ELM GENETIC DIVERSITY AND HYBRIDIZATION IN THE PRESENCE OF DUTCH ELM DISEASE

Johanne Brunet and Raymond P. Guries[1]

Abstract.—The impact of Dutch elm disease (DED) on the genetic diversity of slippery elm (*Ulmus rubra)* is summarized and its potential impact on the genetic diversity of other North American native elms, American elm (*U. americana)*, rock elm (*U. thomasii)*, winged elm (*U. alata*), cedar elm (*U. crassifolia*), and September elm (*U. serotina),* is discussed. The potential for hybridization between the introduced Siberian elm *U. pumila* and the native North American elms is considered given previous findings with *U. rubra*. We do not expect DED to reduce the genetic diversity of these native elms. The only exception may be *U. serotina* if its restricted range leads to genetic discontinuities among populations. We do not expect hybridization between *U. americana* and *U. pumila* due to incompatibility barriers, but hybridization between *U. pumila* and other native elms appears more likely and could have negative effects on the long term conservation of these species. This information is timely given the current efforts to restore American elm across the U.S. landscape.

Introduction

Dutch elm disease (DED), caused by the introduced fungal pathogen *Ophiostoma ulmi*, has devastated North American elm populations for more than 75 years. The first wave of DED in North America began around 1930 (Smalley and Guries 1993) and quickly decimated both rural and urban populations. Currently, a new sub-species, *O. novo-ulmi*, even more virulent than the first, continues to ravage native elm populations (Brasier 2000). All native North American elm species, including the iconic American elm, *Ulmus americana*, have been affected by DED. Although elms have not been eliminated from forest settings (Richardson and Cares 1976), there was tremendous mortality due to DED, which resulted in a smaller percentage of large diameter native elm trees in eastern hardwood forests, based on reports after 20 years of DED pressure in an east-central Indiana forest (Parker and Leopold 1983). Although precise estimates of the numbers of elms lost to DED are unknown, the loss has been estimated into the hundreds of millions of trees based on their former abundance (Bey 1990).

In response to the disease, resistance to DED was examined in different elm species worldwide. Little resistance was detected in most native elm species of Europe and North America but it was identified in some Asian elm species, including the Siberian elm, *U. pumila*. Resistance to DED is variable in *U. pumila* and some accessions have been used in attempts to breed DED resistance into native North American elm species. In fact, *U. pumila* has served as the source of DED resistance in virtually every new Eurasian elm cultivar released in the United States since the 1960s (Smalley and Guries 1993). The development of DED-resistant hybrid elms led to the replacement of North American elms with Eurasian hybrids on urban boulevards, but forest losses to the disease continue due to the lack of DED resistance in North American elms (Smalley and Guries 1993).

[1] Ecologist (JB), USDA Agriculture Research Service, Department of Entomology, 1630 Linden Drive, University of Wisconsin, Madison, WI; Professor Emeritus (RPG), University of Wisconsin, Madison. JB is corresponding author: to contact, call 608-265-3587 or email at Johanne.Brunet@ars.usda.gov or jbrunet@wisc.edu.

Breeding programs in the United States failed to introduce DED resistance from Eurasian species into *U. americana*. While many believed ploidy level differences accounted for the inability to cross *U. americana* with DED-resistant species, an incompatibility barrier may provide a more likely explanation (Ager and Guries 1982). *Ulmus americana* is a tetraploid (4n=56) while other elms are diploid (2n=28), although Whittemore and Olsen (2011) have recently reported that more than 20 percent of 81 *U. americana* trees from a range-wide collection were diploid. What progress has been made in developing putatively disease resistant *U. americana* trees has come from selections drawn from *U. americana* seedling populations. This finding suggests that development of DED-resistant *U. americana* may be possible without recourse to breeding with resistant Eurasian elms.

Dutch elm disease could have decreased the genetic diversity of native North American elm species. The demographic changes and reduction in population sizes associated with DED losses could create genetic bottlenecks and alter the level of genetic diversity within elm populations and also modify how such genetic diversity is distributed over the landscape (genetic differentiation) (Bouzat 2010). In addition, although *U. pumila* was introduced to the United States prior to the first DED pandemic (Ware 1995), it was largely unaffected by it due to its resistance to DED and its tolerance of dry climatic conditions (Ding et al. 2006, Leopold 1980). *Ulmus pumila* trees were planted to serve as wind breaks along highways, near farms, villages and towns, and in urban landscapes to replace dead or dying DED-susceptible native elms (Ware 1995). *Ulmus pumila* has naturalized (Zalapa et al. 2009) and now occurs throughout the range of native U.S. elms and has been declared invasive in some states (Kartesz 2015, NRCS 2017). Given the ability of *U. pumila* to interbreed with some North American elms (e.g., slippery elm or *U. rubra*; Zalapa et al. 2009, 2010), we expect the risk of hybridizing with native elm species to increase over time.

We have previously examined the impact of DED on the genetic diversity of one native North American elm species, *U. rubra*, common in Wisconsin and much of the eastern United States (Brunet et al. 2016). We have also examined the levels of hybridization between the native *U. rubra* and invasive *U. pumila* in Wisconsin (Zalapa et al. 2009, 2010). In the current study, we summarize these results and discuss how we expect DED and the spread of *U. pumila* across the U.S. landscape to influence other native elm species, besides *U. rubra*. We address the potential impact of DED on the genetic diversity of these native elms and also discuss the potential for hybridization between other native elm species and the introduced *U. pumila*. These questions are timely, given the current program to restore *U. americana* across the U.S. landscape. This study also illustrates how efforts to combat the negative impacts of a disease epidemic may have unforeseen consequences for native elm populations.

Materials and Methods

Plant Materials

Elm accessions used in this research were drawn from a variety of sources and represent both fresh and herbarium specimens (Table 1). Fresh specimens were collected relatively recently (2005-2007) from wild populations; collection of herbarium specimens took place between 1890 and 2004. Identification of species and their putative hybrids were made at the time of collection using a suite of morphological traits. The leaves, buds, twigs, and seed characteristics used for identification of each parental species, *U. rubra* or *U. pumila*, are described in Table 2 of Zalapa et al. (2010). The *U. pumila* accessions comprised samples from Asia, live trees collected throughout the United States, and live trees and herbarium specimens from Wisconsin (Table 1). Accessions of *U. rubra* originated mostly from Wisconsin and represented both live trees and herbarium specimens (Table 1).

Table 1.—Locations, sample sizes, and reference for *Ulmus pumila* accessions collected in the United States and East Asia and of *U. rubra* accessions collected in Wisconsin

Location	N	Reference
Ulmus pumila		
1. United States accessions		
Live trees from KY, KS, OR, CO, AZ, IA, OK, OH, PA, UT, TX, DE, GA, NJ, MN, AK, IL, IN, VA, WA, TN, SD, MO, MA, NV, LA, NY, MD.	37	Zalapa et al. 2010
2. Wisconsin accessions		
a) Live trees from 6 wild U. pumila populations with morphological hybrids.	95	Zalapa et al. 2009
b) Live trees from 8 wild populations from WI, SD, IL with no morphological hybrids	171	Zalapa et al. 2010
c) Wisconsin herbarium accessions collected throughout WI between 1948 and 2001	52	Zalapa et al. 2010
3. Accessions from East Asia		
72 China (15 Henan, 13 Shanxi, 10 Hebei, 7 Xinjiang, 6 Hubei, 5 Beijing, 5 Heilongjiang, 3 Ganzu, 3 Shandong, 2 Liaoning, 2 Guizhou, and 1 Shaanxi), 9 Russia, 1 Korea, and 4 Morton Arboretum.	86	Zalapa et al. 2010
Ulmus rubra		
Wisconsin accessions		
UW-Herbarium specimens, Madison, WI. Collected throughout WI between 1890 and 2004. Thirty-eight specimens were collected before 1960 (pre-DED) and 39 between 1961 and 2004 (post-DED)	77	Brunet et al. 2016
Leaves from 100 living trees with 20 trees sampled in each of five wild U. rubra populations in Wisconsin	100	Zalapa et al. 2009, Brunet et al. 2016

Genetic Analysis

Elm accessions were genotyped using 9 to 13 microsatellite loci, previously described in Zalapa et al. (2009, 2010) and Brunet et al. (2016). The impact of DED on the genetic diversity of *U. rubra* was examined by comparing the genetic diversity of herbarium specimens pre- and post-DED. We also examined and compared the levels of genetic diversity of herbarium specimens to that of wild *U. rubra* populations (Brunet et al. 2016). Genetic diversity was quantified by the number of alleles and the level of heterozygosity, both calculated using Gene AlEx 6 (Peakall and Smouse 2006). In addition, we looked for evidence of genetic differentiation among wild *U. rubra* populations. Here, we used analyses of molecular variance (AMOVA) (Excoffier et al. 1992) and calculated pairwise F_{ST} in Gene AlEx to determine how the genetic diversity was distributed within and among populations. We also used a Bayesian clustering method available in the program STRUCTURE (Pritchard et al. 2000) to identify the number of genetic clusters in the data and identify potential genetic discontinuities among groups or populations. Principal coordinate analyses (PCoA) illustrated the distribution of genetic diversity among specific groups (Brunet et al. 2016).

Three different methods were used to identify genetic hybrids in naturalized *U. pumila* populations. First, the nine loci with species-specific alleles permitted direct identification of the genetic hybrids (Zalapa et al. 2010). Second, Bayesian clustering analyses implemented in the program STRUCTURE separated the two pure parental species of *U. pumila* and *U. rubra* from the hybrids (Zalapa et al. 2009, 2010). Finally, principal coordinate analyses (PCoA) helped illustrate the pattern of introgression (Zalapa et al. 2009). Details on the genetic analyses and the specific software employed can be found in Zalapa et al. (2009, 2010) and Brunet et al. (2016).

Table 2.—Genetic diversity of *Ulmus rubra* accessions. These data represent a subsample of the data presented in Table 3 of Brunet et al. 2016. Populations 1-5 are wild populations. N is sample size; H_e is the expected level of heterozygosity.

Accession	N	Number of alleles	Alleles per locus (SE)	H_e (SE)/
Population 1	20	62	4.8 (0.7)	0.51 (0.08)
Population 2	20	62	4.8 (0.8)	0.53 (0.07)
Population 3	20	79	6.1 (1.0)	0.60 (0.08)
Population 4	20	73	5.6 (0.9)	0.52 (0.08)
Population 5	20	75	5.8 (1.0)	0.56 (0.08)
All populations combined	100	106	8.1 (1.4)	0.56 (0.08)
All herbarium specimens	77	108	8.3 (1.4)	0.57 (0.08)
Pre-DED	38	92	7.1 (1.2)	0.57 (0.08)
Post-DED	39	97	7.5 (1.2)	0.58 (0.08)

Results

We obtained no evidence that DED reduced the genetic diversity within *U. rubra*. First, levels of genetic diversity did not change between the groups of pre- and post-DED herbarium specimens (Table 2). Second, levels of genetic diversity were similar between wild and herbarium specimens (Table 2). Finally, the levels of genetic diversity remained high in wild *U. rubra* populations (Table 2). Moreover, we found no evidence of genetic differentiation among wild *U. rubra* populations. The AMOVA results indicated low levels of genetic differentiation, where 96 percent of the genetic variation was observed within relative to 4 percent among populations. In addition, overall FST was low at 0.04 and FST values ranged between 0.018 and 0.055 among pairs of populations. Finally, the STRUCTURE results indicated the presence of three genetic clusters (K=3) in the five wild *U. rubra* populations, with ample mixing of the clusters within each population (See Fig. 2 in Brunet et al. 2016).

Our results support widespread hybridization between *U. pumila* and *U. rubra* in Wisconsin (Zalapa et al., 2009, 2010) and an asymmetric pattern of introgression toward *U. pumila* (Fig. 2 in Zalapa et al. 2009). Such a pattern of introgression indicates most of the first-generation (F1) hybrids backcross to *U. pumila* rather than to *U. rubra*. We also observed greater genetic diversity and new combination of alleles in *U. pumila* populations containing hybrids (Zalapa et al. 2009, 2010). Hybrids were common not only in *U. pumila* populations where we had originally detected morphological hybrids (Zalapa et al. 2009) but also in populations where no hybrids were suspected based on morphological observations (Zalapa et al. 2010). Out of 92 trees sampled from naturalized *U. pumila* populations in Zalapa et al. (2009), 51 trees were determined to be hybrids, and of these, 35 were first-generation hybrids and 16 were backcrosses, 14 back to *U. pumila* and 2 back to *U. rubra* (Table 2 in Zalapa et al. 2009). The hybrids had more alleles and greater heterozygosity than the pure *U. pumila* individuals (hybrids: 7.22 alleles and He = 0.90 vs. *U. pumila*: 2.78 alleles and He = 0.26). Moreover, in the 171 naturalized *U. pumila* trees collected for the Zalapa et al. 2010 study, 44 were identified as genetic hybrids and of these, 30 individuals were identified as F1 hybrids, 7 as first-generation backcross to *U. pumila* (BC1Pu) and 7 as second-generation backcross to *U. pumil*a (BC2Pu). The presence of hybrids always increased the genetic diversity of *U. pumila* populations, both the number of alleles and the level of heterozygosity (Table 2 in Zalapa et al. 2009 and Table 4 in Zalapa et al. 2010).

Discussion

Dutch elm disease did not decrease the level of genetic diversity within *U. rubra* (see Brunet et al. 2016 for details). Both the number of alleles and the level of heterozygosity remained essentially unchanged in the herbarium specimen post-DED collection relative to pre-DED levels, and these levels were also similar to those in present day natural *U. rubra* populations. Moreover, there was little genetic differentiation among natural *U. rubra* populations such that each population harbored similar levels of genetic diversity. A search of the literature on other forest trees indicated that species subjected to a serious disease epidemic experienced little or no impact on levels of genetic diversity (reviewed in Brunet et al. 2016).

The lack of reduction in genetic diversity following the introduction of a serious disease may be due to the fact that these trees are wind-pollinated. Pollen can move long-distances in wind-pollinated trees reducing population differentiation and allowing each population to harbor most of the genetic diversity characteristic of the species (Burczyk et al. 2004, Loveless and Hamrick 1984, Slatkin 1987). Under such conditions, even if many elm populations were decimated by disease, even a single remaining population would maintain most of the genetic diversity characteristic of the species. In general, levels of genetic diversity tend to be high in wind-pollinated trees (Brunet et al. 2016). In addition, the high level of heterozygosity observed within populations suggests that all (or most) of the alleles could be recovered from fewer individuals than if trees were more inbred. The high level of heterozygosity also reflects the fact that little selfing or biparental inbreeding (mating between close relatives) occurs in these elm populations (Brunet et al. 2016, Glémin et al. 2006). Therefore, high gene flow and low levels of inbreeding have allowed for the maintenance of high levels of genetic diversity in *U. rubra* despite the loss of a large number of *U. rubra* trees to DED. Despite long-term exposure of *U. rubra* to DED, the species remains relatively unchanged in its genetic constitution.

Of the six native elm species present in the United States, *U. americana* has the largest historic range, followed closely by *U. rubra;* both occur in the eastern and midwestern regions of the United States (NRCS 2017). The range of rock elm (*U. thomasii*) is more limited, as it is found primarily in the north-central United States and it is less common than *U. americana* or *U. rubra* in their shared range. Winged elm (*U. alata*) and cedar elm (*U. crassifolia)* are found in the southern parts of the United States while the range of September elm (*U. serotina)* is very limited in several southern states (NRCS 2017). Because all North American elm species are wind-pollinated and pollen has the potential to move long distances, we expect little genetic differentiation among populations. For genetic differentiation to occur, populations should be isolated by large geographic distances that limit gene flow, be small in size, or be exposed to different selection regimes (Slatkin 1987). The species where substantial genetic differentiation may be present is *U. serotina*, given its restricted range. Future studies should assess the level of genetic differentiation among populations of this native elm species. Overall, given the distribution of North American elms and their wind-pollination system, and based on genetic structure data previously collected in *U. rubra*, we expect little genetic differentiation among populations of the North American native elm species. We therefore expect much—perhaps most—of the genetic diversity of these native elm species to be maintained within a single or a few populations.

Selfing is low in the native U.S. elm species where it has been examined (Lester 1971). We also expect low levels of biparental inbreeding (matings among close relatives) given the potentially high gene flow via pollen in these highly outcrossed species. Although we do not have data on outcrossing rates for many North American elm species, we expect their populations to be strongly outcrossed and to have high levels of heterozygosity as was observed in *U. rubra* (Zalapa et al. 2010). In highly heterozygous populations, the total number of alleles can be recovered

in fewer individuals than for inbred populations. The number of individuals needed to recover all the alleles would be even less in tetraploid individuals of *U. americana* where each individual can carry up to four distinct alleles (Whittemore and Olsen 2011). Based on the mating system and pollen dispersal mechanism of these North American elm species, and on the results obtained from genetic studies of *U. rubra*, we predict little impact of DED on the level of genetic diversity within, and the pattern of distribution of genetic diversity among, populations of North American elm species. *Ulmus serotina* may be an exception if genetic differentiation is strong and populations are decimated by disease. Future genetic studies should test these predictions.

In previous studies, we detected frequent hybridization between *U. rubra* and *U. pumila* (Zalapa et al. 2009, 2010). Hybridization increased the genetic diversity of *U. pumila* populations and we hypothesized introgressed *U. rubra* genes and new gene combinations following hybridization may have facilitated adaptation of *U. pumila* to a wider range of environmental conditions (Zalapa et al. 2010). We expect *U.rubra-U.pumila* hybrids to be common where both species coexist and their number to increase over time. Moreover, given the observed introgression predominantly back to *U. pumila* (rather than *U. rubra)* the *U. rubra* genes are expected to mostly get eliminated over time in the hybrids (Zalapa et al. 2009). In addition, the directional pattern of introgression toward *U. pumila* suggests that DED resistance is unlikely to get transmitted to *U. rubra* in the wild. Hybridization and introgression back to *U. pumila* can therefore be added to forest fragmentation by humans and DED as factors affecting the long-term conservation of *U. rubra* in the United States.

The recent discovery of diploid *U. americana* trees throughout its range (Whittemore and Olsen 2011) raises the question of whether these diploid trees could also hybridize with *U. pumila*. If the incompatibility between *U. americana* and *U. pumila* resulted simply from differences in ploidy levels, with tetraploid *U. americana* not crossing with diploid *U. pumila*, then the diploid *U. americana* would raise concern about potential hybridization with *U. pumila.* However, other factors besides ploidy levels seem to contribute to the incompatibility observed between these two species (Ager and Guries 1982). The absence of morphological hybrids between *U. pumila* and *U. americana* across the landscape, despite the widespread range of *U. americana*, supports the presence of some incompatibility barrier between these two species (Ager and Guries 1982). This situation differs sharply from the extensive hybridization observed between *U. pumila* and *U. rubra* across the range of *U. rubra.* Based on the available evidence, we predict that *U. americana* is not likely to hybridize and introgress with *U. pumila* and we do not expect hybridization to negatively affect *U. americana*.

We hypothesize that hybridization between *U. pumila* and the other native elm species is more likely because no incompatibility barriers are known between these species. An older study reported mortality of seedlings from crosses between *U. pumila* and *U. thomasii* at the Lake States Forestry Experimental Station in the 1950s (Sholtz 1957). While this was attributed to "hybrid lethality," more research is needed to confirm whether pre- or post-zygotic barriers exist between these two elm species. Hybridization between *U. pumila* and four other native elm species (i.e., *U. thomasii*, *U. alata, U. crassifolia,* and *U. serotina*) may be more difficult to detect across the landscape given the smaller range of these native elm species relative to *U. rubra*. However, any hybridization between *U. pumila* and these wild elm species is likely to have negative effects and could engender conservation concerns (Ellstrand and Schierenbeck 2000, Rieseberg et al. 2003). The threat is greatest for small populations already at risk from other stresses where continued hybridization could lead to genetic assimilation and eventual loss of a native taxon (Prentis et al. 2007, Rhymer and Simberloff 1996). More studies are needed to determine the size and distribution of *U. serotina* populations because, given its most restricted range and the increased stress imposed by DED, this native elm species could be the most threatened by hybridization.

Conclusions

Despite the impact of DED, large numbers of elms survive to reach reproductive maturity, and as a result of their wind-pollination system generating strong gene flow and the fact that they are strongly outcrossed, we expect the disease not to have diminished the genetic diversity of North American native elm species. One exception could be *U. serotina* if strong fragmentation occurs among its scarcer populations. While hybridization is extensive between *U. rubra* and *U. pumila*, we do not expect much hybridization between *U. americana* and *U. pumila* because of incompatibility barriers, in addition to differences in ploidy levels. Hybridization between *U. pumila* and the other four native elm species is more likely because no incompatibility barriers have yet been identified. Although hybrids may be more difficult to detect across the landscape, because these species are not as common as *U. rubra*, hybridization could have negative impact on the genetic integrity of these native elm species. The greatest threat would be for *U. serotina* given its more restricted range. While DED may be unlikely to reduce the genetic diversity of North American native elm species, the planting of more *U. pumila* across the landscape, partly in response to DED, increases the probability of hybridization for five North American native elm species with potential negative impact on the long-term conservation of these native species. Taken together, our work emphasizes the need to understand the long-term impacts of an invasive disease on native species to help determine if any intervention is needed such as a conservation program or an extensive breeding program. If planting an exotic species or hybrids between a native and an exotic species in response to an invasive disease threat, we must understand the potential risks of hybridization with our native species as well as impacts of hybridization on the long-term conservation of our native species. Finally, hybridization could transfer resistance to the native species, and, in addition, because hybridization can increase genetic diversity and create new genotypes, it could facilitate adaptation over time to an invasive threat.

Acknowledgments

Juan E. Zalapa was instrumental in initiating this line of research while a joint postdoctoral associate in our respective laboratories. Comments from anonymous reviewers improved the manuscript. J.B. gratefully acknowledges financial support from the Agricultural Research Service (ARS) of the U.S. Department of Agriculture.

Literature Cited

Ager, A.; Guries, R.P. 1982. **Barriers to interspecific hybridization in *Ulmus americana*.** Euphytica. 31: 909-920.

Bey, C.F. 1990. ***Ulmus americana*.** In: Burns, R.M.; Honkala, B.H., eds. Silvics of North America, vol. 2 hardwoods. Agric. Handb. 654. Washington, DC: U.S. Department of Agriculture, Forest Service: 801-807.

Bouzat, J.L. 2010. **Conservation genetics of population bottlenecks: the role of chance, selection, and history.** Conservation Genetics. 11: 463-478.

Brasier, C.M. 2000. **Intercontinental spread and continuing evolution of the Dutch elm disease pathogens.** In: Dunn, C.P., ed. The elms: breeding, conservation and disease management. Boston, MA: Kluwer Academic Publishers: 61-72.

Brunet, J.; Zalapa, J.E.; Guries R.P. 2016. **Conservation of genetic diversity in slippery elm (*Ulmus rubra*) in Wisconsin despite the devastating impact of Dutch elm disease.** Conservation Genetics. 17: 1001-1010.

Burczyk, J.; DiFazio, S.P.; Adams, W.T. 2004. **Gene flow in forest trees: how far do genes really travel?** Forest Genetics. 11: 179-192.

Ding, J.; Reardon, R.; Wu, Y.; Zheng, H.; Fu, W. 2006. **Biological control of invasive plants through collaboration between China and the United States of America: a perspective.** Biological Invasions. 8: 1439-1450.

Ellstrand, N.C.; **Schierenbeck, K.A. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants?** Proceedings of the National Academy of Sciences. 97(13): 7043-7050.

Excoffier, L.; Smouse, P.E.; Quattro J.M. 1992. **Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data.** Genetics. 131: 479-491.

Glémin, S.; Bazin, E.; Charlesworth, D. 2006. **Impact of mating systems on patterns of sequence polymorphism in flowering plants.** Proceedings of the Royal Society of London B. 276: 3011-3019.

Kartesz J.T. 2015 **North American plant atlas.** Chapel Hill, NC: The Biota of North America Program (BONAP). http://bonap.net/napa.

Leopold, D.J. 1980. **Chinese and Siberian elms.** Journal of Arboriculture. 6: 175-179.

Lester, D.T. 1971. **Self-compatibility and inbreeding depression in American elm.** Forest Science. 17: 321-322.

Loveless, M.D.; Hamrick, J.L. 1984. **Ecological determinants of genetic structure in plant populations.** Annual Review of Ecology and Systematics. 15: 65-95.

Natural Resources Conservation Service (NRCS). 2017. **The PLANTS database.** Greensboro, NC: U.S. Department of Agriculture, Natural Resources Conservation Service. http://plants.usda.gov.

Parker, G.R.; Leopold, D.J. 1983. **Replacement of *Ulmus americana* L. in a mature east-central Indiana Woods.** Bulletin of the Torrey Botanical Club. 110: 482-488.

Peakall, R.; Smouse, P.E. 2006. **GenAlEx 6: Genetic analysis in Excel. Population genetic software for teaching and research.** Molecular Ecology Notes. 6: 288-295.

Prentis, P.J.; White, E.M.; Radford, I.J.; Lowe, A.J.; Clarke, A.R. 2007. **Can hybridization cause local extinction: A case for demographic swamping of the Australian native *Senecio pinnatifolius* by the invasive *Senecio madagascariensis*?** New Phytologist. 176: 902-912.

Pritchard, J.K.; Stephens, M.; Donnelly, P. 2000. **Inference of population structure using multilocus genotype data.** Genetics. 155: 945-959.

Richardson, C.J.; Cares, C.W. 1976. **An analysis of elm (*Ulmus americana*) mortality in a second-growth hardwood forest in southeastern Michigan.** Canadian Journal of Botany. 54: 1120-1125.

Rieseberg, L.H.; Raymond, O.; Rosenthal, D.M.; Lai, Z.; Livingstone, K. [et al.]. 2003. **Major ecological transitions in wild sunflowers facilitated by hybridization.** Science. 301: 1211-1216.

Rhymer, J.M.; Simberloff, D. 1996. **Extinction by hybridization and introgression.** Annual Review of Ecology and Systematics. 27: 83-109.

Sholtz, H.F. 1957. **Silvicultural characteristics of rock elm (*Ulmus thomasii*).** Station Paper 47. St. Paul, MN: U.S. Department of Agriculture, Forest Service, Lake States Forest Experiment Station.16 p.

Slatkin, M. 1987. **Gene flow and the geographic structure of natural populations.** Science. 236: 787-792.

Smalley, E.B.; Guries, R.P. 1993. **Breeding elms for resistance to Dutch elm disease.** Annual Review of Phytopathology. 31: 325-352.

Ware, G.H. 1995. **Little-known elms from China: landscape tree possibilities.** Journal of Arboriculture. 21: 284-288.

Whittemore, A.T.; Olsen R.T. 2011. ***Ulmus americana* (Ulmaceae) is a polyploid complex.** American Journal of Botany. 98: 754-760.

Zalapa, J.E.; Brunet, J.; Guries, R.P. 2009. **Patterns of hybridization and introgression between invasive *Ulmus pumila* (Ulmaceae) and native *U. rubra*.** American Journal of Botany. 96: 1116-1128.

Zalapa, J.E.; Brunet, J.; Guries, R.P. 2010. **The extent of hybridization and its impact on the genetic diversity and population structure of an invasive tree, *Ulmus pumila* (Ulmaceae).** Evolutionary Applications. 3: 157-168.