FINAL REPORT

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Report Completed by: Dayle Saar
Project Objective: Using molecular markers, the project objective was to determine the "true" range for Red Mulberry (*Morus rubra*) within the state.

Project Summary

Introduction:

The mulberry genus, *Morus*, consists of about 16 species. Two species are native to North America, *Morus rubra* (Red Mulberry) and *M. microphylla* (Little-leaved or Texas Mulberry); only *M. rubra* is found in Illinois (Fig. 1). White Mulberry (*M. alba*) is native to eastern Europe and Asia, and is the sole food for the silk caterpillar. White Mulberry was introduced to the US around 1780 (Webster, 1790), and on several subsequent occasions, in failed attempts to start a silk industry in this country. White mulberry was also planted along city streets, as part of a mulberry "craze" during the 1820s and 1930s (Cole, 1926). It was used as shelterbelt plantings during the 1930s and beyond (Dahl, 1940). Altogether, many thousands of White Mulberry trees were planted. This species has since escaped cultivation and is a common weed across most of eastern North America, and in pockets farther west. It readily hybridizes with our native Red Mulberry.

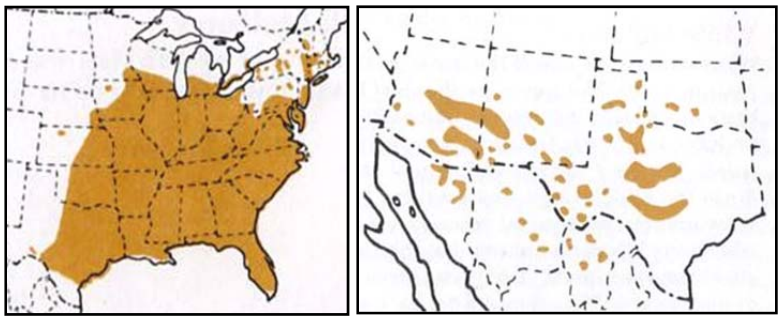

Figure 1. Range maps for the two native mulberry species in North America. Left: Red Mulberry (*Morus rubra*), and right: Little-leafed or Texas Mulberry (*M. microphylla*). Adapted from Elias, 1989.

Fruits of Red Mulberry are blackish-purple when ripe. Fruits of White Mulberry are variable; ripe fruit may be pure white or pink through red to blackish-purple. Leaves of Red Mulberry are generally larger than White Mulberry, but size ranges overlap. Based on the oldest plant keys available, through present, these species have been distinguished almost exclusively on the basis of leaf pubescence (e.g., Jones, 2005; Mohlenbrock, 2002; Wunderlin, 1997; Swink & Wilhelm, 1994; Gleason & Cronquist, 1991; Radford et al., 1968; Steyermark, 1963; and Britton & Brown, 1913) (Table 1).

Table 1. Past and currently accepted leaf characters to distinguish between Red and White Mulberry species.

LEAF SURFACE	RED MULBERRY	WHITE MULBERRY
upper	scabrous	glabrous & often lustrous
lower	pubescent throughout	glabrous or w/ tufts @ vein junctions

Prior to this study, we observed that Red Mulberry trees in southern Illinois are markedly different than those in northern Illinois. Although the northern Red Mulberries have the pubescence described in the keys for Red Mulberry, we have noted that they have the same straight vein pattern as the White Mulberry. The secondary veins extend out from the midvein to the leaf margin, and end in a tooth. The southern Red Mulberries, however, have a uniquely curved pattern. Their secondary veins curve towards the leaf apex before reaching the margin and join the next secondary vein, resulting in a looped pattern just inside the leaf margin.

"Northern" Red Mulberries have the generally smaller leaves of the White Mulberry, and often have a slight luster to the leaves; White Mulberry leaves usually have high luster, while those of "southern" Red Mulberry are large and dull. However, because the existing keys are essentially limited to only one character, both the northern and southern trees are indistinguishable, taxonomically. We have studied many herbarium specimens, and species identification has followed plant keys on leaf pubescence, regardless of luster or vein pattern.

Based on DNA sequences, it appears that the White Mulberry can, in fact, produce more pubescence that previously thought, resulting in many White Mulberry trees being miss-identified as Red Mulberry. A number of these pubescent White Mulberry trees were growing in relatively undisturbed woodlands. We plotted the results of all *M. rubra* (both northern and southern types) on the range map. No obviously White Mulberry trees (based on keys) are included (Fig. 2).

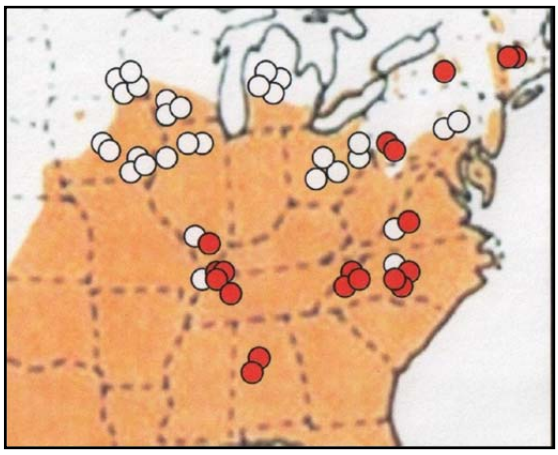

Figure 2. Location of Red Mulberry (dark circles) vs. White Mulberry (white circles), based on ITS sequences. All trees would be identified as Red Mulberry, based on current taxonomic criteria.

Materials and Methods:

Herbarium specimens and topology maps from the Illinois *Gazetteer* (Delorme Mapping Company) were utilized to identify potential habitat for Red Mulberry. Potential habitats were surveyed on foot. Trees that appeared to be Red Mulberry were sampled (Fig. 7). If no "pure" Red Mulberry could be located, trees that appeared to by hybrids were sampled. Obvious White Mulberry trees (based on high leaf luster and vein pattern) were noted but most were not sampled. Mulberry trees were observed in 52 Illinois counties; 40 IL counties were sampled across the state, while 12 others were visited, but only White Mulberry trees were located (not sampled) (Table 2.).

Sampling consisted of herbarium vouchers and 1-2 young leaves for DNA, dried in silica gel. If young leaf material was unavailable, 6-10 winter buds were collected and dried. DNA material was kept refrigerated prior to extraction in the lab.

DNA was extracted using DNeasy (Qiagen) following the manufacturer's instructions. PCR parameters followed Galla et al. (2009) and Saar et al. (2012). Results were visualized on 1.0% agarose gels. DNA was sequenced at the core lab at Northern Illinois University, DeKalb, IL. Sequences were aligned with Clustal X software (Thompson et al., 1997). Species identification was based on nuclear molecular markers developed by Galla et al. (2009) and chloroplast markers developed by Saar et al. (2012).

Results:

White Mulberry was located in every county surveyed, in moderate to highly disturbed habitats. Red Mulberry was found only in wooded habitats with low to moderately low disturbance. Although considered a "riparian" species, Red Mulberry generally does not occur in true bottomland forests, where soils are inundated with water for at least a few weeks during the year. It most often occurs in a transition zone where bottomland species, such as Boxelder Maple (*Acer negundo*) and Silver Maple (*A. saccharinum*), are found only occasionally in the overstory among the upland hardwood species (oaks, hickories, etc.).

In the southern third of the state, Red Mulberry was easy to locate in most suitable habitats with sufficient isolation from White Mulberry (large forested tracts) (Fig. 3). Areas of counties surveyed in extreme SE IL were heavily cultivated. Red Mulberry was not located in parts of central Illinois due to large areas in cultivation. Some areas along the Mississippi R. in NW IL may be warm enough to support Red Mulberry, but urbanization and other anthropomorphic disturbances do not allow for suitable habitat.

Red Mulberry in northern IL is rare and occurs only in exceptional microclimates of winter warmth. In each case, the small outlying populations are associated with geographical features that trap or direct warmer air associated with bodies of water in winter. In MS Palisades State Park (Carroll Co.), Red Mulberry trees were located up on the bluffs (Figs. 4 and 5). Apparently, relatively warm arm air rising from the MS R. during winter is "funneled" up west-facing limestone bluffs. Only White Mulberry and hybrids occur down behind the bluffs away from the river. The population of Red Mulberry located in Starved Rock State Park (LaSalle Co.) occurs along a permanent creek (vs. ephemeral) in LaSalle Canyon (Fig. 6). This canyon has two right-angle turns before emptying into the windswept IL R. and floodplain, which forms wind barrier. This species could not be located in nearby canyons with straighter canyon runs. These other canyons also had shallow creek beds without permanent water. Plant material representing the population from Will Co. was obtained from a tree grown from seed at the Morton Arboretum, Lisle, IL. The seeds were collected in 1978 from trees on an island in the Des Plaines R. in McKinley Woods Forest Preserve. Only one individual of Red Mulberry was located in Grundy Co., above the IL R. It was a sapling that may have survived during the warmer winters of the last few years.

Table 2. Counties surveyed by this project. “X” indicates trees were sampled; “(X)” indicates trees were observed but not sampled.

IL COUNTY	RED MUL-BERRY	HYBRID (Red & White)	WHITE MUL-BERRY
Bureau		X	(X)
Calhoun	X		(X)
Carroll	X	X	(X)
Christian			(X)
Cook			(X)
De Witt			(X)
DeKalb			(X)
DuPage			(X)
Fayette	X	X	X
Franklin	X	X	(X)
Gallatin			(X)
Grundy	X	X	(X)
Henderson	X		(X)
Henry			(X)
Jackson			(X)
Jefferson		X	(X)
Jersey	X		(X)
Jo Daviess		X	(X)
Johnson	X	X	(X)
Kane			(X)
Kankakee		X	(X)
Kendall		X	X
La Salle	X	X	(X)
Lawrence		X	(X)
Lee		X	(X)
Logan			(X)
Macon			(X)
Marion	X	X	(X)
Marshall	X	X	(X)
Massac	X	X	(X)
McClean		X	(X)
Mercer		X	(X)

Table 2 continues.

Figure 3. Counties corresponding to Table 2. Red circles indicate Red Mulberry; pink circles indicate interspecific hybrids; white figures depict White Mulberry – circles where collected and squares where observed but not collected. * See text for habitat descriptions of these sites.

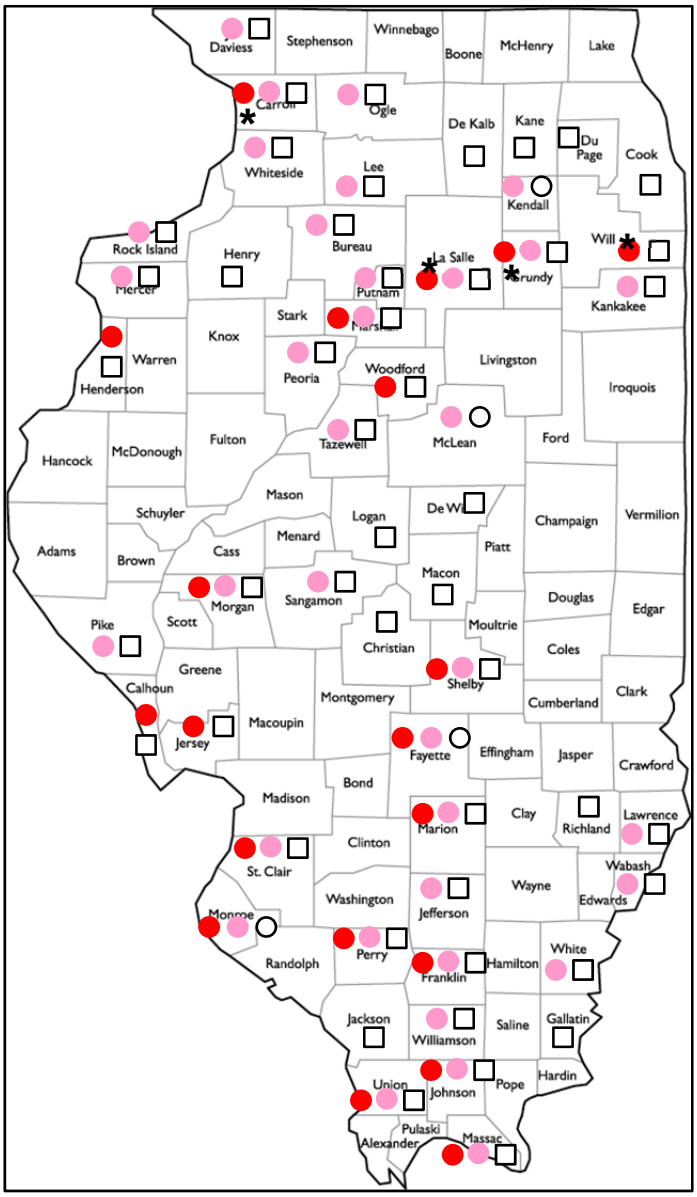

Table 2, continued.

Monroe	X	X	X
Morgan	X	X	(X)
Ogle		X	(X)
Peoria		X	(X)
Perry	X	X	(X)
Pike		X	(X)
Putman		X	(X)
Richland			(X)
Rock Island		X	X
Sangamon		X	(X)
Shelby	X	X	(X)
St. Clair	X	X	(X)
Tazewell		X	(X)
Union	X	X	(X)
Wabash		X	(X)
White		X	(X)
Whiteside		X	(X)
Will	X		(X)
Williamson		X	(X)
Woodford	X		(X)

Summary:

Red Mulberry in the southern third of the state is frequent where disturbance is low and where it is isolated from White Mulberry to prevent interspecific hybridization. However, in northern IL, Red Mulberry is rare and occurs only in exceptional microclimates of winter warmth. To maintain these populations, they must be isolated from hybridization with White Mulberry.

Figure 4. Red Mulberry growing on bluffs at MS Palisades State Park.

Figure 5. Bluffs overlooking MS R. at MS Palisades State Park. Outlying population of Red Mulberry was located on these bluffs.

Figure 6. Starved Rock State Park, view up creek in LaSalle Canyon; trees of Red Mulberry are on right bank.

Figure 7. Sampling Red Mulberry in southern IL.

Publications and Presentations – Completed and in Progress:

The following has been presented, with credit given to IDNR and the IL Wildlife Preservation Fund.

Thomas, S.G., N.T. Huynh, and D.E. Saar. April 2013. *Determining the Northern Limit for Red Mulberry* (Morus rubra) *Using DNA-identified Individuals,* poster presented during Scholars' Week at Murray State University, Murray, KY.

Saar, D.E., N.C. Bundy, L.J. Potts, and M.O. Saar. 2012. Status of *Morus murrayana* (Moraceae). *Phytologia* 94: 245-252.

Two additional publications are in preparation. One will focus in the Illinois populations. The second publication will include data from IL but in less detail. It will be incorporated with a larger project that included much of the eastern US.

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Murray State project looks at mulberry trees

Dr. Dayle Saar, a Yorkville resident, and her students from Murray State University, have developed molecular markers to distinguish the two species of mulberry found in Illinois based on differences in their DNA sequences. The identification method of using leaf hairs to determine species, and native vs. nonnative, is not always consistent with DNA identification. Dr. Saar and her students have found that differing vein patterns in the leaves of the two species provide a much more reliable field character.

Two species of mulberry occur naturally in Illinois: Red Mulberry and White Mulberry. White Mulberry was first introduced from Asia shortly after the Revolutionary War as part of several failed attempts to start a silk industry in this country. White Mulberry leaves are the sole food source of the silkworm caterpillar. Descendants of these weedy trees are common today in fencerows and backyards.

Only a small percentage of White Mulberry trees actually produce white fruits. Most individuals produce purplish-black fruits that are indistinguishable from fruits of Red Mulberry. Red Mulberry, which does not behave like a weed, is our only species of mulberry that is native to Eastern North America. These two species have been distinguished from each other by the location and amount of hairs on leaf undersides.

Using DNA-identified individuals plotted on a map, it became obvious to these researchers that White Mulberry is much more cold hardy than Red Mulberry. The two species readily hybridize, and the cold hardiness trait is imparted to the hybrid offspring. Results of their statewide study show that Red Mulberry is quite rare in the northern third of the state, restricted to isolated pockets along rivers that provide a microclimate of winter warmth.

Reports of Red Mulberry along the Fox River in Yorkville and Silver Springs State Park were investigated. All trees found were either White Mulberry or hybrids between the two species. No Red Mulberry trees were located. Farther south in the state, however, Red Mulberry is readily found in wooded river habitats with minimal disturbance from human activities. Saar's research was funded through a grant from the IL Wildlife preservation Fund.

Dr. Saar is an Associate Professor and Curator of the Herbarium in the Department of Biological Sciences at Murray State University in Murray, KY.