011; Greenlee, 2009; Ogden and Springer-Ogden, 2008). Interest among horticultural consumers in native plants is also high (McMahan, 2006; Brzuszek and Harkess, 2009). Recent research also supports native plant enthusiasts' claims that native taxa support greater levels of biodiversity than non-native taxa (Tallamy and Shropshire, 2009; Burghardt et al., 2009). But despite heady appraisals of their functional and aesthetic uses as landscape plants, many native plant species remain poorly understood horticulturally with little known about their propagation or ecophysiological tolerances in the managed landscape. With such a vast reservoir of germplasm to evaluate, horticulturists would do well to spend time exploring the frontiers of North American flora in an effort to promote progressive landscape design installations and practices.

At this juncture, horticulture and ecology intersect, requiring interdisciplinary inquiries into the study of native plants *in situ* and *ex situ*. Many native communities have long been reported to be under duress from biological invasions (e.g. Goldman et al., 2008), lessening the number of opportunities horticulturists have to survey and collect germplasm for anthropogenic use. Despite a growing record of research, few researchers have characterized the direct impacts of invasion on native taxa and their communities or the mechanisms of invasiveness (Flory and Clay, 2010; Lavergne and Molofsky, 2007). A recent study has documented significant reductions in native biomass, lower species richness, diversity, and native-plant competitiveness

as the direct result of the incursion of a non-native species under experimental conditions (Flory and Clay, 2010). Others have documented shifts in arthropod community composition in native and managed landscapes of mixed species composition (Hartley et al., 2010; Stewart et al., 2002). These studies highlight only a handful of the issues faced by ecosystem managers today. From a horticultural perspective, plant breeders need to embrace ecologically conscious ideotypes for the development of non-invasive crops (Anderson et al., 2006) and promote the increased planting of native taxa in managed landscapes (Burghardt et al., 2009). From an ecological perspective, more research is needed to understand the physiological tolerances of native species in the wild and the managed landscape (O'Brien, 1996), while employing horticultural techniques for use in ecological restorations (Franco, et al., 2006; Young, 2000; Dreesen and Harrington, 1998).

## **Literature Review**

The following review is comprised of four general sections. In the first section, I explore the intersections between biogeography and horticulture and reviews the literature pertinent to the biogeography of the genus *Dirca* and its family Thymelaeaceae. In the second section, I review the horticultural relevance of provenance and the variation among provenances for responses to various forms of environmental stress. In the third section, I briefly examine research with recalcitrant species and the successful use of etiolation as a propagation strategy.

### ***Horticulture and Biogeography: A Biological Odd Couple?***

Phytogeography is the branch of biogeography that is concerned with the distributions of plant taxa and the factors that contribute to those distributions (Lomolino et al., 2006).

Biogeographers pursue questions related to where organisms occur across space and time as well

as why species occur in given areas and why others do not. Biogeographical analyses require a holistic consideration of geology, ecology, climatology, and paleobotany because of the potential complexity of species' contemporary and historical distributions. Little is known about the biogeography of many horticultural plants beyond basic, qualitative descriptions of their native habitats, occurrences, and populations. Knowing and understanding the biogeographies of horticulturally important species would aid their breeding, development, and utilization in the landscape, particularly in light of heightening ecological awareness in the horticulture industry.

The scope of this interface is diverse and multi-disciplinary. For example, agro-horticultural interactions with native ecosystems have negatively altered the biodiversity of many regions like the Mediterranean, the southeastern and mid-continent of North America due to the destruction of habitat or the introduction of invasive species (Pemberton and Liu, 2009; Grixti et al., 2009; Kaniewski et al., 2007). Kaniewski et al., (2007) demonstrated the impact of long-term effects of human interactions on mountain ecosystems in Turkey noting the fluctuations in the distribution of forest communities, species composition within those communities, and the presence of arboricultural, Hellenistic-Roman cultivars of *Juniperus* L., *Corylus* L., *Vitis* L., *Corylus* L., and *Olea* L. They concluded that human activities seem to be the driving factor in the distribution and composition of vegetation in the region through the Hellenistic-Roman occupation and after 650 cal. yr AD. Similarly, in the United States, horticultural introductions have come to define human impact on the native landscape. Pemberton and Liu (2009) studied the amount of time horticultural crops were marketed in Florida from (1887-1930) relative to their rate of naturalization as a risk-assessment model. Their study confirmed reported global patterns of invasiveness in a number of families including Poaceae, Araceae, Convolvulaceae, and Euphorbiaceae as well as patterns of invasiveness in particular plant life forms. Vines and

aquatic plants were found to naturalize significantly more than other life forms like succulents and palms. They also found that plants with cosmopolitan and paleotropical origins had higher rates of naturalization than those plants from other small geographic regions, supporting the notion that plants with larger native ranges are more apt to invade and colonize than those with smaller native ranges (Daehler and Strong, 1993). Inferences from horticulture like these can lead biogeographers to better understand historical distributions of domesticated crop species and ecological situations involving non-native species.

Conversely, inferences from biogeography can lead horticulturists to make regionally adapted selections for cultivation and utilize genetic resources from a particular geographic area to enhance the productivity or adaptability of a crop species. For example, plant explorers have traveled the world for centuries in search of plants to domesticate. Plant selection for ornament is linked to developed societies, a certain level of disposable income, and an appreciation for subtle, intangible qualities of beauty in human surroundings (Kingsbury, 2009). The centers of origins of domesticated plants correlate with the centers of earliest human civilization (Creech, 1966). Understanding the ecological distribution, genetic structure, provenance of collected forms, and systematic relationships of the wild-type progenitors of these crops potentially furthers their horticultural usage. An understanding of ecological distribution is particularly important in development of food crops. Lebeda et al. (2009) assessed the current status of wild lettuce (*Lactuca* L. spp.) germplasm concluding that “detailed floristic, biogeographic and ecologic delimitation of the distributions of known *Lactuca* spp.,” are currently lacking and constitute a major challenge for lettuce breeders in light of “complex taxonomic and phylogenetic relationships within the genus.” Future breeding and development of lettuce risk stagnation in the absence of an appreciable understanding of the genus’s biogeography.

Similarly the genetic diversity of plants in the cultivated landscape has implications on *ex situ* biodiversity preservation. Jin and Li (2007) discussed the conservation status and imperiled genetic structure of *Heptacodium miconioides* Rehder, a species introduced to horticulture from the 1980 Sino-American Botanical Expedition (SABE) (Dosmann and Del Tredici, 2003). *Heptacodium miconioides* is a species of concern because of its highly fragmented, endemic distribution in China's Zhejiang, Anhui, and Hubei Provinces. To date, all plants in commerce stem from the original germplasm collection in Hangzhou Botanical Garden made on the 1980 SABE (Koller, 1986), representing a very narrow gene pool in the cultivated landscape and underscoring the need for increased *ex situ* preservation of germplasm from other provenances (Falk and Thibodeau, 1986). Provenance-based selections within a single species' range can result in cultivars with discriminate differences (Escribano and Lazaro, 2009; Ramesh and Khurana, 2008). The variation may be defined phenotypically, as in possessing a novel floral feature, or ecotypically, as in possessing adaptations to particular edaphic conditions. Biogeographical research could help horticulturists characterize this variation.

Many concepts in biogeography, like endemism and disjunction, have great relevance to horticulturists. Disparate biogeographies interest researchers because marginal populations often have unusual phenotypic traits due to morphological variation as a result of limited gene flow with other populations (Garcia-Ramos and Kirkpatrick, 1997). Populations occurring at the extremes of environmental gradients such as pH or soil moisture availability are also of interest when these novel environments effect morphology and stress tolerances (Lesica and Allendorf, 1995). This geographic disjunction can on some scales lead to speciation, pending the development of reproductively isolating barriers (Grant, 1971). Recent research suggests that rapid evolution of plant species introduced to environments that foster different phenotypic

expressions are much more common than previously thought (Buswell et al., 2010), though this study reported responses in short-lived herbaceous species. The implications of such research on long-lived, long-generational woody species are unknown.

The daphne family (Thymelaeaceae) is a cosmopolitan family of approximately 48 genera of mainly tropical or subtropical shrubs and small trees with centers of origins in prehistoric Gondwanaland, which includes modern-day Africa, the Indonesian islands, and Australia (Yinzheng et al., 1999; Galicia-Herbada, 2006). Estimates of the number of species vary by author from as few as about 500 (Van der Bank et al., 2002) to about 900 (Rogers, 2009). Thymelaeaceae is in the order Malvales, though there is little agreement about its relationships with other genera in the order (Bayer et al., 1999). The family is subject to taxonomic scrutiny due to few distinguishing morphological characteristics and unresolved molecular analyses, particularly in the two largest genera, *Gnidia* Gilg. and *Pimelea* Banks & Sol. ex Gaertn., which recent studies show are not monophyletic (Van der Bank et al., 2002; Beaumont et al., 2009). Classification of the family has primarily depended on the interpretation of three floral organs: the gynoecium, the nature of the floral tube, and the characterization of petaloid structures (Van der Bank et al., 2002). Endemism and disjunction are common throughout Thymelaeaceae (Graves and Schrader, 2008; Bredenkamp and Van Wyk, 2006; Yinzheng et al., 1999; Tawan, 1999; Mayer, 1991). The family has not garnered extensive horticultural appreciation beyond the genus *Daphne* L.

### ***Phylogeny and Systematics of Dirca***

The genus *Dirca*, described by Linnaeus in 1753 is the only representative of Thymelaeaceae in North America (Nevling, 1964) with its closest relatives most likely in the

genera *Daphnopsis* and *Ovidia* (Van der Bank et al., 2002). MacPhail and Cantrill (2006) reported finding *Ovidia*-type pollen in the Forest Bed of the Falkland Islands where no present-day occurrences are known. Considering the Gondwanic origins of the family and widespread occurrence of *Daphnopsis*, the largest American genus in the family (Nevling, 1959), the discovery of *Ovidia*-type pollen poses interesting questions to biogeographers namely a) what was the historic distribution of these genera and/or their ancestors and b) what relationship exists between *Dirca*, *Ovidia*, and *Daphnopsis*, and c) what climatic or ecological factors contributed to those disparate ranges? Future researchers that consider the systematic relationships between these Western Hemispheric species of Thymelaeaceae will no doubt add resolution to the literature with regards to the evolutionary patterns of the family, while possibly arriving at a tentative explanation for the extant distribution of the genus *Dirca*.

The genus *Dirca* contains four species, including one recently published taxon (Floden et al., 2009) (Fig. 1). *Dirca palustris* L. occurs in highly localized populations across the eastern one-third of the United States from Maine and Ontario, west to North Dakota, south to Oklahoma and Louisiana, and east to northern Florida (Nevling, 1964; Peterson et al., 2009a). *Dirca mexicana* Nesom & Mayfield is known from a single reported population in Tamaulipas, Mexico (Nesom and Mayfield, 1995) and remains un-introduced to horticultural commerce. *Dirca occidentalis* Gray occurs in six counties north, east, and south of the San Francisco Bay in California. *Dirca decipiens* A. Floden was described from one population in Johnson County, Kansas and two populations in Carroll County, Arkansas, occurring sympatrically within the range of *D. palustris* (Floden et al., 2009). Differences between *D. decipiens* and *D. palustris* were first reported by Floden and Mayfield in 2006. They concluded that *D. decipiens*, then regarded as a geographically disjunct population of *D. palustris* in eastern Kansas, was

taxonomically distinct from the latter because of a more distal filament insertion (shared with *D. mexicana*), larger, lobed calyces, sessile flowers and fruits, dense hoary pubescence on the bud scales and persistently pubescent leaves and stems (shared with *D. occidentalis* and *D. mexicana*).

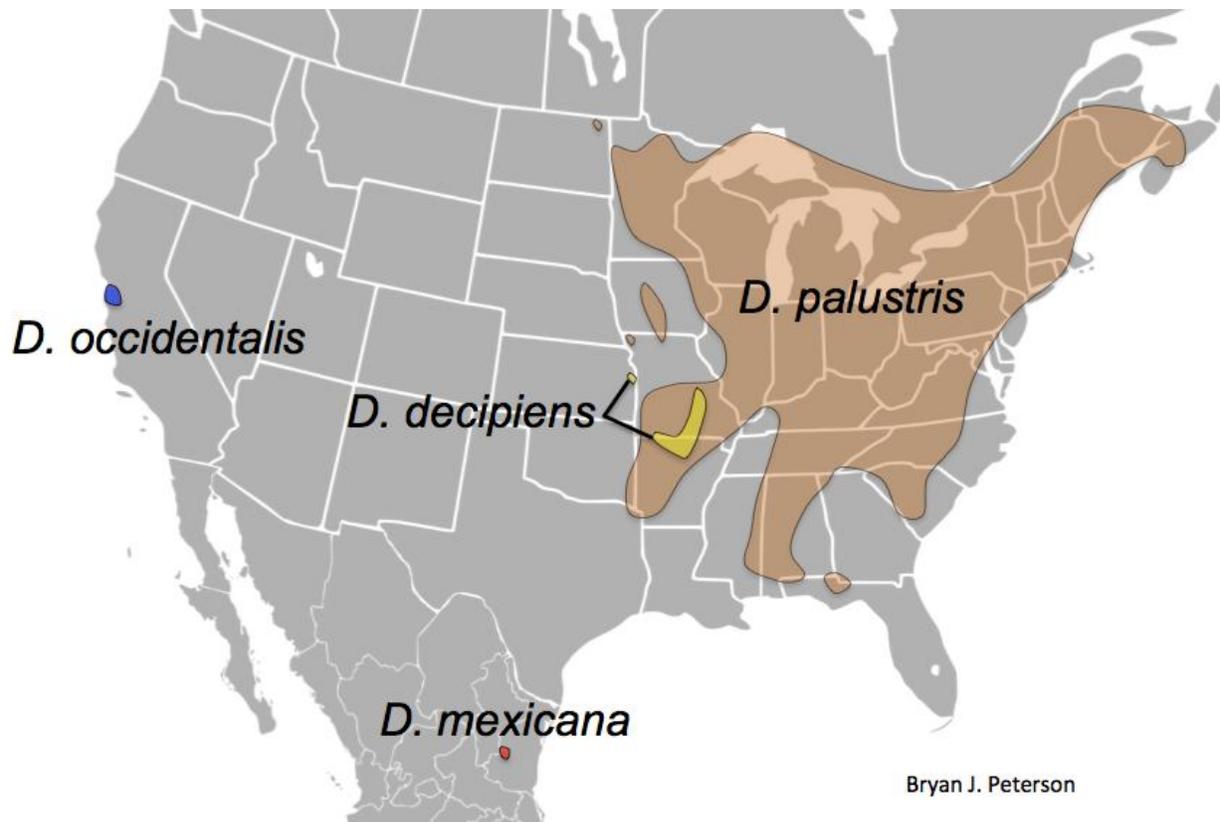


Fig. 1. Distributions of four species of *Dirca* in North America.

Schrader and Graves (2004) analyzed the systematics of *Dirca* using Internal Transcribed Spacer (ITS) and Inter-Simple Sequence Repeats (ISSR) polymorphisms to confirm species level divergence. Nesom and Mayfield (1995) in describing *D. mexicana* noted its morphological affinity to *D. occidentalis* but acknowledged a “well-known pattern of close relationship and disjunction between species of the eastern and southeastern United States and the sierra of northeastern Mexico.” Schrader and Graves (2004) aimed to resolve this inconsistency between morphological and biogeographical data. They concluded that although *D. mexicana* more

closely resembles *D. occidentalis* in appearance, it is more closely related genetically to *D. palustris*. Concurring with Nesom and Mayfield (1995), they hypothesized that *Dirca* was probably continuous across the mid-latitudes of North America during the Eocene and that ancestral conditions of modern-day *D. occidentalis* and *D. palustris/D. mexicana* were separated geographically by climate change during the middle Miocene. Fossil data from MacGinitie (1953) supports this hypothesis. Schrader and Graves further suggested that the divergence of *D. palustris* and *D. mexicana* occurred during the Pliocene when elements of the broad-leaved deciduous forest in eastern Mexico became isolated from that in eastern North America.

This analysis raises questions about the origins of *Dirca* and other Thymelaeaceae ancestors in North America considering the accepted Gondwanic origins of the family. Though fossil and palynological evidence is scant, it seems logical to hypothesize that the presence of Thymelaeaceae in the Americas originated in present-day South America following the breakup of Gondwanaland in the Mesozoic. Fossil evidence noted by MacGinitie (1953) predates *Ovidia*-type pollen found on the Falkland Islands as reported by MacPhail and Cantrill (2006), but lacking corroborating evidence these data can't be interpreted as evidence of a pattern of migration or distribution.

Schrader and Graves (2004) cited the development of obligate summer dormancy in *D. occidentalis* as consistent with its high level of differentiation. Due to its disparate occurrence around the San Francisco Bay in California, the biogeographic history of *Dirca occidentalis* is particularly interesting given its occurrence in an ecological niche different than that of its relatives. Schrader and Graves contend that the development of summer dormancy is the most plausible explanation for its migration to and continued existence in California. Citing Axelrod (1986), they noted that the development of a rain shadow brought on by the orogeny of the

Cascade-Sierra Nevada and Coast Ranges led to a xerification of the climate, likely confining *D. occidentalis* to the narrow geographic area where it occurs today and naturally selecting for summer dormancy within the population.

### ***Provenance and Ecophysiology***

Studying provenance from a horticultural perspective is valuable when considering how plant diversity functions in managed ornamental and edible landscapes, both for phenotypic and genotypic traits that might be improved using artificial selection (Bagley, 1980). Hällfors et al. (2011) confirmed that provenance in tandem with site factors such as climate and edaphic conditions is an informative factor in plant performance in the landscape. Additionally, provenance variation in response to environmental stress provides researchers with a powerful set of tools for examining a suite of issues at the crossroads of horticulture and ecology. A perusal of the literature reveals several dominant themes examined through the provenance lens—unusual distributions in part due to edaphic conditions; anatomical differences among populations and patterns of phenotypic plasticity across geographical gradients; the links between cold hardiness, altitude, and/or clinality; the potential of populations from different provenances to yield horticulturally or ecologically valuable genotypes for commercial propagation.

Variation for traits linked to edaphic tolerances plays a role in reproductive isolation and even speciation (Dawson et al., 2007; Rajakaruna, 2004). Rajakaruna (2004) reviewed several studies documenting a variety of edaphically differentiated taxa in unique situations like serpentinite and limestone outcrops, guano deposits, and mine tailings. His review revealed a variety of pre- and post-zygotic factors developed due to these substrate conditions including

difference in flowering times on heavy-metal soils in grass species, switches to self-compatibility and the development of autogamy in *Lasthenia* Cass., and hybrid inviability in *Mimulus* L. Another study suggests that such environmentally mediated divergences can occur with only a change in a few loci, and thus in a rather shortened period of evolutionary time (Rajakaruna et al., 2003). The biology of edaphically restricted taxa suggests that the evolution of edaphic adaptations may often have effects on gene flow between derivative populations and their progenitors, a potentially fertile area of research at the intersections of speciation and ecophysiology (Rajakaruna and Whitton, 2004).

Curiously, this genre of sympatric speciation arises in *Dirca* with the recent description of *D. decipiens* (Floden et al., 2009); in one instance in Arkansas populations of *D. decipiens* and *D. palustris* are located less than one kilometer apart (Lowman, 2010). Such relative closeness in similar habitats and no apparent intermediate populations or individuals suggests a couple of hypothetical possibilities for their apparent speciation—a reduction in hybrid fitness or the establishment of pre-zygotic barriers due to microedaphic conditions affecting timing of anthesis. Speciation isn't a linear, uniform, or organized process, and further research is needed to examine the degree of taxonomic divergence between *D. decipiens* and *D. palustris*.

Studies of anatomical differences and phenotypic plasticity related to provenance shed insights into the relationship between wild populations and their environments, insights that inform conservation strategies, taxonomic and systematic inquiries, and potentially horticultural selection. Numerous investigators of adaptive anatomy have concluded that species across their range, when occurring in disparate environments, often show cellular and anatomical differences (e.g. Esteban et al., 2010; Lopez et al., 2010; Bischoff and Müller-Schärer, 2010; Gao et al., 2010; Sandquist and Ehleringer, 1997). Studies of pecan (*Carya illinoensis* (Wangenh.)

K.Koch) have revealed significant provenance variation resulting into distinct sub-taxonomic groupings on a north-south gradient within the native range (Wood et al., 1998), and underscore the importance of maintaining genetic diversity in crop species for future horticultural or ecological uses (Ruter et al., 1999). A similar climatic pattern of provenance variation was documented in red-osier dogwood (*Cornus sericea* L.) by Smithberg and Weiser (1968) in part due to morphological characters like plant form and change of bark color and physiological traits like growth rate and timing of leaf abscission.

Phenotypic plasticity is a classical mechanism for explaining how plants migrate and distribute themselves geographically (Valladares et al., 2007; Weinig, 2000). Phenotypic plasticity promotes the development of novel, locally adapted phenotypes that better enable plants to establish themselves throughout range expansions (Godoy et al., 2011; Magi et al., 2011). Sandquist and Ehleringer (1997) documented intraspecific variation of leaf pubescence and drought response in *Encelia farinosa* A.Gray ex Torr., a desert perennial shrub. They concluded that the presence of opposing character states in a common garden might have a genetic basis that would explain the plastic environmental response of the species over its desert distribution. High-stress environments, like deserts and forests with dense canopies, can lead to a significant reduction in active plasticity at the margins of distributions, resulting in phenotypic and physiological differences in those populations (Magi et al., 2011). The degree of phenotypic plasticity coupled with an understanding of genetic diversity can result in conservation policy recommendations and an increased understanding of the inherent value of populations at the extremes of broad distributions (Peterson et al., 2011; Pfeifer et al., 2010).

Differences in cold hardiness of provenances across north-south gradients is well documented in a variety of woody taxa (Repo et al., 2000; Hannerz and Westin, 2005; Chunyang

et al., 2005; Graves et al., 2006; Weng and Parker, 2008; Hällfors et al., 2011). Weng and Parker (2008) investigated adaptive variation in fall cold hardiness among 20 provenances of aspen (*Populus tremuloides* Michx.) and found that provenance accounted for over 40% of the total variation in cold injury. Support for altitudinal gradients in coniferous species exists as well (Oleksyn et al., 1998). Provenance differences like this have been well studied by horticulturists in an effort to supply the horticultural trade with products that can be appropriately marketed to specific regional and national audiences (McNamara et al., 2002). Studies like Lenahan et al. (2010) offer templates for future inquiries into the landscape value of underused native taxa by testing and recommending provenances best suited for particular climates. McNamara et al. (2002) reiterated as others have that cold-hardiness trials should be conducted on regionally grown plants due to the strong influence of cold acclimation and deacclimation and maximum midwinter hardiness levels by local photoperiod and temperature conditions (Smithberg and Weisner, 1968; Donselman and Flint, 1982; Alexander et al., 1984; Chunyang et al., 2005).

Adaptive variation in response to environmental stressors like cold hardiness, drought tolerance, and edaphic tolerances also has implications for the response of wild populations to climate change and can be potentially informative factors when attempting to understand life histories (Weng and Parker, 2008; Graves and Schrader, 2008). Species that occur in transitional geographic and ecotypic zones are excellent subjects for future research to examine differences in genetic variation among provenances in response to climate change and habitat degradation (Weber and Montes, 2010). Species like *Picea abies* (L.) H. Karst., which vary along altitudinal and latitudinal gradients, are potentially useful modeling systems for montane environments subject to the brunt of anthropogenic environmental change (Oleksyn et al., 1998). While it's often assumed that climate change has deleterious effects on biodiversity, it also can permit

range expansions in species that quickly adapt to rapid environmental change. Population trends of a European orchid (*Himantoglossum hircinum* (L.) Spreng.) reveal two areas of its broad distribution worthy of conservation in light of increasing annual temperatures and human land use, a leading edge and a lagging edge, each with locally adapted, novel genetic traits (Pfeifer et al., 2010). Climate change and habitat fragmentation impact natural selection on reproductive traits (Weber and Kolb, 2011; Castilla et al., 2011), highlighting the importance of holistic studies of provenance effects throughout species' distributions.

Exploiting the variation among provenances for breeding and development purposes has been alluded to in a number of horticultural and agronomic species. Lauridsen (2004) reported that germplasm of *Gmelina arborea* Roxb., a fast-growing deciduous tree used in timber plantations in Africa and Asia, from disparate provenances could positively impact selection for traits like wood density and wood quality properties. Genetic improvement for ecophysiological traits like tolerance of saline soils has been suggested from data acquired from provenance trials (Mahmood et al., 2002). Other provenance trials with forest species like *Balanites aegyptiaca* (L.) Delile revealed that provenances from drier areas were better adapted to drought than provenances from wetter areas and that tree growth increased across a gradient from humid areas to drier areas (Weber and Montes, 2010). Intraspecific hybridization between provenances is a potential tool for plant breeders working with plants with large geographic distributions, as noted by Johnston et al. (2003) in their studies of *Pinus caribaea* Morelet var. *hondurensis* (Sénéclauze) W. H. G. Barrett & Golfari. This technique has been used by tree breeders to enhance genetic gain among suites of desirable traits, increase hybrid heterozygosity, improve overall tree productivity and vigor, and in studies of quantitative-trait architecture in tree genomes (Johnston et al., 2003).

Selecting valuable genotypes for ecological restorations from provenance analyses is another often-reported theme in the literature. Bischoff et al. (2010) most recently suggested that locally adapted, site-specific traits exist, but others have proffered that the success of ecological restoration seems to hinge equally on the environment and the conditions of the restoration site because local adaptation varies by taxa (O'Brien and Krauss, 2010). Such findings don't rule out the value of genotypic and phenotypic differences of disparate populations at the margins of distributions, but suggest that specifically for ecological restoration the degree of effects of adaptive variation is less when juxtaposed with the environmental variation present at the restoration site (O'Brien and Krauss, 2010). Despite the effects of environmental variation, seed germination and seedling recruitment have a strong provenance effect (Gao et al., 2010; O'Brien and Krauss, 2010; Gustafson et al., 2004), and in the cases of rare taxa are vital for ensuring an effective conservation strategy to preserve the unique integrities of local populations (Gibson et al., 2008).

### ***Etiolation: A Propagation Strategy for Recalcitrant Species***

Genera recalcitrant to asexual propagation like *Dirca* pose a formidable challenge to horticulturists keen on distributing them in horticultural commerce and to ecologists interested in *ex situ* forms of conservation. Etiolation, or the exclusion of light, can enable adventitious root formation on stems of taxa that otherwise are highly recalcitrant to asexual propagation (Husen, 2008; Maynard and Bassuk, 1996; Maynard and Bassuk, 1987).

Maynard and Bassuk (1987) provide a brief review of the history of etiolation as a stockplant pretreatment. The earliest study cited was Reid (1923), who utilized etiolation to propagate camphor (*Cinnamomum camphora* (L.) J. Presl) from stem cuttings. Gardner (1936)

first employed the use of bands in the form of black insulation tape at the proximal ends of apple (*Malus Tourn. ex L.*) stems, resulting in a 70% increase in rooting compared to non-banded stems. Delargy and Wright (1979) investigated root formation in apple cuttings and the interaction of auxin and etiolation treatments, showing that extensive rooting only occurred when both were used. More recent uses of etiolation in high-value forestry crops include teak (*Tectona grandis* L.f.) (e.g. Husen, 2008) and *Pinus pinaster* Aiton (Majada et al., 2011).

Methodology for etiolation has remained relatively consistent since its earliest uses (Maynard and Bassuk, 1987). Common to all methodologies is a near or total exclusion of light for blanching developing shoots, banding of etiolated stems at the site of rooting hormone application and allowance for chlorophyll formation at the apical end of the shoot, followed by excision of the shoot at the site of banding for cutting propagation (Hansen and Potter, 1997; Maynard and Bassuk, 1987). Some have documented significant rooting from only etiolated, unbanded stems (Anderson, 1981; Blakesley et al., 1992; Patience and Alderson, 1984), leading Maynard and Bassuk (1991) to conclude that exact treatment combinations are taxa- and even cultivar-specific.

The anatomical and hormonal changes due to etiolation don't seem to have a universal basis across all taxa, but are instead much debated and discussed in the literature. Naalamle et al. (2008) reviewed several earlier studies, reporting anatomical changes associated with etiolation including delayed lignification of pericyclic cells, the presence of undifferentiated parenchyma in root initiation sites, and softer tissues in the pith. Maynard and Bassuk (1996) in a study of *Carpinus* L. proposed that sclereid formation reduces rooting potential, but that the formation of sclereids was staved off up to 12 weeks by etiolation. Naalamle et al. (2008) corroborated those results in an analysis of the root-development sequence in *Quercus* L. cuttings. However, they

concluded that ultimately the reduced rooting capacity of *Quercus macrocarpa* Michx. is due to an inability of the species to form root primordia than mechanical restrictions to the growth of that primordia.

The relationships between plant hormones in and exogenously applied synthetic plant hormones to etiolated tissues have been the source of much inquiry. Delargy and Wright (1979) discussed the role of light in the degradation of indole-3-acetic acid (IAA), positing that the principle role of indole-3-butyric acid (IBA) is to favor the conjugation of amino acids and endogenous IAA, which would lead to the formation of root initials. Disagreement and uncertainty exist in the literature though for exactly how cooperative or antagonistic light is to endogenous IAA at various stages of etiolation and de-etiolation (Husen, 2008; Symons and Reid, 2003; Hartmann et al., 2001; Maynard and Bassuk, 1988). Regardless, the synergism of etiolation coupled with IBA application is essential to rooting success in many species as opposed to singular applications of IBA or etiolation without exogenous hormone applications (Husen, 2008; Pacholczak et al., 2005; Husen and Pal, 2003; Delargy and Wright, 1979), though there is much variation among species and even cultivars (Hansen and Potter, 1997). IBA manipulates IAA oxidase and peroxidase activities as a mechanism for the generation of adventitious roots (Qaddoury and Amssa, 2004). IBA treatment can also induce a rise in the levels of endogenous auxin in sharp contrast to untreated cuttings (Qaddoury and Amssa, 2004; Fett-Neto et al., 2001). A recent review highlighted the increasing body of research into plant hormones, light, and their roles in etiolation and de-etiolation in part due to advancements in genetic technology (Symons and Reid, 2003), underscoring our relatively vacant understanding of the complex mechanisms governing adventitious root formation in etiolated tissues.

While advantageous for rooting difficult-to-propagate species, etiolation is labor-intensive (Hansen and Potter, 1997). The value of etiolation in commercial plant propagation will come from a thorough assessment of the market value of the crop under production, the cost-to-benefit ratio, and the availability of trained labor to perform the procedure.

### Literature Cited

- Alexander, N.L., Flint, H.L., and Hammer, P.A. (1984). Variation in cold-hardiness of *Fraxinus americana* stem tissue according to geographic origin. *Ecology*, 65, 1087–1092.
- Anderson, N., Gomez, N., & Galatowitsch, S. (2006). A non-invasive crop ideotype to reduce invasive potential. *Euphytica*, 148, 185-202.
- Anderson, W. C. (1981). Etiolation as an aid to rooting. *Proceedings of the International Plant Propagator's Society*, 31, 138-141.
- Axelrod, D. I. (1986). Analysis of some palaeogeographic and palaeoecologic problems of palaeobotany. *Palaeobotanist*, 35, 115-129.
- Bagley, W.T. (1980). Provenance research for tree crops: *Populus*, *Platanus*, *Juglans*, *Carya*, *Quercus*, etc. Tree crops for energy co-production on farms. *The Institute for Plants*, 191-196.
- Bayer, C., Fay, M. F., De Bruijn, A. Y., Savolainen, V., Morton, C. M., Kubitzki, K., & Chase, M. W. (1999). Support for an expanded family concept of Malvaceae within a recircumscribed order Malvales: a combined analysis of plastid atpB and rbcL DNA sequences. *Botanical Journal of the Linnean Society*, 129, 267-303.

- Beaumont, A. J., Edwards, T. J., Manning, J., Maurin, O., Rautenbach, M., Motsi, M. C., Chase, M. W. (2009). *Gnidia* (Thymelaeaceae) is not monophyletic: taxonomic implications for Thymelaeoideae and a partial new generic taxonomy for *Gnidia*. *Botanical Journal of the Linnean Society*, 160, 402-417.
- Bischoff, A., & Muller-Scharer, H. (2010). Testing population differentiation in plant species- how important are environmental maternal effects. *Oikos*, 119, 445-454.
- Blakesley, D., Weston, G. D., & Elliott, M. C. (1992). Increased rooting and survival of *Cotinus coggygria* cuttings from etiolated stock plants. *Journal of Horticulture Science*, 67, 33-37.
- Bredenkamp, C. L., & Van Wyk, A. E. (2006). Phytogeography of *Passerina* (Thymelaeaceae). *Bothalia*, 36(2), 191-199.
- Brzuszek, R. F., & Harkess, R. L. (2009). Green industry survey of native plant marketing in the southeastern United States. *HortTechnology*, 19(1), 168-172.
- Burghardt, K. T., Tallamy, D. W., & Shriver, W. G. (2009). Impact of native plants on bird and butterfly biodiversity in suburban landscapes. *Conservation Biology*, 23(1), 219-224.
- Castilla, A. R., Alonso, C., & Herrera, C. M. (2011). Exploring local borders of distribution in the shrub *Daphne laureola*: Individual and populations traits. *Acta Oecologia*, 37(37), 269-271.
- Christopher, T. (Ed.). (2011). *The new American landscape*. Portland, OR: Timber Press.
- Creech, J. L. (1966). Expeditions for new horticultural plants. *Arnoldia*, 26(8), 49-53.

- Daehler, C. C., & Strong, D. R. (1993). Prediction and biological invasions. *Trends in Ecology and Evolution*, 8, 380.
- Dawson, K., Veblen, K.E., and Young, T.P. (2007). Experimental evidence for an alkali ecotype of *Lolium multiflorum*, an exotic invasive annual grass in the Central Valley, CA, USA. *Biological Invasions*, 9, 327-334.
- Delargy, J. A., & Wright, C. E. (1979). Root formation in cuttings of apple in relation to auxin application and to etiolation. *New Phytologist*, 82, 341-347.
- Donselman, H.M. and Flint, H.L. (1982). Genecology of eastern redbud (*Cercis canadensis*). *Ecology*, 63(4), 962-971.
- Dosmann, M., & Del Tredici, P. (2003). Plant Introduction, Distribution, and Survival: A Case Study of the 1980 Sino-American Botanical Expedition. *BioScience*, 53(6), 588-597.
- Dreesen, D.R., and Harrington, J.T. (1998). Propagation of native plants for restoration projects in the southwestern U.S. – preliminary investigations. In: *Proc. Joint Meeting of the Western Forest and Conservation Nursery Association. Aug. 19-21, 1997, Boise, ID.*
- Escribano, S., & Lazaro, A. (2009). Agro-morphological diversity of Spanish traditional melons (*Cucumis melo* L.) of the Madrid provenance. *Genetic Resources and Crop Evolution*, 56, 481-497.

- Esteban, L. G., Martin, J. A., de Palacios, P., Fernandez, F. G., & Lopez, R. (2010). Adaptive anatomy of *Pinus halepensis* trees from different Mediterranean environments in Spain. *Trees*, 24, 19-30.
- Falk, D. A., & Thibodeau, F. R. (1986). Saving the rarest. *Arnoldia*, 46(3), 3-16.
- Fett-Neto, A.G., Fett, P.S., Goulart, L.W.V., Pasquali, G., Termignoni, R.R., and Ferreira, A.G.. (2001). Distinct effect of auxin and light on adventitious root development in *Eucalyptus saligna* and *Eucalyptus globules*. *Tree Physiology*, 21, 457-464.
- Floden A. and Mayfield, M.H. (2006). Leatherwood in Kansas: A morphological assessment of an anomalous population of *Dirca palustris* (Thymelaeaceae) [Abstract]. Botany Conference 2006, Chico, California. Retrieved from:  
<http://www.2006.botanyconference.org/engine/search/index.php?func=detail&aid=1115>.  
(Accessed 14 November 2010).
- Floden, A., Mayfield, M., & Ferguson, J. (2009). A new narrowly endemic species of *Dirca* (Thymelaeaceae) from Kansas and Arkansas, with a phylogenetic overview and taxonomic synopsis of the genus. *Journal of the Botanical Research Institute of Texas*, 3(2), 485-499.
- Flory, S. L., & Clay, K. (2010). Non-native grass invasion alters native plant composition in experimental communities. *Biological Invasions*, 12(5), 1285-1294.
- Franco, J.A., Martínez-Sánchez, J.J., Fernández, J.A., and Bañón, S. (2006). Selection and nursery production of ornamental plants for landscaping and xerogardening in semiarid

- environments. *Journal of Horticultural Science*, 81, 3-17.
- Galicia-Herbada, D. (2006). Origin and diversification of *Thymelaea* (Thymelaeaceae): Inferences from a phylogenetic study based on ITS (rDNA) sequences. *Plant Systematics and Evolution*, 257, 159-187.
- Gao, Z., Zhang, C., & Milne, R. I. (2010). Size-class structure and variation in seed and seedling traits in relation to population size of an endangered species *Craigia yunnanensis* (Tiliaceae). *Australian Journal of Botany*, 58, 214-223.
- García-Ramos, G. and Kirkpatrick, M. 1997. Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, 51, 21-28.
- Gardner, F. E. (1937). Etiolation as a method of rooting apple variety stem cuttings. *Proceedings of the American Society for Horticultural Science*, 34, 323-329.
- Gibson, J. P., Rice, S. A., & Stucke, C. M. (2008). Comparison of population genetic diversity between a rare, narrowly distributed species and a common, widespread species of *Alnus* (Betulaceae). *American Journal of Botany*, 95(5), 588-596.
- Godoy, O., Saldano, A., Fuentes, N., Valladares, F., & Gianoli, E. (2011). Forests are not immune to plant invasions: phenotypic plasticity and local adaptation allow *Prunella vulgaris* to colonize a temperate evergreen rainforest. *Biological Invasions*, 13(7), 1615-1625 .
- Goldman, R. L., Goldstein, L. P., & Daily, G. C. (2008). Assessing the conservation value of a human-dominated island landscape: Plant diversity in Hawaii. *Biodiversity and Conservation*, 17(7), 1765-1781.

- Grant, V. (1971). *Plant speciation*. New York: Columbia University Press.
- Graves, W. R., & Schrader, J. A. (2008). At the interface of phylogenetics and population genetics, the phylogeography of *Dirca occidentalis* (Thymelaeaceae). *American Journal of Botany*, 95, 1454-1465.
- Graves, W. R., Schrader, J. A., & Sharma, J. (2006). Cold hardiness of the rare *Dirca occidentalis*: Comparisons to *Dirca palustris* from disparate provenances. *Journal of Environmental Horticulture*, 24(3), 169-172.
- Grixti, J. C., Wong, L. T., Cameron, S. A., & Favret, C. (2009). Decline of bumble bees (*Bombus*) in the North American Midwest. *Biological Conservation*, 142(1), 75-84.
- Greenlee, J. (2009). *The American Meadow Garden: Creating a Natural Alternative*. Portland, OR: Timber Press.
- Gustafson, D. J., Gibson, D. J., & Nickrent, D. L. (2004). Competitive relationships of *Andropogon gerardii* (Big Bluestem) from remnant and restored native populations and select cultivated varieties. *Functional Ecology*, 18, 451-457.
- Hall, C. R., Hodges, A. W., & Haydu, J. J. (2006). The economic impact of the green industry in the United States. *HortTechnology*, 16(2), 345-353.
- Hällfors, M.H., Linden, L., Rita, H., & Schulman, L.E. (2011). Using a botanic garden collection to test a bioclimatic hypothesis. *Biodiversity Conservation*, 20, 259-275.
- Hannerz, M. and Westin, J. (2005). Autumn frost hardiness in Norway spruce plus tree progeny and trees of the local and transferred provenances in central Sweden. *Tree Physiology*, 25, 1181-1186.

- Hansen, O. B., & Potter, J. R. (1997). Rooting of apple, rhododendron, and mountain laurel cuttings from stock plants etiolated under two temperatures. *HortScience*, 32(2), 304-306.
- Hartley, M. K., Rogers, W. E., & Siemann, E. (2010). Comparisons of arthropod assemblages on an invasive and native trees: abundance, diversity and damage. *Arthropod-Plant Interactions*, 4(4), 237-245.
- Hartmann, H. T., Kester, D. E., Davies, F. T., Jr., & Geneve, R. L. (2001). *Hartmann and Kester's Plant Propagation: Principles and Practices* (7th ed.). Prentice Hall.
- Haynes, C. L., VanDerZanden, A. M., & Iles, J. K. (2007). A survey of the ornamental horticulture industry in Iowa. *HortTechnology*, 17(4), 513-517.
- Husen, A. (2008). Stock-plant etiolation causes drifts in total soluble sugars and anthraquinones, and promotes adventitious root formation in teak (*Tectona grandis* L. f.) coppice shoots. *Plant Growth Regulation*, 54, 13-21.
- Husen, A., & Pal, M. (2003). Effect of serial bud grafting and etiolation on rejuvenation and rooting cuttings of mature trees of *Tectona grandis* Linn. f. *Silvae Genetica*, 52(2), 84-88.
- Jin, Z., & Li, J. (2007). Genetic differentiation in endangered *Heptacodium miconioides* Rehd. based on ISSR polymorphism and implications for its conservation. *Forest Ecology and Management*, 245, 130-136.
- Johnston, A. J., Dieters, M. J., Dungey, H. S., & Wallace, H. M. (2003). Intraspecific hybridization in *Pinus caribaea* var. *hondurensis* I. Performance for growth and form traits. *Euphytica*, 129, 147-157.

- Kaniewski, D., De Laet, V., Paulissen, E., & Waelkens, M. (2007). Long-term effects of human impact on mountainous ecosystems, western Taurus Mountains, Turkey. *Journal of Biogeography*, *34*, 1975-1997.
- Kingsbury, N. (2009). *Hybrid*. Chicago: University of Chicago Press.
- Koller, G. (1986). Seven-son flower from Zhejiang: Introducing the versatile ornamental shrub *Heptacodium jasminoides*. *Arnoldia*, *46*(4), 2-14.
- Lauridsen, E. B. (2004). Features of some provenances in an international provenance experiment of *Gmelina arborea*. *New Forest*, *28*, 127-145.
- Lavergne, S., & Molofsky, J. (2007). Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Science*, *104*(10), 3883-3888.
- Lebeda, A., Dolezalova, I., Kristkova, E., Kitner, M., Petrzelova, I., Mieslerova, B., & Novotna, A. (2009). Wild *Lactuca* germplasm for lettuce breeding: current status, gaps, and challenges. *Euphytica*, *170*, 15-34.
- Lenahan, O., Graves, W. R., & Arora, R. (2010). Cold hardiness and deacclimation within three Populations of *Styrax americanus*. *HortScience*, *45*(12), 1819-1823.
- Lesica, P. and Allendorf, F.W. (1995). When are peripheral populations valuable for conservation? *Conservation Biology*, *9*, 753-760.
- Li, C., Welling, A., Puhakainen, T., Vihera-Aarnio, A., Ernstsén, A., Junttila, O., . . . Palva, E. (2005). Differential responses of silver birch (*Betula pendula*) ecotypes to short-day photoperiod and low temperature. *Tree Physiology*, *25*, 1563-1569.

- Lomolino, M. V., Riddle, B. R., & Brown, J. H. (2006). *Biogeography* (3rd ed.). Sunderland, MA: Sinauer Associates.
- Lopez, R., Climent, J., & Gil, L. (2010). Intraspecific variation and plasticity in growth and foliar morphology along a climate gradient in the Canary Island pine. *Trees*, *24*, 343-350.
- Lowman, L. (2010, Spring). A new woody species in my backyard. *Claytonia*, *30*(1), 4-5.
- MacGinitie, H. D. (1953). Fossil plants of the Florissant Beds, Colorado. *Contributions to Paleontology*, (599), 1-386.
- Macphail, M., & Cantrill, D. J. (2006). Age and implications of the Forest Bed, Falkland Islands, southwest Atlantic Ocean: Evidence from fossil pollen and spores. *Palaeogeography, Palaeoclimatology; Palaeoecology*, *240*, 602-629.
- Magi, M., Semchenko, M., Kalamees, R., & Zobel, K. (2011). Limited phenotypic plasticity in range-edge populations: a comparison of co-occurring populations of two *Agrimonia* species with different geographical distributions. *Plant Biology*, *13*(1), 177-184.
- Mahmood, K., Marcar, N. E., Naqvi, M. H., Arnold, R. J., Crawford, D. F., Iqbal, S., & Aken, K. M. (2003). Genetic variation in *Eucalyptus camaldulensis* Dehnh. for growth and stem straightness in a provenance-family trial of saltland in Pakistan. *Forest Ecology and Management*, *176*, 405-416.
- Majada, J., Martinez-Alonso, C., Feito, I., Kidelman, A., Aranda, I., & Alia, R. (2011). Mini-cuttings: an effective technique for the propagation of *Pinus pinaster* Ait. *New Forests*, *41*(3), 399-412.

- Mayer, S. S. (1991). Artificial Hybridization in Hawaiian *Wikstroemia* (Thymelaeaceae). *American Journal of Botany*, 78(1), 122-130.
- Maynard, B. K., & Bassuk, N. L. (1987). Stockplant etiolation and blanching of woody plants prior to cutting propagation. *Journal of the American Society for Horticultural Science*, 112(2), 273-276.
- Maynard, B.K. and Bassuk N.L. (1988). Etiolation and banding effects on adventitious root formation. In: Davis T.D., Haissig B.E., Sankhla N. (eds.) *Adventitious root formation by cuttings*. Dioscorides Press, Portland, OR.
- Maynard, B. K., & Bassuk, N. L. (1991). Stock plant etiolation and stem banding effect on the auxin-dose response of rooting in stem cuttings of *Carpinus betulus* L. 'Fastigiata'. *Plant Growth Regulation*, 10, 305-311.
- Maynard, B. K., & Bassuk, N. L. (1996). Effects of stock plant etiolation, shading, banding, and shoot development on histology and cutting propagation of *Carpinus betulus* L. *Journal of the American Society for Horticultural Science*, 121(5), 853-860.
- McMahan, L. R. (2006). Understanding cultural reasons for the increase in both restoration efforts and gardening with native plants. *Native Plants Journal*, 7(1), 31-34.
- McNamara, S., Pellett, H., Florkowska, M., & Lindstrom, O., Jr. (2002). Comparison of the cold hardiness of landscape tree and shrub cultivars growing at two disparate geographic locations. *Journal of Environmental Horticulture*, 20(2), 77-81.

- Naalamle, J. A., Paolillo, D. J., Jr., & Bassuk, N. (2008). Adventitious root formation in stem cuttings of *Quercus bicolor* and *Quercus macrocarpa* and its relationship to stem anatomy. *Journal of the American Society for Horticultural Science*, 133(4), 479-486.
- Nesom, G. L., & Mayfield, M. H. (1995). A new species of *Dirca* (Thymelaeaceae) from the Sierra of northeastern Mexico. *Sida*, 16(3), 459-467.
- Nevling, L.I., Jr. (1959). A revision of the genus *Daphnopsis*. *Annals of the Missouri Botanical Garden*, 46(4), 257-358.
- Nevling, L. I., Jr. (1964). Typification in *Dirca*. *Journal of the Arnold Arboretum*, 45(1), 158-159.
- Norton, M., & Norton, C. (2008). Ecological restoration and change in biodiversity with and without horticultural intervention. *HortScience*, 43(4), 1091.
- O'Brien, B.C. (1996). Xeriscaping: sources of new native ornamental plants. p. 536-539. In: J. Janick (ed.), *Progress in new crops*. VA, Arlington: ASHS Press.
- O'Brien, E.K. and Krauss, S.L. (2010). Testing the home-site advantage in forest trees on disturbed and undisturbed sites. *Restoration Ecology*, 18, 359-372.
- Ogden, S., & Springer Ogden, L. (2008). *Plant-driven design: Creating gardens that honor plants, place, and spirit*. Portland, OR: Timber Press.

- Oleksyn, J., Modrzyński, J., Tjoelker, M. G., Zytowski, R., Reich, P. B., & Karolewski, P. (1998). Growth and physiology of *Picea abies* populations from elevational transects: Common garden evidence for altitudinal ecotypes and cold adaptation. *Functional Ecology*, *12*, 573-590.
- Pachloczak, A. Z., Szydło, W. I., & Łukaszewska, A. S. (2005). The effect of etiolation and shading of stock plants on rhizogenesis in stem cuttings of *Cotinus coggygria*. *Acta Physiologiae Plantarum*, *27*(4A), 417-428.
- Patience, P. A., & Alderson, P. G. (1984). Improving the rooting of *Syringa vulgaris* cuttings by etiolation. *Proceedings of the International Plant Propagator's Society*, *34*, 316-327.
- Pemberton, R. W., & Liu, H. (2009). Marketing time predicts naturalization of horticultural plants. *Ecology*, *90*(1), 69-8-.
- Peterson, B. J., & Graves, W. R. (2009). Variation in development and response to root-zone pH among seedlings of *Dirca palustris* (Thymelaeaceae) from three provenances. *HortScience*, *44*(5), 1319-1322.
- Peterson, B. J., Graves, W. R., and Sharma, J. (2011). Phenotypic and genotypic diversity of eastern leatherwood in five populations that span its geographic distribution. *American Midland Naturalist*, *165*, 1-21.
- Pfeifer, M., Passalacqua, N. G., Bartram, S., Schatz, B., Croce, A., Carey, P. D., Jeltsch, F. (2010). Conservation priorities differ at opposing species borders of a European orchid. *Biological Conservation*, *143*(9), 2207-2220.

- Qaddoury, A., & Amssa, M. (2004). Effect of exogenous indole butyric acid on root formation and peroxidase and indole-3-acetic acid oxidase activities and phenolic contents in date palm offshoots. *Botanical Bulletin of Academia Sinica*, 45, 127-131.
- Rajakaruna, N. (2004). The edaphic factor in the origin of plant species. *International Geological Review*, 46, 471-478.
- Rajakaruna, N., Baldwin, B. G., Chan, R., Desrochers, A. M., Bohm, B. A., & Whitton, J. (2003). Edaphic races and phylogenetic taxa in the *Lasthenia californica* complex (Asteraceae: Heliantheae): An hypothesis of parallel evolution. *Molecular Ecology*, 12, 1675-1679.
- Rajakurana, N., & Whitton, J. (2004). Trends in the evolution of edaphic specialists with an example of parallel evolution in the *Lasthenia californica* complex. *Plant Adaptation: Molecular Genetics and Ecology*, 103-110.
- Ramesh, K. R., & Khurana, D. K. (2008). Clonal provenance variation of *Populus alba* Linn. in nursery. *Indian Journal of Forestry*, 31(4), 599-610.
- Repo, T., Zhang, G., Ryypö, A., Rikala, R., and Vuorinen, M. (2000). The relation between growth cessation and frost hardening in Scots pines of different origins. *Trees*, 14, 456-464.
- Reid, O. (1923). The propagation of camphor by stem cuttings. *Transactions and Proceedings of the Botanical Society of Edinburgh*, 28, 184-188.
- Rogers, Z. S. (2009). *A World Checklist of Thymelaeaceae*. Retrieved November 27, 2009, from Missouri Botanical Garden website: <http://www.tropicos.org/Project/Thymelaeaceae>

- Ruter, B., Hamrick, J. L., & Wood, B. W. (1999). Genetic diversity within provenance and cultivar germplasm of collections versus natural populations of pecan (*Carya illinoensis*). *The Journal of Heredity*, 90(5), 521-528.
- Sandquist, D. R., & Ehleringer, J. R. (1997). Intraspecific variation of leaf pubescence and drought response in *Encelia farinosa* associated with contrasting desert environments. *New Phytologist*, 135(4), 635-644.
- Schrader, J. A., & Graves, W. R. (2004). Systematics of *Dirca* (Thymelaeaceae) based on ITS sequences and ISSR polymorphisms. *Sida*, 21(2), 511-524.
- Smithberg, M. H., & Weiser, C. J. (1968). Patterns of variation among climatic races of red-osier dogwood. *Ecology*, 49(3), 495-505.
- Symons, G. M., & Reid, J. B. (2003). Interactions between light and plant hormones during de-etiolation. *Journal of Plant Growth Regulation*, 22, 3-14.
- Tallamy, D. W., & Shropshire, K. J. (2009). Ranking lepidopteran use of native versus introduced plants. *Conservation Biology*, 23(4), 941-947.
- Tawan, C. S. (1999). A new species of *Gonystylus* (Thymelaeaceae) from Sarawak, Borneo. *Botanical Journal of the Linnean Society*, 130, 65-68.
- Valladares F., Gianoli E., & Gomez J.M. (2007). Ecological limits to plant phenotypic plasticity. *New Phytologist*, 176, 749-763.
- Weber, A., & Kolb, A. (2011). Evolutionary consequences of habitat fragmentation: population size and density affect selection on inflorescence size in a perennial herb. *Evolutionary Ecology*, 25(2), 417-428.

- Weber, J. C., & Montes, C. S. (2010). Correlations and clines in tree growth and wood density of *Balanites aegyptiaca* (L.) Delile provenances in Niger. *New Forests*, 39, 39-49.
- Weinig, C. (2000). Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. *Evolution*, 54, 124–136.
- Weng, Y.H. and Parker, W.H. (2008). Adaptive variation in fall cold hardiness of aspen from northwestern Ontario. *Tree Physiology*, 28, 143-150.
- Wood, B. W., Grauke, L. J., & Payne, J. A. (1998). Provenance variation in pecan. *Journal of the American Society for Horticultural Science*, 123(6), 1023-1028.
- Van der Bank, M., Fay, M. F., & Chase, M. W. (2002). Molecular phylogenetics of Thymelaeaceae with particular reference to African and Australian genera. *Taxon*, 51, 329-339.
- Yinzheng, W., Gilbert, M. G., Mathew, B., Brickell, C., & Nevling, L. I. (1999). 17. Thymelaeaceae . In *Flora of China* (Vol. 13, p. 213). Retrieved from [http://www.efloras.org/florataxon.aspx?flora\\_id=2&taxon\\_id=10894](http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=10894). Accessed 14 November 2010.
- Young, T.P. (2000). Restoration ecology and conservation biology. *Biological Conservation*, 92,73-83.

## ***Dirca*: An Introduction to the Leatherwoods**

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Horticulturists have overlooked *Dirca* spp. despite the many ornamental characteristics of these shade-tolerant but difficult-to-propagate shrubs. The genus is characterized by slow growth, shade tolerance, flowers in late winter or spring, and mature growth approximately 1 to 2 meters tall. Despite a long history of favorable descriptions in the literature (Kirkegaard, 1912; Anderson, 1933; Del Tredici, 1984; Dirr, 2005; Buffin, 2005), *Dirca* spp. remain rare in commerce and limited mostly to arboreta or collector gardens.

Commonly known as leatherwoods, *Dirca* spp. are valued horticulturally for their tolerance to shade, late winter and early spring flowering time, yellow fall color, and arborescent stature (Esson, 1949; Dirr, 1998). In cultivation, *Dirca* spp. are best suited to partial or full shade in rich, organic soils. Seed-propagated plants of all species may bloom after three to five years. Challenges to marketing and producing *Dirca* for horticultural use include its recalcitrance to sexual and asexual propagation methods and the need to characterize differences in provenances for sensitivity to edaphic conditions and cold hardiness (Peterson and Graves, 2009; Graves et al., 2006), particularly in *Dirca palustris*. The recalcitrance of *Dirca* spp. to both sexual and asexual propagation methods has been well documented in the literature (Dirr and Heuser, 1987; Del Tredici, 1984). Endodormancy and paradormancy cause sporadic

germination among all three species and limit the production of nursery stock (Schrader and Graves, 2005). An asexual approach could be a more viable way to propagate plants for commercial production. However, Dirr and Heuser (1987) noted minimal success in propagating *D. palustris* from softwood stem cuttings taken in early summer. Research presently undertaken by the authors is designed to explore whether etiolating stems used for cuttings will improve rooting.

Three species are described here. A group of researchers has published evidence for a fourth species *Dirca decipiens* (Floden et al., 2009) known from one population in Johnson County, Kansas, and two populations in Carroll County, Arkansas, occurring sympatrically within the range of *D. palustris*.

### ***Dirca palustris* L. (Eastern leatherwood)**

*Dirca palustris* L. occurs in highly localized populations across the eastern one-third of the United States from Maine and Ontario, west to North Dakota, south to Oklahoma and Louisiana, and east to northern Florida (Nevling, 1964; Peterson et al., 2009). The species is identified by its alternate, entire and obovate leaves, and pedicellate, axillary trios of yellow, tubular flowers borne in early spring. Despite a broad range, the plant is uncommon in the wild and listed as endangered in Florida and threatened in Maryland. *Dirca palustris* occurs in rich mesic forest understories with a particular affinity for streamsides (Williams, 2004), but has been documented from a wide range of soil types and profiles. Comparisons of plants from different provenances within the species' distribution are needed to select commercially viable forms for introduction into the horticultural trade and to understand the species' phylogeographic adaptations to irradiance and edaphic conditions.

Plants from two provenances have survived since 2007 in a trial at Ames, Iowa, on the boundary between USDA Zones 4 and 5. Plants of *D. palustris* from Florida have been more vigorous and have shown more stem growth than plants from Thunder Bay, Ontario. Plants of *Dirca occidentalis* and *Dirca mexicana* have also been evaluated since 2007 in the same trial. Of the three species, *D. mexicana* exhibits the highest survival rate, and stem growth and health ratings similar to those of *D. palustris* from Florida. *Dirca occidentalis* is intermediate in survival rate, stem growth, and plant health.

#### ***Dirca occidentalis* Gray (Western leatherwood)**

*Dirca occidentalis* Gray occurs in six counties near the San Francisco Bay in California. It's utilized to limited extent horticulturally in California, but is largely uncultivated outside of its range. This species is identified by its whitish bud scales, obovate leaves and comparatively larger, yellow funnellform flowers with long-exserted stigmas. Graves et al., 2006 suggested that more information is needed to assess the cold-hardiness and suitability of *D. occidentalis* for planting outside of its native range, which is restricted to portions of USDA Hardiness Zone 9 within a Mediterranean climate. Plants in a trial at Ames, Iowa, have survived at a rate similar to the survival rate of *D. palustris* from Thunder Bay, Ontario, but by midsummer evaluation exhibit varying degrees of foliar chlorosis. A plant of this species has flowered in Ames, Iowa, and remains alive as of this report, demonstrating cold hardiness greater than necessary to persist within its native range.

### ***Dirca mexicana* Nesom & Mayfield (Mexican leatherwood)**

*Dirca mexicana* Nesom & Mayfield is known from a single reported population in Tamaulipas, Mexico (Nesom and Mayfield, 1995), and has not been introduced to horticultural commerce. This report represents the first publication of this species in the popular literature. This species is identified by its alternate, entire and ovate to elliptic leaves, and sessile, axillary trios of yellow flowers. A propagation protocol is needed for *D. mexicana* to promote its use as an ornamental and to foster efforts to conserve the species in the wild (Schrader and Graves, 2005). Initial trials in Ames, Iowa, suggest that *Dirca mexicana* may be the most commercially viable species in the genus, due to its high survival rate (higher than its native congener *D. palustris*), annual growth rate, and excellent health ratings.

### **Literature Cited**

- Anderson, E. (1933). Leatherwood (*Dirca palustris*). *Arnold Arboretum Bulletin Popular Information*, 1(5), 25-27.
- Buffin, M. W. (2005). *Winter-flowering shrubs*. Portland, OR: Timber Press.
- Del Tredici, P. (1984). Propagating leatherwood: A lesson in humility. *Arnoldia*, 44, 20-24.
- Dirr, M. A. (1998). *Manual of woody landscape plants: Their identification, ornamental characteristics, propagation, and uses*. Champaign, IL: Stipes Publishing Company.
- Dirr, M. A. (2005). Ten top trees and shrubs: Tantalizing choices for spring and beyond. *Horticulture, 2005 Spring Planting Issue*, 102(2), 44-48.

- Dirr, M. A., & Heuser, C. W. (1987). *The reference manual of woody plant propagation: From seed to tissue culture*. Athens, GA: Varsity Press.
- Esson, J. G. (1949). Leatherwood for early spring bloom. *Journal of the New York Botanical Garden*, 50, 57-59.
- Floden, A.J., Mayfield, M.H., & Ferguson, C.J. (2009). A new narrowly endemic species of *Dirca* (Thymelaeaceae) from Kansas and Arkansas, with a phylogenetic overview and taxonomic synopsis of the genus. *Journal of the Botanical Research Institute of Texas*, 3, 485-499.
- Graves, W. R., Schrader, J. A., & Sharma, J. (2006). Cold hardiness of the rare *Dirca occidentalis*: Comparisons to *Dirca palustris* from disparate provenances. *Journal of Environmental Horticulture*, 24(3), 169-172.
- Kirkegaard, J. (1912). *Trees, Shrubs, Vines and Herbaceous Perennials*. Boston, MA: The Bullard Company.
- Nesom, G. L., & Mayfield, M. H. (1995). A new species of *Dirca* (Thymelaeaceae) from the Sierra of northeastern Mexico. *Sida*, 16(3), 459-467.
- Nevling, L. I., Jr. (1964). Typification in *Dirca*. *Journal of the Arnold Arboretum*, 45(1), 158-159.
- Peterson, B. J., & Graves, W. R. (2009). Variation in development and response to root-zone pH among seedlings of *Dirca palustris* (Thymelaeaceae) from three provenances. *HortScience*, 44(5), 1319-1322.

Peterson, B. J., Graves, W. R., & Sharma, J. (2009). Color of pubescence on bud scales conflicts with keys for identifying species of *Dirca* (Thymelaeaceae). *Rhodora*, *111*(945), 126-130.

Schrader, J. A., & Graves, W. R. (2005). Seed germination of *Dirca* (Leatherwood): Pretreatments and interspecific comparisons. *HortScience*, *40*(6), 1838-1842.

Williams, C. E. (2004). Mating system and pollination biology of the spring-flowering shrub, *Dirca palustris*. *Plant Species Biology*, *19*, 101-106.

### **CHAPTER 3. Survival, Health, and Growth of Three Species of Leatherwood in the Upper Midwest**

*A paper prepared for HortScience*

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**ABSTRACT.** Horticulturists have overlooked the genus *Dirca* despite the many ornamental characteristics of these shade-tolerant but difficult-to-propagate shrubs. A trial of three species was established at Ames, Iowa, in 2007 to evaluate winter hardiness, health, and annual stem extension. *Dirca mexicana* had the greatest survival rate with 89% alive as of 2010. Within *Dirca palustris*, plants from Thunder Bay survived 45% better than plants from Florida but exhibited poor vigor and achieved low health ratings. In 2009 and 2010, plants of *D. palustris* from Thunder Bay grew 59% and 40% less, respectively, than *D. mexicana* and *D. palustris* from Florida. *Dirca mexicana* and *D. palustris* from Florida were rated the healthiest, and their stems extended the most annually. Qualitatively, plants of both species exhibited uniform growth and abundant foliage rarely blemished by herbivory or foliar diseases. Our results demonstrate *D. mexicana* has the potential to be used as a landscape plant in USDA Hardiness Zones 4 and 5, whereas provenance selections from within *D. palustris* evince a selection gradient for commercially viable traits. Plants from Florida show the most potential for horticultural use because of exceptional vigor and health, despite initially poor survivability. We

do not recommend *D. occidentalis* for broader landscape use in the Upper Midwest unless suitable genotypes are discovered.

Many plants native to North America remain underused in landscapes despite public interest in the cultivation of native plants (McMahan, 2006; Brzuszek and Harkess, 2009) and the conservation of these plants in the wild. Three species in the genus *Dirca* L., commonly known as leatherwoods, are indigenous to North America. Despite a long history of favorable descriptions (Kirkegaard, 1912; Anderson, 1933; Del Tredici, 1984; Buffin, 2005), *Dirca* spp. remain rare in commerce and underutilized as landscape plants.

Eastern leatherwood (*Dirca palustris* L.) occurs in localized populations across the eastern one-third of the United States from Maine and Ontario, west to North Dakota, south to Oklahoma and Louisiana, and east to northern Florida (Nevling, 1964; Peterson et al., 2009). Despite a broad range, the plant is uncommon in the wild and listed as endangered in Florida and threatened in Maryland (Wunderlin and Hansen, 2008). *Dirca palustris* occurs in understories of rich, mesic forests and has a particular affinity for north-facing slopes near waterways (Williams, 2004), but it has been documented in a wide range of soil types and profiles. Western leatherwood (*Dirca occidentalis* Gray) occurs in six counties near the San Francisco Bay in California. Graves et al. (2006) suggested that more information is needed to assess the cold hardiness and suitability of *D. occidentalis* for planting outside its native range, which is restricted to portions of USDA Hardiness Zone 9, a Mediterranean climate. Mexican leatherwood (*Dirca mexicana* Nesom and Mayfield) is known from a single reported population in Tamaulipas, Mexico, (Nesom and Mayfield, 1995) and has not been introduced to horticultural commerce.

Horticulturists value *Dirca* spp. for their tolerance of shade, yellow flowers in late winter and early spring, yellow foliage in autumn, and arborescent stature (Esson, 1949; Dirr, 1998). In cultivation, *Dirca* spp. are best suited to partial or full shade in rich, organic soils (Dirr, 1998). Little is known about the edaphic affinities of the species, and the literature is conflicted about tolerances in the wild, suggesting that *Dirca* spp. grow in a range of pH and soil types (Cooperrider, 1962; Clark, 1971; Del Tredici, 1984; Graves and Schrader, 2008). Challenges to marketing and producing *Dirca* spp. for horticultural use include their recalcitrance to sexual and asexual propagation (Dirr and Heuser, 1987; Del Tredici, 1984), slow growth (Esson, 1949; Dirr, 1998), and the need to evaluate differences among taxa and genotypes within taxa in environmental tolerances, vigor, and ornamental traits. Peterson and Graves (2009) documented differences between provenances of *D. palustris* in response to soil pH for stem elongation and biomass accrual. However, further comparisons of *D. palustris* from different provenances within its range are needed to select commercially superior forms for introduction into the horticultural trade and to document the extent of variation in environmental tolerances within the species (Graves et al., 2006; Peterson and Graves, 2009). Such information is needed for the other species of *Dirca* also.

This report describes the first trial to compare directly *D. palustris*, *D. occidentalis*, and *D. mexicana*. Our specific objectives were to document the capacity of these species to survive near the boundary of U.S.D.A. cold-hardiness zones 4 and 5 (U.S. Department of Agriculture, 1990) in the Upper Midwest, to rate the health of surviving plants, and to track annual stem elongation for three growing seasons.

## Materials and Methods

**Sources of germplasm.** Three species were included in this trial; two provenances of *Dirca palustris* (nine plants from Thunder Bay, Ontario, and 18 plants from Torreya State Park, Liberty County, Florida), *D. mexicana* collected from the Sierra Madre Oriental in Tamaulipas, Mexico (Nesom and Mayfield, 1995), and *D. occidentalis* from San Mateo County, CA. All plants were grown from seeds we collected in 2005 that germinated in early 2006. Seedlings were raised in pots in the greenhouse before to installation in the trial.

**Experimental sites.** Three experimental plots were established in Ames, Iowa (42° 1'30.47"N, 93°38'31.14"W). The sites were chosen for their similarity to conditions in which *Dirca* might naturally occur in the wild; along a watercourse, in shade, with overhead canopy. Distances separating the plots ranged from 26 m to 135 m, respectively. Overstory vegetation at the sites is predominantly deciduous hardwoods such as *Acer negundo* L., *Acer nigrum* F. Michx., *Acer platanoides* L., *Fraxinus* L. spp., *Juglans nigra* L., and *Ulmus* L. spp. *Lonicera tatarica* L. and *Parthenocissus quinquefolia* (L.) Planch. are adjacent to all three sites. The understory contains various woodland ephemerals like *Asarum canadense* L., *Viola sororia* Willd., *Viola pubescens* Aiton, in addition to *Ageratina altissima* (L.) King & H. Rob., *Menispermum canadense* L., *Polygonatum biflorum* (Walter) Elliot, two species of *Smilax* L., *Verbesina alternifolia* (L.) Britton ex Kearney, and at least three species of *Carex* L. Soil analyses of the plots indicated pH values of 7.75, 7.65, and 7.45 and percent organic matter contents of 9, 11, and 14, respectively. Means of measurements of photosynthetically active radiation (PAR) at solar noon on cloudless days varied within and among sites, but were within a range of 45 to 150  $\mu\text{mol m}^{-1} \text{s}^{-1}$ .

**Experimental design.** Eighteen-month-old plants (81 total, 27 each of three species) grown in from 6 cm diameter, square band pots (Anderson Die & Mfg. Co., Portland, OR) were planted on 24 October 2007 in three plots in Ames, Iowa. Each plot was approximately 5.5 m by 1.8 m and had been cleared of brushy vegetation the day before planting. Vegetation removed during the clearing included both native and non-native woody species. Plants were installed randomly in three rows of nine, spaced approximately 0.5 m apart with approximately 1 m between rows. Each site contained 27 plants with nine plants of each species represented; within *D. palustris*, six plants from Florida and three from Thunder Bay were installed. The sites were irrigated from the adjacent stream and mulched with chips of wood and bark immediately after planting. No other irrigation was provided, nor was fertilizer applied.

**Environmental monitoring.** Air temperature was logged with an iButton Data Logger (Maxim Integrated Products, Sunnyvale, CA) at each plot, and data on annual precipitation were obtained from an Iowa State Agclimate Automated Weather station 4.8 km from the plots. Annual rates of precipitation (measured November through October) ranged from a low of 96.3 cm in 2008-2009 to a high of 136.9 cm in 2009-2010. Mean daily winter minima always occurred in January and ranged from a low of -32 °C in 2008-2009 to a high of -26 °C in 2007-2008. Mean daytime summer maxima during the peak growing season (1 May through 31 August) were similar throughout the study and ranged from a low of 31 °C in 2008-2009 to a high of 33 °C in 2009-2010.

**Data collection and analysis.** Data were collected twice annually from 2008 to 2010. On 1 May, percentage survival was calculated from a count of living plants. On 1 July, health assessments were made using a qualitative scale of 0 to 3. A 0 rating meant dead or missing; a 1 rating indicated the plant was suffering from serious chlorosis, had distorted leaves, or was

growing poorly; a 2 rating indicated the plant displayed moderate problematic symptoms intermediate between a 1 and a 3 rating; a 3 rating indicated the plant had excellent foliar quality and appeared vigorous. Stem extension was measured in millimeters as the length of new wood on the longest branch only. In addition to these quantitative data, a database of qualitative appraisals of plant health was maintained from notes made in May and July of each year. Mean separation tests were conducted using Statistical Analysis System 9.1.3 (SAS Institute Inc., Cary, NC). The LSMEANS option of the general linear models (GLM) procedure was used to generate mean-separation statistics.

## Results

Plants of *D. mexicana* had the highest survival rate over the three-year trial period (Fig. 1). As of July 2010, 89% of *D. mexicana* plants remained alive. Within *D. palustris*, plants from Thunder Bay survived better than plants from Florida, but they often exhibited poor stem extension and health (Figs. 2, 3). *Dirca palustris* from Florida exhibited the poorest survival in both winter and summer seasons with a 33% decline between October 2007 and May 2008 and a 22% decline between May 2008 and July 2008. Survival of *D. occidentalis* and *D. palustris* from Thunder Bay was similar, and trends of decline in survival over time were similar in all taxa except *D. mexicana* (Fig. 1).

*Dirca mexicana* and *D. palustris* from Florida consistently exhibited the best health ratings (Fig. 2). Our qualitative observations indicated that plants of both species exhibited uniform growth and abundant foliage rarely blemished by herbivory or foliar diseases. Plants of *D. palustris* from Florida had qualitatively similar health appraisals to *D. mexicana* in both May and July. In contrast, many plants of *D. palustris* from Thunder Bay exhibited varying degrees of chlorosis, particularly in May. *Dirca occidentalis* showed mild symptoms of chlorosis in both

May and July, and in 2009 and 2010 earned the lowest health ratings. Health ratings of the surviving plants of all species improved over time.

Stems of *Dirca mexicana* and *D. palustris* from Florida extended the most annually (Fig. 3), despite poor survivability of the latter. While there were no differences in stem extension across taxa in 2008, stem extension in *D. mexicana* and *D. palustris* Florida increased over time (Fig. 3). Stem extension of *D. occidentalis* was consistently  $80 \pm 3$  mm.

## Discussion

Collection and evaluation of horticulturally uncommon taxa facilitates the process of introducing plants to commerce (Creech, 1966; Dosmann and Del Tredici, 2003). Despite favorable appraisals in the literature and many ornamental traits, *Dirca* spp., like many taxa native to North America, remain underused in managed landscapes (Del Tredici, 1984; Buffin, 2005). Evaluation of provenances from species with large distributions plays a similar role in the new plant development process. While only two provenances of *D. palustris* were evaluated in this study, the methodology could be applied to future studies of provenances of this and other species. Hällfors et al. (2011) confirm that provenance is an informative factor in plant performance in the landscape. However, Schrader and Graves (2003) acknowledge that conserved genetic functions for traits like hardiness can inform how or why a particular taxon performs in the landscape regardless of its contemporary geographic origin.

*Dirca mexicana* had the highest survival rate (Fig. 1). Described in 1994 by Nesom and Mayfield, *D. mexicana* remains un-introduced to horticulture. Our results show that *D. mexicana* possesses the greatest horticultural potential of any of the leatherwoods in the Upper Midwest, given its superior survival and health ratings, and annual stem extension (Figs. 1, 2, 3). Our observational appraisals of *D. mexicana* in other locations on the Iowa State University campus

suggest that the species has the potential for yellow fall color lasting well into November depending on weather conditions.

Within *D. palustris*, plants from Thunder Bay survived better than plants from Florida, but plants from Thunder Bay exhibited poor stem elongation and health (Figs. 2, 3). Differences in cold hardiness of provenances across north-south gradients is well documented in other woody taxa (Repo et al., 2000; Hannerz and Westin, 2005; Graves et al., 2006; Weng and Parker, 2008; Hällfors et al., 2011). Despite poor survival, plants of *D. palustris* from Florida earned health ratings equivalent to those of *D. mexicana*, suggesting that either a tolerance of environmental stresses increases over time in the surviving plants or confirming variation among genotypes for traits like survivability. Regardless, this overall durability of the survivors suggests that a selection differential exists to develop fast-growing clones from this provenance after initial evaluations for cold hardiness. Provenance-based selections within species can result in cultivars with discriminate differences (Escribano and Lazaro, 2009; Ramesh and Khurana, 2008) in part due to infraspecific variation. Peterson et al. (2011) found considerable variation among and within populations of *D. palustris* for phenotypic traits, including floral traits that were negatively correlated with latitude. Further research is needed to evaluate the comparative successes and ornamental traits of other provenances of *D. palustris* to achieve a greater understanding of the exploitable genetic variation present within the species across its distribution.

Personal communications with nursery professionals suggest that *D. palustris* can be an intermittent performer in the nursery and managed landscape due to its slow growth rate. *Dirca mexicana* and *D. palustris* from Florida grew the most annually (Fig. 3), despite poor survivability of the latter. Other studies also have shown that southerly provenances of woody

taxa grow more when transferred to northern sites (Hannerz and Westin, 2005; Weng and Parker, 2008). Given the rapid growth rate of *D. mexicana* in comparison to *D. palustris* from Thunder Bay, it seems that the former would offer horticulturists a good alternative for situations where *Dirca* spp. might be used in the landscape.

We employed a simple, qualitative rating scale for the evaluation of plant health. Mean health ratings of plants from all three species have improved over time, possibly due to adaptation to the local environment in surviving plants or the success of thriving plants at the end of the data collection period. In 2009 and 2010, *D. mexicana* and *D. palustris* from Florida showed more stem extensions than either *D. palustris* from Thunder Bay or *D. occidentalis* (Fig. 3). This demonstrates provenance difference within *D. palustris* and also suggests that *D. occidentalis* is not well adapted for horticultural use in the upper Midwest.

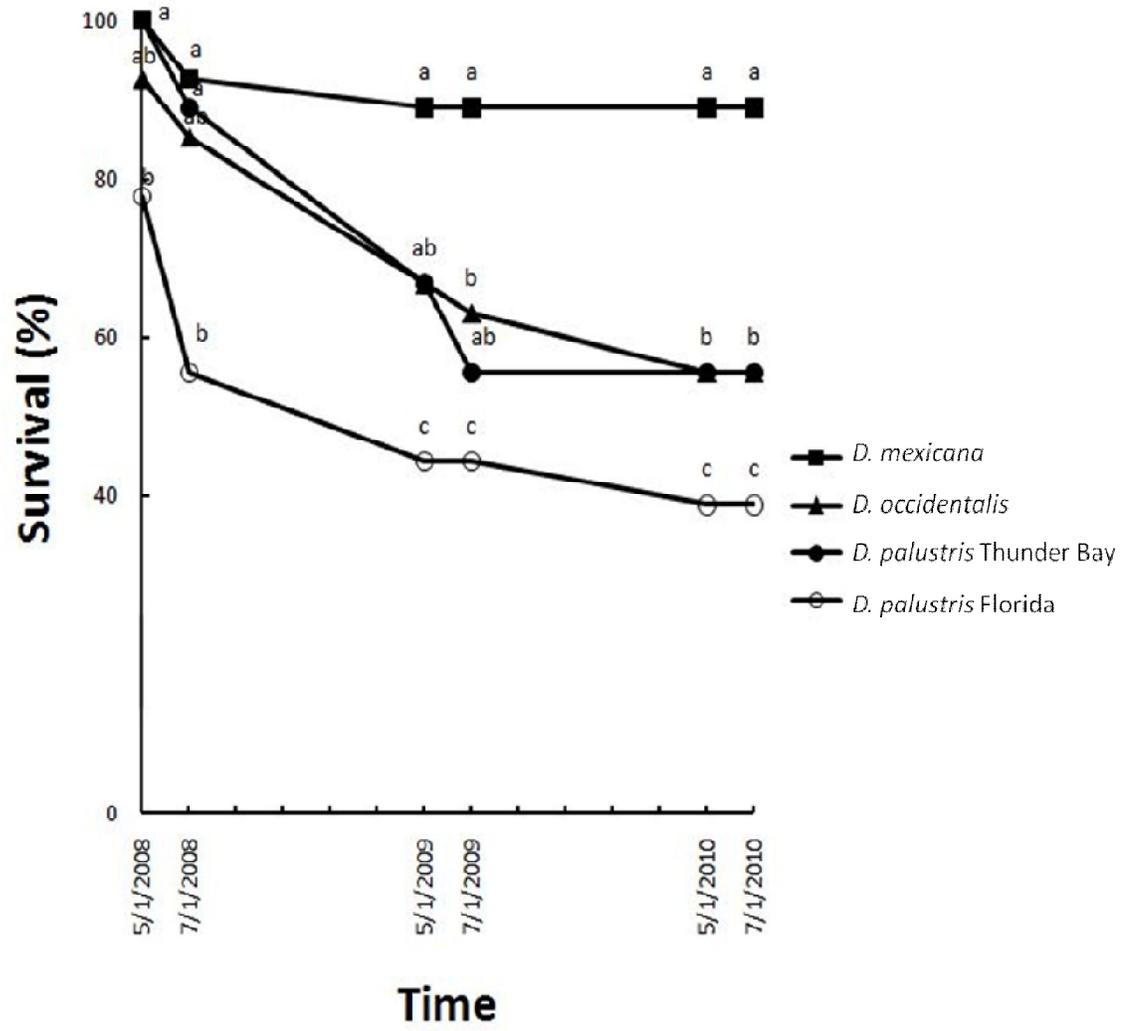
Surviving plants of *D. occidentalis* grow slowly and exhibit poor health, but persisted in the trial, suggesting that plants adapt to their environment over time. Graves et al. (2006) suggested that if *D. occidentalis* could resist low temperature stress, it might have commercial potential in hardiness zones outside its native California distribution. Whereas our results suggest that its capacity to survive at the border of USDA zones 4 and 5 is similar to that of *D. palustris* from Thunder Bay, its poor health ratings undermine its aesthetic value for horticultural production. O'Brien and Krauss (2010) suggest that adaptive differences may become more pronounced at later life history stages, particularly in long-lived species. Long-term monitoring of species in these trials will likely yield additional insights into their ability to either adapt and thrive or suffer from environmental stress and eventually perish. We have observed one plant in a protected microclimate on the Iowa State University campus that has survived for seven years and flowered in 2010 and 2011. Future observations could demonstrate whether this plant and

others in our trial improve in growth and health ratings in succeeding years or whether the stresses of the environment coupled with poor annual growth ultimately foster their decline and eventual death. At present, we do not recommend *D. occidentalis* for broader landscape use in the Midwest without a thorough germplasm evaluation for hardy, healthy genotypes.

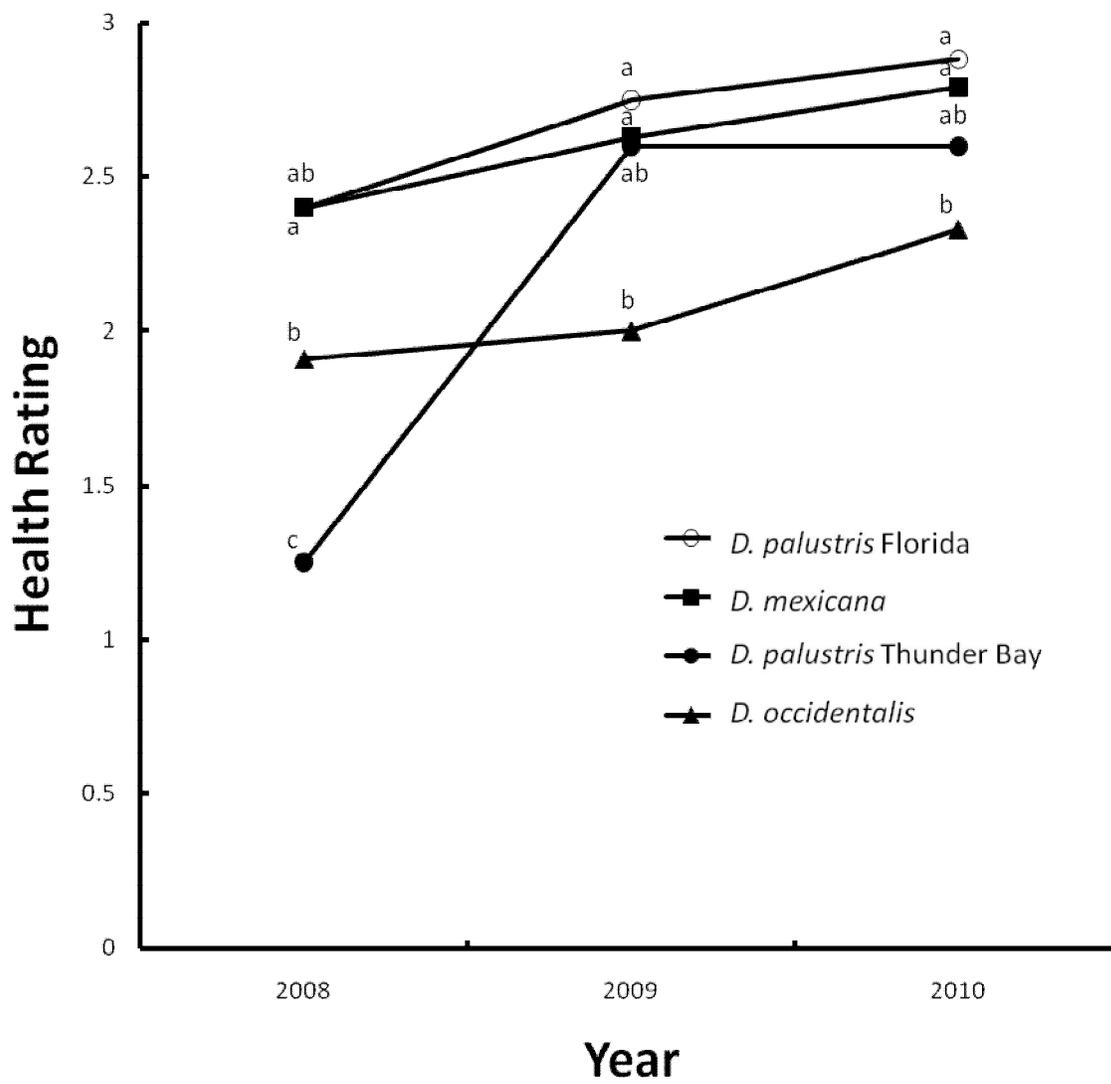
Fig. 1. Survival from 2008 to 2010 of *Dirca mexicana* (n = 27), *D. palustris* Florida (n = 18), *D. palustris* Thunder Bay (n = 9), and *D. occidentalis* (n = 27) in a common garden setting in central Iowa. Means with the same letter at a given date are not different at  $\alpha = 0.05$  according to the Waller-Duncan *k*-ratio *t*-test.

Fig. 2. July health ratings from 2008 to 2010 of *Dirca* spp. in a common garden setting in central Iowa. In 2008 through 2010, respectively, for *Dirca mexicana* n = 25, 24, 24; for *D. palustris* Florida n = 10, 8, 7; for *D. palustris* Thunder Bay n = 8, 5, 5; and for *D. occidentalis* n = 23, 17, 15. Means with the same letter at a given date are not different at  $\alpha = 0.05$  according to the Waller-Duncan *k*-ratio *t*-test.

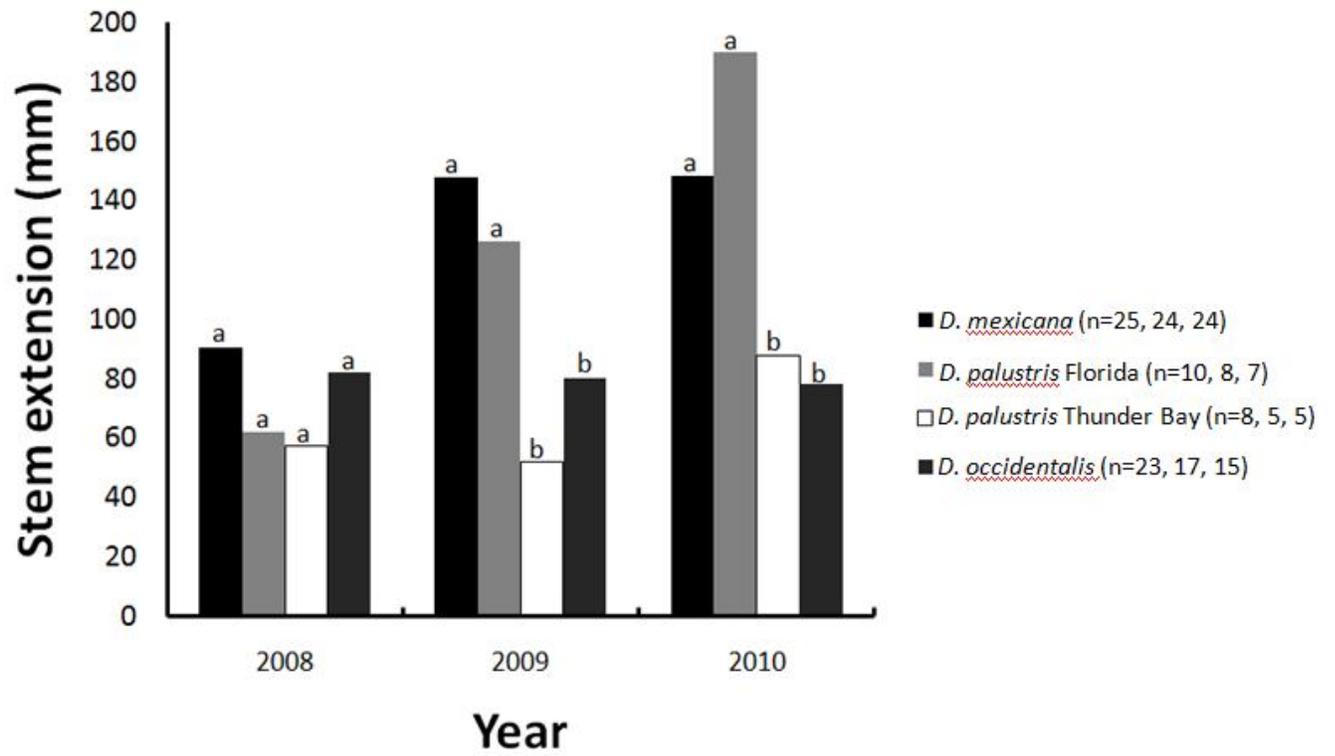
Fig. 3. Stem extension (mm) from 2008 to 2010 of *Dirca* spp. in a common garden setting in central Iowa. In 2008 through 2010, respectively, for *Dirca mexicana* n = 25, 24, 24; for *D. palustris* Florida n = 10, 8, 7; for *D. palustris* Thunder Bay n = 8, 5, 5; and for *D. occidentalis* n = 23, 17, 15. Means with the same letter at a given date are not different at  $\alpha = 0.05$  according to the Waller-Duncan *k*-ratio *t*-test.



(Fig. 1)



(Fig. 2)



(Fig. 3)

## Literature Cited

- Anderson, E. 1933. Leatherwood (*Dirca palustris*). *Arnold Arb. Bull.* 1(5): 25-27.
- Brzuszek, R.F., and R.L. Harkess. 2009. Green industry survey of native plant marketing in the southeastern United States. *HortTechnology* 19:168-172.
- Buffin, M.W. 2005. Winter-flowering shrubs. Timber Press, Portland, OR.
- Clark, R. C. 1971. The woody plants of Alabama. *Ann. Missouri Bot. Gard.* 58:99-242.
- Cooperrider, T. S. 1962. The flora of north-facing slopes compared to that of the surrounding area in eastern Iowa. *Amer. Midland Nat.* 67:368-372.
- Creech, J. L. 1966. Expeditions for new horticultural plants. *Arnoldia* 26:49-53.
- Del Tredici, P. 1984. Propagating leatherwood: A lesson in humility. *Arnoldia* 44:20-24.
- Dirr, M. A. 1998. Manual of woody landscape plants: Their identification, ornamental characteristics, propagation, and uses. Stipes Publishing Company, Champaign, IL.
- Dirr, M.A. and C.W. Heuser, Jr. 1987. The reference manual of woody plant propagation: From seed to tissue culture. Varsity Press, Athens, GA.
- Dosmann, M.S. and P. Del Tredici. 2003. Plant introduction, distribution, and survival: A case study of the 1980 Sino-American botanical expedition. *BioScience* 53:588-597.
- Escribano, S. and A. Lazaro. 2009. Agro-morphological diversity of Spanish traditional melons (*Cucumis melo* L.) of the Madrid provenance. *Gen. Res. and Crop Evol.* 56: 481-497.
- Esson, J. G. 1949. Leatherwood for early spring bloom. *J. New York Bot. Gard.* 50:57-59.
- Graves, W.R. and J.A. Schrader. 2008. At the interface of phylogenetics and population genetics, the phylogeography of *Dirca occidentalis* (Thymelaeaceae). *Amer. J. Bot.* 95:1454-1465.

- Graves, W. R., J.A. Schrader, and J. Sharma. 2006. Cold hardiness of the rare *Dirca occidentalis*: Comparisons to *Dirca palustris* from disparate provenances. *J. Environ. Hort.* 24:169-172.
- Hällfors, M.H., L. Linden, H. Rita, and L.E. Schulman. 2011. Using a botanic garden collection to test a bioclimatic hypothesis. *Biodivers. Conserv.* 20:259-275.
- Hannerz, M. and J. Westin. 2005. Autumn frost hardiness in Norway spruce plus tree progeny and trees of the local and transferred provenances in central Sweden. *Tree Physiol.* 25:1181-1186.
- Kirkegaard, J. 1912. Trees, shrubs, vines and herbaceous perennials. The Bullard Company, Boston, MA.
- McMahan, L. R. 2006. Understanding cultural reasons for the increase in both restoration efforts and gardening with native plants. *Native Plants J.* 7:31-34.
- Nesom, G. L. and M.H. Mayfield. 1995. A new species of *Dirca* (Thymelaeaceae) from the Sierra of northeastern Mexico. *Sida* 16:459-467.
- Nevling, L. I., Jr. 1964. Typification in *Dirca*. *J. of the Arnold Arb.* 45:158-159.
- O'Brien, E.K. and S.L. Krauss. 2010. Testing the home-site advantage in forest trees on disturbed and undisturbed sites. *Rest. Ecol.* 18:359-372.
- Peterson, B. J. and W.R. Graves. 2009. Variation in development and response to root-zone pH among seedlings of *Dirca palustris* (Thymelaeaceae) from three provenances. *HortScience* 44:1319-1322.
- Peterson, B. J., W.R. Graves, and J. Sharma. 2009. Color of pubescence on bud scales conflicts with keys for identifying species of *Dirca* (Thymelaeaceae). *Rhodora* 111:126-130.

- Peterson, B. J., W.R. Graves, and J. Sharma. 2011. Phenotypic and genotypic diversity of eastern leatherwood in five populations that span its geographic distribution. *Amer. Midland Nat.* 165:1-21.
- Ramesh, K. R. and D.K. Khurana. 2008. Clonal provenance variation of *Populus alba* Linn. in nursery. *Ind. J. For.* 3:599-610.
- Repo, T., G. Zhang, A. Ryyppö, R. Rikala, and M. Vuorinen. 2000. The relation between growth cessation and frost hardening in Scots pines of different origins. *Trees* 14:456-464.
- SAS Institute Inc. 2002. SAS/STAT User's Guide. Release 9.1.3 ed. Cary, N.C.
- Schrader, J.A. and W.R. Graves. 2003. Phenology and depth of cold acclimation in the three subspecies of *Alnus maritima*. *J. Amer. Soc. Hort. Sci.* 128:330-336.
- United States Department of Agriculture. 1990. USDA plant hardiness zone map. Misc. Publ. 1475, U.S. Dept. of Agr. Washington, DC.
- Weng, Y.H. and W.H. Parker. 2008. Adaptive variation in fall cold hardiness of aspen from northwestern Ontario. *Tree Physiol.* 28:143-150.
- Williams, C. E. 2004. Mating system and pollination biology of the spring-flowering shrub, *Dirca palustris*. *Plant Species Biol.* 19:101-106.
- Wunderlin, R.P. and B.F. Hansen. 2008. Atlas of Florida vascular plants. Institute for Systematic Botany, University of South Florida, Tampa. <http://www.plantatlas.usf.edu/>. Accessed 14 April 2011.

## CHAPTER 4. Responses to Root-Zone pH of Three Species of Leatherwood (*Dirca* spp.)

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**ABSTRACT.** Beyond a single study of pH responses in three provenances of *Dirca palustris* (Peterson and Graves, 2009), little is known about the edaphic tolerances of the genus *Dirca*. This genus of shade-tolerant shrubs possesses many ornamental characteristics that merit consideration of their use in the managed landscape. In this study, we evaluated pH responses of three species under field conditions. Stems of *Dirca palustris* extended the most in acidic root zones (111 mm), but mean health ratings were highest in the slightly acidic and control treatments, suggesting a range of optimal growth and performance from pH 6.0 to 7.65. *Dirca occidentalis* performed best for all response variables under slightly acidic conditions. Stems of *Dirca mexicana* extended the most (139 mm) in slightly basic root zones, and the species was rated the healthiest of all species evaluated. For *D. occidentalis* and *D. mexicana*, SPAD values predicted optimum relative greenness at a more acidic pH than that at which stem length was maximized. We have documented that *Dirca* spp. perform well in a range of soils from pH 6.5 to 7.5, offering initial insights into the proper use of these shrubs in managed landscapes.

**Significance to the Nursery Industry** New plants are the lifeblood of the horticulture industry.

Given abundant consumer interest in native plants, an underappreciated genus like *Dirca*

warrants evaluation and consideration for their aesthetic characteristics. Additionally, promotion of native plants should be accompanied by information about their responses to the environment. We offer clarifications to the literature in regards to the edaphic sensitivities of *D. palustris* and the first insights of pH responses of *D. mexicana* and *D. occidentalis*.

## Introduction

Beyond a single study of pH responses in three provenances of *Dirca palustris* (Peterson and Graves, 2009), little is known about the edaphic tolerances of the genus *Dirca*. *Dirca spp.* are unusual members of the North American woody flora and have many ornamental traits that warrant their inclusion in ornamental landscapes (Dirr 1998; Esson, 1949). Despite consumer interest in native species (McMahan, 2006; Brzuszek and Harkess, 2009), little is known about the performance of these species in the landscape (O'Brien, 1996) despite promotions of their adaptation to climates and soils local to their native range (Price et al., 2009). Soil pH effects phenological, physiological, and morphological traits in plants (Price et al., 2009; Wielgolaski, 2001) and impacts their use in managed landscapes, underscoring the importance of inquiries into edaphic sensitivities and tolerances in plant materials of emerging popularity.

*Dirca palustris* occurs in the wild in discrete, isolated populations, most of which contain few individuals, though over an extensive range from Nova Scotia to North Dakota and south to Florida (Floden et al., 2009). Historically, reports suggest that the species possesses a wide adaptability to in situ conditions including an affinity to water (Anderson, 1933), north or east-facing slopes along watercourses (Steyermark, 1963), beech and sugar maple forests (Fernald, 1950), and rich mesic forests (Ward and Horn, 1998; Cooperrider, 1962). Anderson (1933) noted that *D. palustris* grows in a variety of soils and conditions but states that the species

usually avoids limestone. Contrarily, Cooperrider (1962) described populations in eastern Iowa as limited to sites with limestone bedrock. Adding further confusion, other reports suggested that the species occurs in acidic (Ward and Horn, 2008) and sandy loam soils (Schulz et al., 2004). In the only direct test of pH effects on *D. palustris*, Peterson and Graves (2009) found seedlings responded most favorably to acidic substrates, though the experiments were done with potted plants in a soilless growing medium. Such disagreement in the literature merits investigation so proper prescriptions for horticultural use can be made and to determine if the adaptability is ecotypic or differentially adapted to local edaphic conditions (Rajakaruna, 2004).

There have been no reports of pH effects on *Dirca occidentalis*, a western endemic species restricted to an 8000 km<sup>2</sup> area around the San Francisco Bay (Graves and Schrader, 2008). Nesom and Mayfield (1995) described the area in which *D. mexicana* grows in Tamaulipas, Mexico, as a north-facing, mesic, limestone cove above a rocky watercourse. Graves (2008) reported that the native soils on which *D. mexicana* occurs are acidic with a pH of 6.3. To date, no study has characterized the pH tolerances of these species in the landscape.

In this study we evaluated pH responses between three species in an outdoor trial, following the suggestion by Peterson and Graves (2009) that more information be gathered on the range of pH tolerances of the three species in a common garden setting. Our objectives were to examine the pH sensitivities of *D. palustris* in an effort to clarify confusion in the literature and to document the responses of *D. mexicana* and *D. occidentalis* to various root-zone pH treatments.

## Materials and Methods

**Sources of germplasm.** One-year old plants of *Dirca palustris*, *D. mexicana*, and *D. occidentalis* were randomly assigned to one of three treatment categories. Drupes of *D. palustris* were collected from an isolated garden specimen on 19 June 2007 in Orono, ME at the Lyle E. Littlefield Ornamentals Trial Garden and sown in our greenhouses on 21 June. Drupes of *D. mexicana* were collected from the Sierra Madre Oriental in Tamaulipas, Mexico on 9 May and 10 May and sown 15 May 2007. Drupes of *D. occidentalis* were collected in San Mateo County, CA on 9 June 2007 and sown on 12 June. Drupes of all species were sown in Fafard® 52 soilless, peat-based medium (Fafard® Inc., Agawam, MA) and held in a minimally heated greenhouse until germination during spring 2008 after exposure to warm and cold stratification under ambient conditions.

**Soil collection and sampling.** A composite soil sample comprising 20 subsamples was collected from our research site to determine the native pH before experimental amendments. Soil pH, percent organic matter, and total nitrogen were measured by the Soil and Plant Analysis Laboratory, Iowa State University, Ames, IA, using the 1:1 soil:water suspension method.

**Experimental site.** An experimental plot was established in Ames, IA (42° 1'30.47"N, 93°38'31.14"W). The sites were chosen for their similarity to conditions in which *Dirca* might naturally occur in the wild; a north-facing slope, along a watercourse, in shade, with overhead canopy. Overstory vegetation at the sites is predominantly deciduous hardwoods such as *Acer negundo*, *Acer nigrum*, *Acer platanoides*, *Fraxinus* spp., *Juglans nigra*, and *Ulmus* spp. Initial soil analyses indicated a mean pH value of 7.65 and 13.5 percent organic matter content. Means of measurements of photosynthetically active radiation measured at solar noon on a cloudless day ranged from 40 to 130  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The plot was approximately 12 m by 3 m and had been

cleared of brushy vegetation the day before planting. Vegetation removed during the clearing included both native and non-native woody species. Plants were installed randomly in three rows of 21, spaced approximately 0.5 m apart with approximately 1 m between rows.

**Experimental design.** The experiment was design as a randomized plot of 63 plants with experimental units randomized to treatment and position. Two soil treatments in addition to the native soil were used in this experiment. To obtain the two treatments, we modified the soil (native pH 7.65) with 1 M sulfuric acid ( $\text{H}_2\text{SO}_4$ ) to a pH between 4.5-5.0 and 6.0-6.5 using 200 mL/L medium and 70 mL/L medium, respectively. These ranges of final pH were established to account for potential variation within and between individual soil samples throughout the trial. We chose to modify the soil with sulfuric acid because of its rapid response time versus other acidification methods that require several months to alter the pH effectively and stably (Peterson and Graves, 2009). Approximately 3.78 L (equivalent of 1 U.S. #1 grade nursery container) was excavated from those locations within the trial that required amendment (approximately 79.5 L of soil per treatment). Soil and sulfuric acid were combined in a wheelbarrow with thorough agitation. Approximately 3.78 L of soil-acid mixture was returned to each excavation within its respective treatment.

Plants of each species were planted on October 20, 2009 and randomly assigned before planting to one of three experimental categories described above. Seedlings had four to five true leaves at the time of planting.

**Data collection and analysis.** Temperature data were logged with an iButton Data Logger (Maxim Integrated Products, Sunnyvale, CA). On 1 May, health assessments were made using a qualitative scale of 0 to 3. A 0 rating meant dead or missing; a 1 rating indicated the plant was suffering from serious chlorosis, had distorted leaves, or was growing poorly; a 2

rating indicated the plant displayed problematic symptoms intermediate between a 1 and a 3 rating; a 3 rating indicated the plant had excellent foliar quality and exhibited strong growth. Soil pH was monitored monthly during the 2010 growing season and composite samples were analyzed by the Soil and Plant Analysis Laboratory, Iowa State University, Ames, IA by using the 1:1 soil:water suspension method. On 1 July, stem extension was measured in millimeters as the length of new wood on the longest axis only. Relative greenness of three leaves near the apex of the stem was measured using a SPAD-502 Chlorophyll Meter (Konica Minolta Sensing, Tokyo, Japan). One-way ANOVA and means comparisons for each pair using Student's t test were generated with JMP software (SAS Institute, Cary, NC). Graphing tools and regression functions of Microsoft Excel 2007 (Microsoft, 2007) were used to show the interactions between response variables.

## Results and Discussion

Stem length of *D. palustris* decreased linearly with increasing pH (Fig. 1). Quadratic functions best described stem length responses in *D. mexicana* and *D. occidentalis* with predicted maxima at pH 7.2 and 6.8, respectively (Fig. 1). Relative greenness (SPAD) values for *D. palustris* increased linearly with increasing pH (Fig. 2). Quadratic functions best described SPAD responses in *D. mexicana* and *D. occidentalis* with predicted maxima at pH 6.2 and 6.4, respectively (Fig. 2). None of the mean SPAD values were different at  $\alpha = 0.05$ . Qualitative health ratings for *D. mexicana* were the highest across all treatment groups for all species. All species health ratings increased with increasing pH (Table 1).

Our findings regarding *D. palustris* concur with observations in the wild (Ward and Horn, 2008), previous observations from managed landscapes (Dirr, 1998), and support the findings of Peterson and Graves (2009), for plants in soilless growing media, that the species

grows optimally in acidic soils. But given the great extent to which other reports document its occurrence on alkaline, limestone-predominating substrates (Cooperrider, 1962; Clark, 1971), it's apparent that *D. palustris* possesses some degree of phenotypic plasticity for edaphic conditions. This however raises questions about the degree of infrequency of *D. palustris* in the wild. Our findings, coupled with other reports, suggest that the species isn't limited to a particular set of soil characteristics or properties. A future study might consider a complete survey of pH from across the distribution of *D. palustris* and further analysis of the relationship between soil chemistry, population occurrences, and morphometric traits. While stems extended optimally in acidic soils, the optimum relative greenness value didn't directly corroborate these findings, though this disagreement in relative growth rates and greenness data has been reported in another study (McNamara and Pellett, 2001). The mathematical optima reported in this study do not necessarily correlate with the optima for physiological processes. Low-pH stress-induced symptoms led us to assign *D. palustris* low health ratings in the most acidic treatment group, a result in contrast to Peterson and Graves (2009) where plants from Maine, as in this study, grew maximally in soils of pH lower than 5.5. Health ratings of plants in the slightly acidic treatment and the control treatment were not different, suggesting acceptable growth and health for *D. palustris* can be realized in soils that range from 6.0 to 7.65 in pH. From a horticultural standpoint, species with expansive geographic distributions and broad adaptations to soil conditions, like *D. palustris*, should logically warrant consideration from horticulturists for their ability to survive and potentially thrive across a range of environmental variables in the managed landscape.

This report provides some of the first edaphic tolerance data for *D. occidentalis* and predicts that the species accrues the most stem growth in slightly acidic pH soils. The pH for

optimum relative greenness value for this species was approximate to the pH value where stem growth was maximized, adding credibility to our initial assertion that the species performs best on acidic substrates. Regarding *D. mexicana*, published reports from the wild disagree in their characterization of the edaphic context in which the species occurs in the wild. Graves (2008) reported an acidic pH of the soils, while Nesom and Mayfield (1995) described the site by its limestone topography. We find no evidence in the literature to support that soils on limestone topographies are necessarily always basic, but acknowledge that complex soil chemical interactions could alter pH beyond the influence of its parent material. We found that stems of *D. mexicana* extend the most in slightly basic pH soils. The optimum relative greenness value for *D. mexicana* didn't correspond with the pH at which stem length was maximized. *Dirca mexicana* was, however, rated as the healthiest species on average across the study, corresponding to similar findings from another study (see Chapter 3). The general lack of corroboration between SPAD and stem lengths may be due to lack of data resolution, given that we considered only three different pH treatment levels in our study. In the case of *D. occidentalis* and *D. mexicana*, the SPAD values predicted optimum relative greenness at a more acidic pH than that at which stem length was maximized.

On a final note, it's worth mentioning that while pH throughout the study period increased across all three treatment groups, the pH during the two months of primary stem extension (May and June) was stable to treatment condition (5.3, 6.5, and 7.6, respectively). Future research employing our study design should consider ways to maintain pH if data are to be collected for more than one season or for traits with continuous responses throughout one growing season.

Table 1. Mean health ratings of *Dirca mexicana* (n = 18), *D. occidentalis* (n = 16), and *D. palustris* (n = 15) under three treatment conditions.

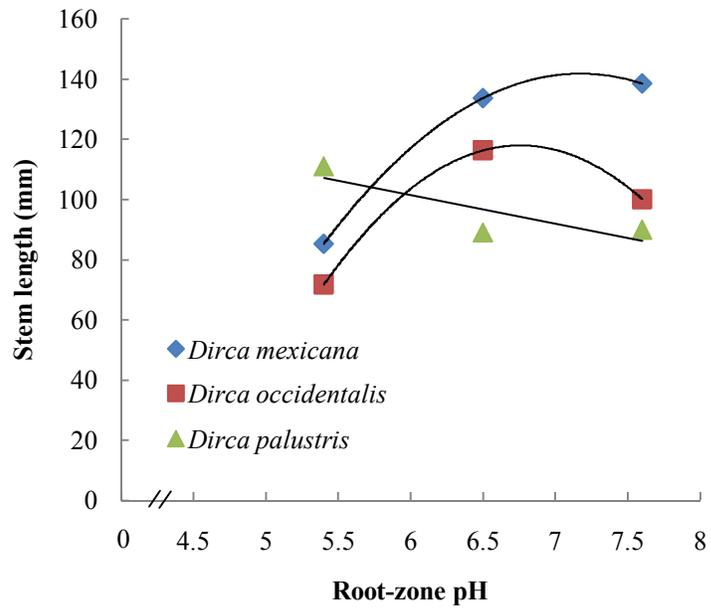
Treatment Group	Health ratings (0-3)		
	<i>D. mexicana</i>	<i>D. occidentalis</i>	<i>D. palustris</i>
4.5-5.0	2.3 a (b)	1.5 a (b)	1.0 a (b)
6.0-6.5	3.0 a (a)	1.9 c (ab)	2.3 b (a)
7.65 (control)	3.0 a (a)	2.1 b (a)	2.4 b (a)
Average	2.8 a	1.8 b	1.9 b

<sup>z</sup> Different letters within rows indicate significance at  $\alpha = 0.05$ .

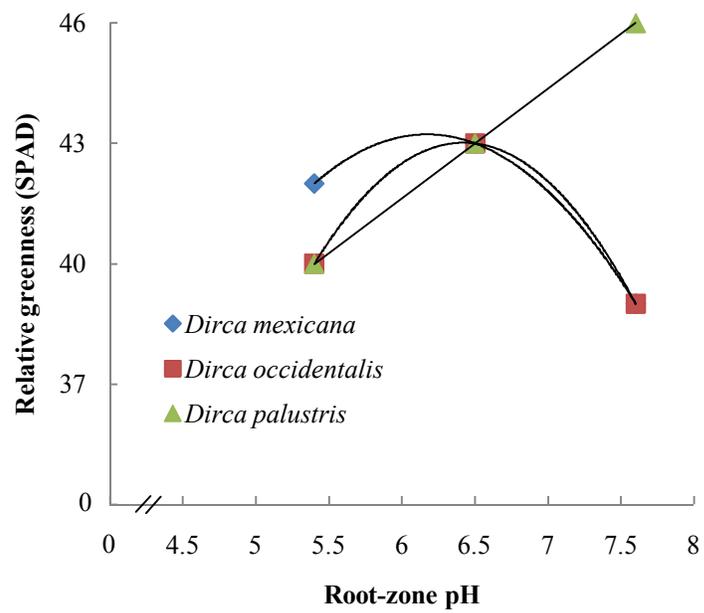
<sup>y</sup> Different letters in parentheses within columns indicate significance at  $\alpha = 0.05$ .

Fig. 1. Interaction of root-zone pH and species for stem extension measured in millimeters as the length of new wood on the longest axis of three species of *Dirca*. Symbols represent means of single-plant replications; *Dirca mexicana* (n = 18), *D. occidentalis* (n = 16), and *D. palustris* (n = 15). Regression functions are: *D. mexicana*: stem length =  $-18.02(\text{pH})^2 + 258.49(\text{pH}) - 785.16$ ,  $R^2 = 1$ ; *D. occidentalis*: stem length =  $-25.145(\text{pH})^2 + 339.82(\text{pH}) - 1030.1$ ,  $R^2 = 1$ ; *D. palustris*: stem length =  $-9.5455(\text{pH}) + 158.76$ ,  $R^2 = 0.7193$ .

Fig. 2. Interaction of root-zone pH and species for relative greenness (SPAD) of three species of *Dirca*. Symbols represent means of single plant replications; *Dirca mexicana* (n = 18), *D. occidentalis* (n = 16), and *D. palustris* (n = 15). Regression functions are: *D. mexicana*: SPAD =  $-2.0661(\text{pH})^2 + 25.496(\text{pH}) - 35.43$ ,  $R^2 = 1$ ; *D. occidentalis*: SPAD =  $-2.8926(\text{pH})^2 + 37.149(\text{pH}) - 76.256$ ,  $R^2 = 1$ ; *D. palustris*: SPAD =  $2.7273x + 25.273$ ,  $R^2 = 1$ .



(Fig. 1)



(Fig. 2)

## Literature Cited

- Anderson, E. 1933. Leatherwood (*Dirca palustris*). Arnold Arb. Bull. 1(5): 25-27.
- Brzuszek, R.F., and R.L. Harkess. 2009. Green industry survey of native plant marketing in the southeastern United States. HortTechnology 19:168-172.
- Clark, R. C. 1971. The woody plants of Alabama. Ann. Missouri Bot. Gard. 58:99-242.
- Cooperrider, T. S. 1962. The flora of north-facing slopes compared to that of the surrounding area in eastern Iowa. Amer. Midland Nat. 67:368-372.
- Dirr, M. A. 1998. Manual of woody landscape plants: Their identification, ornamental characteristics, propagation, and uses. Stipes Publishing Company, Champaign, IL.
- Esson, J. G. 1949. Leatherwood for early spring bloom. J. New York Bot. Gard. 50:57-59.
- Fernald, M.L. 1950. Gray's manual of botany. Dioscorides Press, Portland.
- Floden, A., M. Mayfield, and C.J. Ferguson. (2009). A new narrowly endemic species of *Dirca* (Thymelaeaceae) from Kansas and Arkansas, with a phylogenetic overview and taxonomic synopsis of the genus. J. Bot. Res. Inst. Texas 3:485-499.
- Graves, W.R. 2008. Habitat and reproduction of *Dirca mexicana* (Thymelaeaceae). Rhodora 110: 365-378.
- Graves, W.R. and J.A. Schrader. 2008. At the interface of phylogenetics and population genetics, the phylogeography of *Dirca occidentalis* (Thymelaeaceae). Amer. J. Bot 95:1454-1465.
- McMahan, L. R. 2006. Understanding cultural reasons for the increase in both restoration efforts and gardening with native plants. Native Plants J. 7:31-34.

- McNamara, S. and H. Pellett. 2001. Effect on high pH on foliar chlorosis and growth of five *Betula* species. *J. Environ. Hort.* 19(4):175–179.
- Nesom, G. L. and M.H. Mayfield. 1995. A new species of *Dirca* (Thymelaeaceae) from the Sierra of northeastern Mexico. *Sida* 16:459-467.
- O'Brien, B.C. (1996). Xeriscaping: sources of new native ornamental plants. p. 536-539. In: J. Janick (ed.), *Progress in new crops*. ASHS Press, Arlington, VA.
- Peterson, B. J. and W.R. Graves. 2009. Variation in development and response to root-zone pH among seedlings of *Dirca palustris* (Thymelaeaceae) from three provenances. *HortScience* 44:1319-1322.
- Price, J.G., A.N. Wright, K.M. Tilt, and R.L. Boyd. 2009. Organic matter application improves posttransplant root growth of three native woody shrubs. *HortScience* 44:377-383.
- Rajakaruna, N. 2004. The edaphic factor in the origin of plant species. *Intl. Geol. Rev.* 46:471-478.
- Schulz, K., J. Zasada, and E. Nauertz. 2004. Annual, local, and individual variation in the inflorescence and fruit production of eastern leatherwood (*Dirca palustris* L. Thymelaeaceae). *J. Torrey Bot. Soc.* 131:292–304.
- Steyermark, J.A. 1963. *Flora of Missouri*. Iowa State Univ. Press, Ames, IA.
- Ward, A.B. and C.N. Horn. 1998. A status survey of *Dirca palustris* L. (leatherwood, Thymelaeaceae) in South Carolina. *Castanea* 63:165-173.

Wielgolaski, F.E. 2001. Phenological modifications in plants by various edaphic factors. *Int J Biometeorol* 45:196–202.

## CHAPTER 5. GENERAL CONCLUSIONS

The goal of horticultural plant development is to evaluate, propagate, and distribute new plant products. Evaluating germplasm of native plants is an important frontier for horticulturists to pursue in an effort to promote higher levels of native biodiversity in landscapes. At this juncture, horticulture and ecology intersect and allow researchers to evaluate the responses of plants in their native environments and in the managed landscape, while pursuing assessments of genetic diversity, phenotypic plasticity, and biogeography from a horticulturist's perspective. As the conservation imperative continues into the future, it will be necessary for ecologists and horticulturists to work together to preserve the world's biodiversity both *in situ* and *ex situ*, by employing these multi-disciplinary techniques and strategies.

Taken together, the data presented in this thesis suggest that of the three species considered, *D. palustris* and *D. mexicana* are the best suited for horticultural use in the Midwest or in regions of the country with similar environmental conditions. *Dirca occidentalis*, while arguably the most ornamental member of the genus, is limited at present by its incapacity to persist in the managed landscape over a long period of time. This thesis also includes the first report detailing the responses to root-zone pH of the same three species of *Dirca* in an outdoor trial. Given conflicting or absent data in the literature, this study attempts to resolve the confusion about the edaphic tolerances of *D. palustris* while offering additional resolution to the literature about the otherwise unknown responses of *D. occidentalis* and *D. mexicana* to root-zone pH. Successful strategies for asexually propagating *D. palustris* and *D. mexicana* remain elusive, though additional treatment combinations might offer hope for future researchers. Though it's widely assumed that *Dirca* are shade-tolerant plants, quantifications of that tolerance

to irradiance and shade are absent in the literature. A small study reported here in Appendix 2 suggests further analysis of this topic.

### ***Suggestions for Future Research***

Future researchers might consider further evaluations of *Dirca palustris* from its disparate provenances to explore the range of genetic diversity potentially available for horticulturists to make selections from. Such evaluation and selection work could result in commercially marketable genotypes with cultivar distinctions. Additional research into the perceived limitations of *D. palustris* as a nursery plant would be valuable, since personal communication with nurseryman suggests that the species has limited commercial potential. Expanding upon that idea, a survey of commercial growers about the challenges and benefits of propagating native plants for the retail market would no doubt offer insights and contribute much needed data to the literature where it is otherwise absent.

Additional information is needed on the extent to which *Dirca occidentalis* is able to be promoted as a landscape plant outside its endemic distribution around the San Francisco Bay in California. It boasts the largest flowers of the genus, but overall performed poorly in our trials in the Midwest. Observations of single plants curiously suggest that there is the potential for plants to survive and even flower in the Midwest, but these may represent unique situations in microclimates. Future researchers could expand upon the study outlined in Appendix 4 of this thesis and pursue hybrid development through interspecific crosses to maximize ornamental and physiological characteristics.

Future studies of *Dirca mexicana* should include additional investigation of propagation strategies to facilitate marketing of this promising species. Its relationship to the recently

described *D. decipiens* will no doubt provide future researchers the opportunity to expand our knowledge of the biogeographic origins and systematic relationships of this American genus of Thymelaeaceae. This author certainly hopes to see this species introduced to horticultural commerce sooner than later.

## APPENDIX 1: Propagating Leatherwoods—More Lessons in Humility

Increasing horticultural interest in native North American plant species warrants inquiry into how to best propagate and produce plants for commercial horticulture and conservation. Leatherwoods (*Dirca* spp.) are a genus of three to four shade-tolerant, long-lived shrubs that flower in early spring and often display yellow fall color (Esson, 1949; Del Tredici, 1983; Dirr 1998). Despite their ornamental value, leatherwoods have a history of recalcitrance to propagation via seed and asexual methods or ambiguous reports of success with little documentation for methodology (Del Tredici, 1983), making them rare commodities in horticultural commerce. Esson (1949) reported that leatherwoods were easily raised from seed grown in constantly moist, sandy media but offered no details of his methodology. More recent reports have indicated that endodormancy and paradormancy cause sporadic germination of seeds of all three species and limit the production of nursery stock (Schrader and Graves, 2005). Schrader and Graves (2005) found that endocarp removal, cold stratification, and treatment with GA<sub>3</sub> were reliable methods of increasing germination percentage, rate, and uniformity in *Dirca occidentalis* and *D. palustris*, but not for *D. mexicana* and suggested that further research was needed to document effective propagation strategies for this critically imperiled Mexican native. Results from an initial trial of three species in Ames, Iowa, suggests that *D. mexicana* may be the most commercially viable species in the genus, and because this species is known from only one location in the wild (Nesom and Mayfield, 1995; Graves, 2008), an asexual approach would be a more viable way to propagate plants for commercial and conservation purposes (Hartmann et al., 2001). However, Dirr and Heuser (1987) noted minimal success in propagating *D. palustris* from softwood stem cuttings.

Etiolation, or causing a plant to grow in the absence of light, can enable adventitious root formation on stems of taxa that otherwise are highly recalcitrant to asexual propagation (Husen, 2008; Maynard and Bassuk, 1996; Maynard and Bassuk, 1987). Preliminary research conducted by Schrader (unpublished) demonstrated that softwood cuttings of *D. palustris* rooted successfully (59%) when etiolated and banded prior to rooting hormone application. This report describes two separate attempts in two growing seasons to propagate *D. mexicana* and *D. palustris* from etiolated, softwood stem cuttings. Using Schrader's methodology, the first trial conducted in 2010 attempted to root softwood cuttings of *D. mexicana*. Using a modified version of Schrader's methodology and additional treatments, a second trial in 2011 attempted to root softwood cuttings of *D. mexicana* and *D. palustris*.

## **Materials and Methods**

On 7 February 2010, 36 dormant stock plants of *Dirca mexicana* in #2 nursery containers (with an approximate volume of 7000 cm<sup>3</sup>) previously grown in a 5°C greenhouse were moved into a 25°C greenhouse for bud break. On 23 February, 35 stock plants were randomized to six growth chambers in two categories, light (three chambers) and dark (three chambers), with temperature maintained at 22°C. After 15 days when plants in the dark chambers were qualitatively assessed to be sufficiently etiolated, Velcro bands were placed on stems of plants that underwent etiolation and moved to a shaded greenhouse bench to permit greening without the threat of scorching. Some plants that were etiolated were not banded to comprise another treatment group and also placed on the shaded greenhouse bench. Velcro bands were approximately 2.5 cm<sup>2</sup> and were placed at the proximal end of the shoot at the site of eventual rooting hormone application. On 24 March, 144 cuttings were taken and assigned to four blocks of 36 with 12 replications of each treatment present—control, etiolated-banded, etiolated-

unbanded. Cuttings were made with a razor blade, all fully expanded leaves were halved perpendicularly to the midrib, and base of cuttings were injured before dipping in talc-based, indole-3-butyric acid (IBA) rooting hormone (Rhizoapon®) at 8000 ppm. Cuttings were then placed under a 10-second misting regime every 10 minutes for 24 hours in a temperature-monitored greenhouse. Air temperature was logged with an iButton Data Logger (Maxim Integrated Products, Sunnyvale, CA). The mean daily temperature during the experiment was 23 °C; mean daily minimum and maximum 13 °C and 40.5 °C. Photosynthetically active radiation (PAR) was measured on three dates during the study and averaged 679  $\mu\text{mol m}^{-1} \text{s}^{-1}$ . Cuttings were monitored weekly during the duration of the study. On 16 June, 12 weeks after the inception of the study, an initial check for rooting and callus development was conducted. The final harvest occurred on 8 September, 24 weeks after the inception of the study. One-way ANOVA and means comparisons for each pair using Student's t test were generated with JMP software (SAS Institute, Cary, NC).

On 6 February 2011, 32 dormant stock plants of *Dirca mexicana* in #2 nursery containers with an approximate volume of 7000 cm<sup>3</sup> (Nursery Supplies, Inc., McMinnville, OR) previously held in a 5°C cooler were moved into a 25°C greenhouse for bud break. On 22 February, 30 stock plants of *D. palustris* in six-inch nursery containers, previously grown in a cold greenhouse, along with 32 stock plants of *D. mexicana* were randomly assigned to light and dark conditions. Plants selected for etiolation were placed under 99% light exclusion cloth on a greenhouse bench adjacent to the block of light-exposed plants. On 7 March, Velcro bands were placed on stems of plants that underwent etiolation and moved to a shaded greenhouse bench to permit greening without the threat of scorching. Some plants that were etiolated were not banded in order to comprise another treatment group and also placed on the shaded greenhouse

bench. Velcro bands were approximately 2.5 cm<sup>2</sup> and were placed at the proximal end of the shoot at the site of eventual rooting hormone application. On 23 March, 247 cuttings were taken and assigned to a three-way factorial arrangement of a completely randomized design.

Treatments were a combination of light exposure (control, etiolated-banded, etiolated-unbanded), species, and hormone level (16,000 and 45,000 ppm IBA). Cuttings were made with a razor blade, all fully expanded leaves were halved perpendicularly to the midrib, and base of cuttings were injured before dipping in talc-based, IBA rooting hormone at one of two concentrations. Cuttings were then placed under a 15-second misting regime every 10 minutes for 24 hours in a temperature-monitored greenhouse on a heating mat maintained at 20 °C. Air temperature was logged with an iButton Data Logger (Maxim Integrated Products, Sunnyvale, CA). The mean daily temperature during the experiment was 22 °C; mean daily minimum and maximum 12 °C and 38 °C. Photosynthetically active radiation (PAR) was measured on three dates during the study and averaged 854 μmol m<sup>-1</sup> s<sup>-1</sup>. Cuttings were monitored weekly during the duration of the study. On 25 May 2011, nine weeks after the inception of the study, an initial check for rooting and callus development was conducted and for the purposes of this appendix comprises the final harvest for 2011. One-way ANOVA and means comparisons for each pair using Student's t test were generated with JMP software (SAS Institute, Cary, NC).

## Results

Qualitative assessments of health and condition of cuttings were done weekly in both studies. Desiccation of leaves was first observed in week 2 in both years, though the pace of leaf abscission was slower in 2011. By the 12-week check in 2010, 21.5% of cuttings still had leaves. In contrast, at the nine week check in 2011, 78.5% of cuttings still had leaves.

Of 144 stuck cuttings checked on 16 June 2010, 76 (53%) had formed calluses. At the conclusion of the study on 8 September, one cutting had rooted, or 2.1% of all stuck cuttings. Of 247 stuck cuttings checked on 25 May 2011, six cuttings rooted, or 2.4% of all stuck cuttings. Root length differed ( $\alpha = 0.10$ ) by taxa at the 16,000 ppm treatment level. Roots of *Dirca palustris* averaged 30.5 mm in length with eight primary roots, while those of *D. mexicana* averaged 7.16 mm in length with one primary root. There were no significant differences in means comparisons of root number by taxa or treatment, or root length by treatment level.

In 2010 and 2011, callus formation in *D. mexicana* differed between the controls and etiolated treatment group, but in opposing magnitude between years (Table 1). Leaf retention differed between treatment groups in *D. mexicana* in 2011 (Table 2). *Dirca mexicana* and *D. palustris* differed in response to treatment for leaf retention and callus formation (Tables 1, 2).

## **Discussion**

The title of this appendix is a play on words of the title of Del Tredici's essay (1983), which conveyed the author's humility after concluding that *D. palustris* was best left to its own devices to induce seed germination. This author is similarly humbled after spending two years attempting to propagate *D. mexicana* and *D. palustris* from etiolated stem cuttings with little in the way of success. The 2011 study resulted in a similar total rooting percentage, but the health of the cuttings was better with high rates of leaf retention and callusing in the etiolated treatments than in 2010. The following discussion considers highlights possible alternatives to my methodology and outlines future strategies for the asexual propagation of *Dirca* spp.

McCown (2000) suggested that long-lived perennial plants posed the greatest challenge to plant propagators due to genetic predeterminism, complex growth cycles, and seasonal growth

dynamics dominated by strong episodic or determinant shoot growth. This and previous attempts at propagating *Dirca* asexually seem to corroborate this suggestion (Dirr and Heuser (1987; Peterson, pers. comm.). I chose to use higher concentrations of IBA to establish whether previous researchers simply weren't using enough hormone to induce rooting. Schrader (unpublished) use 8000 ppm IBA in his study that resulted in 59% rooting. Personal communication with Bryan Peterson about a previous unpublished study involving thousands of cuttings of *D. palustris* indicated that the highest concentration of IBA used in that study was 10,000 ppm. Concentrations at or above 40,000 ppm IBA have been used to induce rooting in other recalcitrant woody species (Majada et al., 2011; Barazi and Schwabe, 1982; Gettys et al., 1995). Based on available supplies of IBA, 16,000 ppm was chosen as the second hormone treatment level to be comparatively similar to previous maxima.

Schrader's success in rooting *D. palustris* is intriguing but hasn't been repeated. One possible explanation for his success was that because of his smaller trial (n = 63 cuttings), cuttings were exposed to stressful conditions for a shorter period of time. My two studies utilized greater number of cuttings (n = 144, 247, in 2010 and 2011, respectively), which may have resulted in extended periods of stressful conditions for cuttings before a fully completed block (i.e. one tray) was placed under humid conditions on the mist bench. While not employed by Schrader, Maynard and Bassuk (1987) applied rooting hormone at the time of banding by adhering talc-based IBA to Velcro strips or electrical tape and reported considerably higher rooting percentages with this strategy across nearly all taxa in their study. This pre-treatment might be useful in future attempts at etiolating *Dirca*. Past studies have also examined temperature effects during etiolation and on etiolated stem cuttings under mist and have found differing effects by species, timing of etiolation, and etiolated treatments versus controls

(Patience and Alderson, 1984; Hansen and Potter, 1997). Given evidence for a temperature effect between etiolated stems and control groups (Hansen and Potter, 1997), future studies might introduce temperature treatment variables to confirm the existence of a similar effect in *Dirca*. Life history stage may also play a role in the rooting success of cuttings. Schrader used juvenile plants, whereas both of my studies utilized mature plants. Maynard and Bassuk (1987) reported differences in rooting success between plants of the same species at different life history stages, suggesting that future studies of *Dirca* might compare adventitious root formation in cuttings taken from both juvenile and mature stockplants.

Maynard and Bassuk (1996) in a study of *Carpinus* proposed that sclereid formation reduces rooting potential, but that the formation of sclereids was staved off up to 12 weeks by etiolation. In 2010, cuttings of *D. mexicana* were left under mist conditions for 24 weeks before final harvest. At 24 weeks, many cuttings remained alive and retained leaves across treatment groups, though only one cutting (2.1%) had rooted. Future studies of *Dirca* at the histological level might reveal insights into how stem anatomy impacts adventitious root development and whether etiolation affects the timing of sclereid formation similarly in *Dirca*. Recent research also suggests that plant hormones play a critical role in transitioning from etiolated to de-etiolated states, though it's not known whether the underlying mechanisms are highly conserved throughout angiosperms or whether these vary between taxa (Symons and Reid, 2003).

Another potential propagation strategy for *Dirca* is layering. One early study with admittedly limited inferential power reported rapid growth rate over two growing seasons following winter storm damage, suggesting that injury at least in adult plants resulted in a hormone release and the onset of rapid stem elongation at the injury site (Loew, 1946). Graves (2004) confirmed that *Dirca* do regenerate clonally via rhizomes, alluding to the possibility that

*Dirca* might be propagated from layering. However, Hendricks (1985) failed in a controlled attempt to layer *D. palustris* and an informal attempt by Graves (unpublished) failed to produce anything more than minor callusing at the wounding site of the stem.

In conclusion, these studies represent an attempt to propagate two recalcitrant species using etiolated stem cuttings with little success. Additional strategies and tactics should be employed by future researchers in an effort to develop commercially viable propagation strategies for this genus of horticulturally and ecologically important shrubs.

Table 1. Callus formation of cuttings of *Dirca* over two years. Callus formation was recorded as presence or absence using a scale of 1 or 0, respectively.

Species	2010			2011		
	Control	Etiolated, banded	Etiolated, unbanded	Control	Etiolated, banded	Etiolated, unbanded
<i>D. mexicana</i>	0.79 a	0.38 b	0.40 b	0.28 b (a)	0.60 a (a)	0.62 a (a)
<i>D. palustris</i>	n/a	n/a	n/a	0.38 a (a)	0.37 a (b)	0.33 a (b)

<sup>z</sup> Different letters within rows indicate significance at  $\alpha = 0.05$ .

<sup>y</sup> Different letters in parentheses within columns indicate significance at  $\alpha = 0.05$ .

Table 2. Leaf retention of cuttings of *Dirca* over two years. Leaf retention was recorded as presence or absence using a scale of 1 or 0, respectively.

Species	2010			2011		
	Control	Etiolated, banded	Etiolated, unbanded	Control	Etiolated, banded	Etiolated, unbanded
<i>D. mexicana</i>	0.29 a	0.21 a	0.15 a	0.75 b (a)	0.91 a (a)	0.89 ab (a)
<i>D. palustris</i>	n/a	n/a	n/a	0.72 a (a)	0.73 a (b)	0.63 a (b)

<sup>z</sup> Different letters within rows indicate significance at  $\alpha = 0.05$ .

<sup>y</sup> Different letters in parentheses within columns indicate significance at  $\alpha = 0.05$ .

## Literature Cited

- Barazi, Z.A. and W.W. Schwabe. 1982. Rooted softwood cuttings of adult *Pistacia vera*. J. Hort. Sci. 57:247-252.
- Del Tredici, P. 1984. Propagating leatherwood: A lesson in humility. *Arnoldia* 44:20-24.
- Dirr, M. A. 1998. Manual of woody landscape plants: Their identification, ornamental characteristics, propagation, and uses. Stipes Publishing Company, Champaign, IL.
- Dirr, M.A. and C.W. Heuser, Jr. 1987. The reference manual of woody plant propagation: From seed to tissue culture. Varsity Press, Athens, GA.
- Esson, J. G. 1949. Leatherwood for early spring bloom. J. New York Bot. Gard. 50:57-59.
- Gettys, L., E. Duke, and A. Cox. 1995. Vegetative propagation of a native pawpaw-*Asimina tetramera*. Proc. Fla. State Hort. Soc. 108:389-391.
- Graves, W.R. 2004. Confirmation that *Dirca* spp. (Thymelaeaceae) reproduce from rhizomes. *Rhodora* 106:291-294.
- Graves, W.R. 2008. Habitat and reproduction of *Dirca mexicana* (Thymelaeaceae). *Rhodora* 110: 365-378.
- Hansen, O. B., and J.R. Potter. 1997. Rooting of apple, rhododendron, and mountain laurel cuttings from stock plants etiolated under two temperatures. *HortScience* 32:304-306.
- Hartmann, H. T., D.E. Kester, F.T. Davies, Jr., and R.L. Geneve. 2001. Hartmann and Kester's Plant Propagation: Principles and Practices (7th ed.). Prentice Hall.
- Hendricks, D.R. 1985. Air layering native woody plants. Proc. Int. Pl. Propag. Soc. 34:528-531.

- Husen, A. 2008. Stock-plant etiolation causes drifts in total soluble sugars and anthraquinones, and promotes adventitious root formation in teak (*Tectona grandis* L. f.) coppice shoots. *Pl. Growth Reg.* 54:13-21.
- Loew, E A. 1946. Observations on the growth of an injured plant of *Dirca palustris*. *Proc. Ind. Acad. Sci.* 55: 48-49.
- Majada, J., C. Martinez-Alonso, I. Feito, A. Kidelman, I. Aranda, and R. Alia. 2011. Mini-cuttings: an effective technique for the propagation of *Pinus pinaster* Ait. *New Forests* 41:399-412.
- Maynard, B. K., and N.L. Bassuk. 1987. Stockplant etiolation and blanching of woody plants prior to cutting propagation. *J. Am. Soc. Hort. Sci.* 112: 273-276.
- Maynard, B.K. and N.L. Bassuk. 1988. Etiolation and banding effects on adventitious root formation. In: Davis T.D., Haissig B.E., Sankhla N. (eds.) *Adventitious root formation by cuttings*. Dioscorides Press, Portland, OR.
- Maynard, B. K. and N.L. Bassuk. 1996. Effects of stock plant etiolation, shading, banding, and shoot development on histology and cutting propagation of *Carpinus betulus* L. *J. Am. Soc. Hort. Sci.* 121:853-860.
- McCown, B.H. 2000. Recalcitrance of woody and herbaceous perennial plants: Dealing with genetic predeterminism. *In Vitro Cell. Dev. Biol. Plant* 36: 149-154.
- Nesom, G. L. and M.H. Mayfield. 1995. A new species of *Dirca* (Thymelaeaceae) from the Sierra of northeastern Mexico. *Sida* 16:459-467.
- Patience, P. A., and P.G. Alderson. 1984. Improving the rooting of *Syringa vulgaris* cuttings by etiolation. *Proc. Intl. Pl. Prop. Soc.* 34:316-327.

Schrader, J.A. and W.R. Graves. 2005. Seed germination of *Dirca* (leatherwood): Pretreatments and interspecific comparisons. HortScience 40:1838-1842.

Symons, G. M., and J.B. Reid. 2003. Interactions between light and plant hormones during de-etiolation. J. Plant Growth Reg. 22:3-14.

## APPENDIX 2: Shade Tolerances in Two Provenances of Eastern Leatherwood

Understanding native species' tolerance to the intensity of photosynthetically active radiation (light) is an important tool for promoting their use as landscape plants (Stanton et al., 2010). The genus *Dirca*, commonly known as leatherwood, contains four species of shade-tolerant shrubs found in North America (Floden et al., 2009). *Dirca palustris* has the broadest distribution of the genus, occurring infrequently from Nova Scotia west to North Dakota and south to Louisiana and Florida (Peterson and Graves, 2009). Despite its wide distribution, the species is uncommon in the wild and listed as endangered in Florida and threatened in Maryland (Wunderlin and Hansen, 2008). Authors have varied in their descriptions of habitat for eastern leatherwood, suggesting the species occurs in beech and sugar maple forests (Fernald, 1950), rich mesic forests (Ward and Horn, 1998; Cooperrider, 1962), and along north or east-facing slopes along watercourses (Steyermark, 1963). Despite these disparate habitat descriptions, all settings in which *D. palustris* occurs are characterized by some amount of shade from overstory vegetation. Given the variation in light quantity and timing in suburban landscapes (Kjelgren, 1995), shade occurs throughout much of the managed landscape and underscores the need for shade-tolerant, woody ornamentals.

Light intensity affects morphological and ecophysiological responses in both herbaceous and woody plants (Harbur and Owen, 2004; Hampson et al., 1996). Phenotypic plasticity for light-affected traits such as leaf size, shoot growth rates, and nutrient uptake has been documented in species growing in heterogeneous light conditions in the wild (Steinger et al., 2003; Valladares et al., 2000). Furthermore, responses to light can vary within and among species (Johnson and Cartwright, 2005; Valladares et al., 2000), providing the opportunity to examine the effect of provenance on such ecophysiological phenomena. Provenance-based

selections for shade tolerance could result in cultivars suitable for the horticultural marketplace (Escribano and Lazaro, 2009; Ramesh and Khurana, 2008), while also providing another trait for differentiating between provenances. In this study, our objective was to characterize the differences between provenances of eastern leatherwood at latitudinal extremes of its distribution after initial observations indicated a tendency of the southerly provenance to necrose throughout its lamina when grown in an unshaded greenhouse.

### **Materials and Methods**

Eighteen, two-year old seedlings from each of two provenances (Maine and Florida) grown in #SP4 nursery containers (with an approximate volume of 1000 cm<sup>3</sup>) were randomly assigned to three treatments (heavy shade, light shade, and no shade) in a 3 x 2 factorial block on a greenhouse bench. Air and soil temperatures in each treatment were logged with an iButton Data Logger (Maxim Integrated Products, Sunnyvale, CA). Plants were irrigated to container capacity when the surface of the medium appeared dry, and no fertilizers were used. Data were collected on 18 and 19 May. Measurements of net photosynthetic rates at PAR levels between 1200 and 1400 hours were recorded on 18 May using a LI-COR 6400 Portable Photosynthesis System (LI-COR, Lincoln, NE) on the youngest fully expanded leaf of each plant. Relative greenness of the youngest fully expanded leaf of each plant was measured using a SPAD-502Plus chlorophyll meter (Konica-Minolta, Osaka, Japan). On 19 May, measurements of net photosynthetic rates at afternoon PAR levels (mean PAR between 1100 and 1900 hours, 1500, 1000, 700, 500, 300, 200, 100, 75, 60, 25, and 0  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for all treatments, respectively) were collected using the LI-COR 6400 Portable Photosynthesis System (LI-COR, Lincoln, NE) on the youngest fully expanded leaves. PAR compensation points (PAR level at which photosynthetic and respiration rates are equal) were calculated as the slope of polynomial

regression functions fit to these data points in units of  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Graphing tools and regression functions of Microsoft Excel 2007 (Microsoft, 2007) were used to plot light response curves and to calculate PAR compensation points. All leaves of plants from all treatments were harvested, weighed, and scanned for surface area using LI-COR 3100 Area Meter (LI-COR, Lincoln, NE). One-way ANOVA and means comparisons for each pair using Student's t test were generated with JMP software (SAS Institute, Cary, NC).

## Results and Discussion

Table 1 reports PAR readings recorded at the completion of the study on cloudless days at approximate solar noon. All six plants in the heavy shade treatment from Maine died during treatment, while only one of six plants in that treatment from Florida died, suggesting a possible difference in tolerance to deep shade among plants from different provenances. Qualitative observations during the study indicated that plants under heavy shade required watering less often due to an apparently saturated root zone.

Table 2 summarizes data collected on 18 and 19 May. In the light shade and no shade treatments, photosynthetic rates on 18 May of plants from Maine were greater than those from Florida, though these were based on ambient PAR over a period of two hours. Plants from Maine had greener leaves than those from Florida under light shade and no shade treatments, a condition supported by qualitative observation.. There were no differences between provenances by treatment groups for mean photosynthetic compensation points (Table 2). Polynomial regression functions returned negative PAR values for three plants, which were not included in means comparisons. There were no differences between provenances by treatment groups for leaf weight or leaf surface area.

Qualitative observations of light-induced necrosis suggested that extent of injury was less during the study period than compared to during observations of plants under similar conditions during previous years. Leaves of plants from Florida generally appeared pale green with yellow stippling or small necrotic blotches, while those from Maine were darker green with significant contortion and crinkling of the adaxial laminar surface (Fig. 1). A future study should consider a larger sample size from multiple provenances with tighter environmental controls. Net photosynthesis data collected at light intensities calibrated to mean PAR value of treatment groups would yield a conservative, but potentially more informative data set for comparing differences between provenances (see Schrader et al., 2006).

Table 1. Measurements of photosynthetically active radiation ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) recorded at the completion of the study on bright, cloudless days at approximate solar noon.

Treatment	18 May	19 May <sup>z</sup>	19 May <sup>y</sup>	Average
Heavy shade	3	38	20	20
Light shade	212	506	334	351
No shade	1282	1650	1028	1320

<sup>z</sup>Measurement taken at beginning of data collection.

Table 2. Net photosynthetic rate, relative greenness, mean photosynthetic compensation point, leaf weight and leaf surface area data collected on 18 and 19 May 2010.

Response	Treatment	Provenance	
		Florida	Maine
Net photosynthetic rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Heavy Shade	0.11	N/A
	Light Shade	2.35 b	3.64 a
	No Shade	0.31 b	1.50 a
Relative greenness (SPAD)	Heavy Shade	14.2	N/A
	Light Shade	37.1 b	47.8 a
	No Shade	26.8 b	34.6 a
Mean photosynthetic compensation point ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	Heavy Shade	24.0	N/A
	Light Shade	47.3 a	27.4 a
	No Shade	41.8 a	54.3 a
Leaf weight (g)	Heavy Shade	0.21	N/A
	Light Shade	0.82 a	0.77 a
	No Shade	0.92 a	0.89 a
Leaf surface area ( $\text{cm}^2$ )	Heavy Shade	102	N/A
	Light Shade	222 a	178 a
	No Shade	179 a	145 a

<sup>z</sup>Different letters within rows indicate significance at  $\alpha = 0.05$ .

Figure 1. Composite photograph of leaf morphologies of two provenances of *Dirca palustris*. At left, a photo of a typical leaf of plants from Maine, showing dark green pigmentation and crinkling of the laminar surface. At right, a photo of a typical leaf of plants from Florida, showing pale green pigmentation and chlorotic stippling.



(Fig. 1)

## Literature Cited

- Cooperrider, T. S. 1962. The flora of north-facing slopes compared to that of the surrounding area in eastern Iowa. *Amer. Midland Nat.* 67:368-372.
- Escribano, S. and A. Lazaro. 2009. Agro-morphological diversity of Spanish traditional melons (*Cucumis melo* L.) of the Madrid provenance. *Gen. Res. and Crop Evol.* 56: 481-497.
- Fernald, M.L. 1950. Gray's manual of botany. Dioscorides Press, Portland.
- Floden, A., M. Mayfield, and C.J. Ferguson. 2009. A new narrowly endemic species of *Dirca* (Thymelaeaceae) from Kansas and Arkansas, with a phylogenetic overview and taxonomic synopsis of the genus. *J. Bot. Res. Inst. Texas* 3:485-499.
- Hampson, C.R., A.N. Azarenko, and J.R. Potter. 1996. Photosynthetic rate, flowering, and yield component alteration in hazelnut in response to different light environments. *J. Am. Soc. Hort. Sci.* 121: 1103-1111.
- Harbur, M.M. and M.D.K. Owen. 2004. Light and growth rate effects on crop and weed responses to nitrogen. *Weed Sci.* 52:578-583.
- Johnson, G.R. and C. Cartwright. 2005. Genotype x shade effects for western hemlock. *Can. J. For. Res.* 35: 1496-1501.
- Kjelgren, R. 1995. Variable urban irradiance and shade acclimation in Norway maple street trees. *J. Arbor.* 21:145-149.
- Peterson, B. J. and W.R. Graves. 2009. Variation in development and response to root-zone pH among seedlings of *Dirca palustris* (Thymelaeaceae) from three provenances. *HortScience* 44:1319-1322.

- Ramesh, K. R. and D.K. Khurana. 2008. Clonal provenance variation of *Populus alba* Linn. in nursery. *Ind. J. For.* 3:599-610.
- Schrader, J.A., W.R. Graves, S.A. Rice, and J.P. Gibson. 2006. Differences in shade tolerance help explain varying successes of two sympatric *Alnus* species. *Intl. J. Pl. Sci.* 167: 979-989.
- Steinger, T., B.A. Roy, and M.L. Stanton. 2003. Evolution in stressful environments II: adaptive value and costs of plasticity in response to low light in *Sinapis arvensis*. *J. Evol. Biol.* 16:313-323.
- Steyermark, J.A. 1963. *Flora of Missouri*. Iowa State Univ. Press, Ames, IA.
- Valladares, F., S.J. Wright, E. Lasso, K. Kitajima, and R.W. Pearcy. 2000. Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology* 81:1925-1936.
- Ward, A.B. and C.N. Horn. 1998. A status survey of *Dirca palustris* L. (leatherwood, Thymelaeaceae) in South Carolina. *Castanea* 63:165-173.
- Wunderlin, R.P. and B.F. Hansen. 2008. *Atlas of Florida vascular plants*. Institute for Systematic Botany, University of South Florida, Tampa. <http://www.plantatlas.usf.edu/>. Accessed 14 April 2011.

### **APPENDIX 3: Comparing Three Species of *Dirca* for pH Responses in Soilless Media**

A study was designed to follow similar methodology reported by Peterson and Graves (2009) in an attempt to document pH responses in soilless media for three species of *Dirca*. The aforementioned study only considered *Dirca palustris* from different provenances. Two-year-old seedlings of three species (N = 50, each) were randomly assigned to one of five treatments (N = 10 per treatment) and planted in pH-modified soilless media. Sulfuric acid (1-M H<sub>2</sub>SO<sub>4</sub>) was used to modify pH to three acidic treatment levels. Calcium carbonate (CaCO<sub>3</sub>) was used to modify pH to one basic treatment level. The 6.0 pH of the unadjusted soilless media was considered the fifth treatment. Media were wetted with 2,000 mL of tap water (pH = 7.5) before planting. The media were mixed thoroughly to ensure sufficient distribution of pH-altering agents. Seedlings were de-potted from 6 cm diameter, square band pots (Anderson Die & Mfg. Co., Portland, OR), rinsed in tap water to remove all previous media and potted in #SP4 containers (with an approximate volume of 1000 cm<sup>3</sup>) in the treatment media. Seedlings were irrigated to container capacity with tap water every 7 to 10 days.

Temperatures were logged with an iButton Data Logger (Maxim Integrated Products, Sunnyvale, CA). Soil pH was monitored bi-monthly using the pour-thru method (Cavins et al., 2000) and determined with an IQ160 pH Meter (IQ Scientific Instruments, Carlsbad, CA). Measurements of net photosynthetic rates at PAR levels between 800 and 8400 hours were recorded on 28 July using the LI-COR 6400 Portable Photosynthesis System (LI-COR, Lincoln, NE) on the youngest fully expanded leaf of each plant. Relative greenness of three leaves near the stem apex was measured using a SPAD-502 Chlorophyll Meter (Konica Minolta Sensing, Tokyo, Japan). Means comparisons for each pair using Student's t test were generated with JMP

software (SAS Institute, Cary, NC), though letter groupings were not included in Table 1. Photosynthesis data are not provided due to highly variable and inconclusive results.

Relative greenness values (SPAD) from the three sample dates across the study are shown in Table 1, along with a comparison of target pH treatment value and final pH treatment value. Qualitative results differed approximately by species. *Dirca mexicana* and *Dirca palustris* most often displayed uniformly yellow or chartreuse foliage among treatments. *Dirca occidentalis* variably exhibited chlorosis between the midribs and margins of leaves, though plants in the pH 5.9 treatment tended to be the most uniformly green. Stability of pH of the media throughout the study was poor. The inconsistent pH data rendered this study of little value for demonstrating pH effects on the three species of *Dirca*. Chapter 4 of this thesis provides more useful data obtained in a field study with a similar goal.

Table 1. Relative greenness values (SPAD) of three leaves near the stem apex measured using a SPAD-502 Chlorophyll Meter for three species of *Dirca*.

Target pH treatment (Mean pH on 28 July)	Species	Dates		
		8 June	6 July	28 July
4.5 (6.4)	<i>D. mexicana</i>	19.3	16.1	12.4
	<i>D. occidentalis</i>	20.9	17.2	12.5
	<i>D. palustris</i>	29.6	20.7	11.9
5.3 (6.5)	<i>D. mexicana</i>	18.3	14.5	12.8
	<i>D. occidentalis</i>	21.5	16.8	15.1
	<i>D. palustris</i>	25.6	18.6	11.0
5.9 (6.8)	<i>D. mexicana</i>	16.8	13.6	11.7
	<i>D. occidentalis</i>	20.8	16.7	15.6
	<i>D. palustris</i>	32.3	22.3	15.3
6.0 (6.8)	<i>D. mexicana</i>	18.2	14.6	N/A <sup>z</sup>
	<i>D. occidentalis</i>	20.9	16.2	N/A
	<i>D. palustris</i>	28.9	23.4	N/A
7.3 (7.8)	<i>D. mexicana</i>	16.5	13.4	9.9
	<i>D. occidentalis</i>	15.3	12.8	11.7
	<i>D. palustris</i>	26.8	17.8	12.8

<sup>z</sup> Due to the homogenization of treatment groups, the two groups that had a mean pH of 6.8 on 28 July were treated as one group for results for comparisons.

#### **APPENDIX 4: Documentation of the First Interspecific Crosses in the Genus *Dirca***

Interspecific crosses between three species of *Dirca* were planned in 2010. Each of the three species of *Dirca* possesses novel ornamental characteristics. *Dirca occidentalis* has the largest flowers of the genus (Figure 1) and exhibits bright yellow fall foliar color and coppery, orange bark, but initial trials suggest it lacks the ability to thrive in the Upper Midwest (see Chapter 3). *Dirca palustris* is the most widely distributed of the genus occurring throughout eastern North America in discontinuous, localized populations. *Dirca palustris* has the smallest flowers of the genus (Figure 1), but its widespread geographic occurrence and apparent tolerances for assorted edaphic conditions (see Chapter 4) merits its inclusion in a breeding program to maximize these traits in the hybrid offspring. Its leaves sometimes become yellow in autumn. *Dirca mexicana* warrants inclusion because of its rapid growth characteristics, hardiness, and general plant vigor (see Chapter 3). Observational evidence suggests that *D. mexicana*, like *D. occidentalis*, also has the potential for yellow fall foliar color. Combining these three species in a three-way cross would essentially aim to maximize the best traits of each species. However given the likelihood of sterility in the F1 generation, a series of preliminary two-way crosses was proposed in an effort to achieve the most ornamentally viable F1 population from the closest genetic congeners.

The reproductive biology of *Dirca* has been the subject of only a few studies. Williams (2004) reported that *D. palustris* is self-compatible, an assertion supported by the species' floral morphology, and that pollinator visitation was strongly regulated by temperature. Similar findings have been reported for *D. mexicana* and *D. occidentalis* (Nesom and Mayfield, 1995; Graves, 2006), though with slightly different accounts of the degree of stigma exertion among species. Williams (2004) reported observing a few species of halictid bees frequenting flowers,

while Graves (2008) photographed a butterfly (*Catantixia nimbice nimbice*) visiting flowers of *D. mexicana* in the wild in Tamaulipas, Mexico. Schulz et al. (2004) reported that variation in weather seemed to effect only fruit production of *D. palustris* in years of extreme temperature and rainfall. Inflorescence and drupe number from year to year were relatively stable across their study period and were primarily influenced by plant size and light availability. Populations of *D. occidentalis* reportedly have tremendous variation in fruiting success (Graves, 2006). Both Graves (2008) and Williams (2004) suggested that apomixis has not been ruled out in the genus.

Fifteen replications of the cross between *Dirca mexicana* and *D. occidentalis* were made in the greenhouse on 25 February and 28 February. *Dirca mexicana* was used as the pod parent since it was the only flowering-size stock plant available under greenhouse conditions. Pollen from *D. occidentalis* was wild-collected in San Mateo County, CA and air-transported in glass vials in a cooler. Bud sockets utilized for cross pollination in *D. mexicana* were tagged two days before crossing, emasculated, and reduced to one flower per socket to avoid potential self-pollination. Hand pollinations were conducted by excising stamens from the pollen parent and brushing them gently across the surface of the stigma with the aid of a 10x hand lens.

On 2 April, eight replications of the reciprocal cross between *Dirca occidentalis* and *D. mexicana* was made on a flowering plant in the campus landscape of Iowa State University (ISU) in Ames, IA. Pollen from *D. mexicana* was collected from a nearby trial on the ISU campus. Bud sockets utilized for cross pollination in *D. occidentalis* were tagged 2 days before crossing, emasculated, and reduced to one flower per socket to avoid potential self-pollination. Hand pollinations were conducted by excising stamens from the pollen parent and brushing them gently across the surface of the stigma with the aid of a 10x hand lens.

On 9 April, 13 replications of the cross between *Dirca mexicana* and *D. palustris* were made on flowering plants in a trial on the ISU campus. Pollen from *D. palustris* was collected with permission of the park ranger from Ledges State Park near Boone, IA. Bud sockets utilized for cross pollination in *D. mexicana* were tagged 2 days before crossing and reduced to one flower per socket to avoid potential self-pollination. Hand pollinations were conducted by excising stamens from the pollen parent and brushing them gently across the surface of the stigma with the aid of a 10x hand lens.

On 15 April, one seed was harvested from the February crosses and planted on 24 April in Fafard® 52 soilless potting media (Fafard®, Inc., Agawan, MA) and placed on a bench in approx. 20°C greenhouse. On 15 May, all replications of the April crosses between *Dirca occidentalis* and *D. mexicana* had aborted. Developing fruits of putative crosses between *D. mexicana* and *D. palustris* were wrapped in fine mesh cheesecloth to prevent lost fruits. Nine fruits from the cheesecloth bags were harvested 9 June and stored in a cool dry cabinet until planted 11 October.

Germination was observed in March 2011 after seeds were exposed to stratifying conditions for 24 weeks. One seedling resulted from the cross of *D. mexicana* X *D. occidentalis*; two seedlings resulted from the cross of *D. mexicana* X *D. palustris*. As of this report, hybrid confirmation with inter-simple sequence repeat (ISSR) markers remains uncompleted. Given the possibility of accidental selfing or apomixis, it's possible that none of these seedlings is a hybrid.

Evaluation of any F1 hybrid progeny should center on hardiness, plant architecture and habit, flower size, and floriferousness as primary tools for selecting the most marketable individuals. Asexual propagation of hybrids remains a challenge given inconsistent results in the

use of etiolated, softwood stem cuttings, and limited or untested additional options (see Appendix 1). Given the cold hardiness and aesthetic qualities of *D. mexicana*, it's possible that extensive germplasm assessments could result in candidate specimens for bulk seed harvesting and promotion as a seed strain cultivar. Evaluating germplasm in this manner would require less labor, fewer resources, and potentially result in an equally viable product as one obtained through interspecific hybridization.



Figure 1. Comparisons of floral size between three species of *Dirca*.

**Literature Cited**

- Graves, W.R. 2006. Distribution and reproduction of *Dirca occidentalis* (western leatherwood).  
The Four Seasons (East Bay [Berkeley, California] Regional Parks Botanic Garden)  
12:54-61.
- Graves, W.R. 2008. Habitat and reproduction of *Dirca mexicana* (Thymelaeaceae). *Rhodora*  
110: 365-378.
- Nesom, G. L. and M.H. Mayfield. 1995. A new species of *Dirca* (Thymelaeaceae) from the  
Sierra of northeastern Mexico. *Sida* 16:459-467.
- Schulz, K., J. Zasada, and E. Nauertz. 2004. Annual, local, and individual variation in the  
inflorescence and fruit production of eastern leatherwood (*Dirca palustris* L.  
Thymelaeaceae). *J. Torrey Bot. Soc.* 131:292–304.
- Williams, C.E. 2004. Mating system and pollination biology of the spring-flowering shrub,  
*Dirca palustris*. *Plant Spec. Biol.* 19:101-106.

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