Research Reports

DNA Fingerprinting of Clones and Hybrids of American Elm and Other Elm Species with AFLP Markers[1]

Margaret R. Pooler and A.M. Townsend[2]
USDA/ARS U.S. National Arboretum
3501 New York Ave. NE, Washington, DC 20002

Abstract

The elms represent a diverse group of widely distributed temperate trees that are valued for forest products as well as landscape plants. Genetic diversity was examined among 43 *Ulmus* accessions, including 19 accessions of American elm and representatives of seven other species. Data from 135 markers from five AFLP primer pairs were used to estimate genetic similarity among accessions and to construct a UPGMA-derived dendrogram. While the species clusters were generally well-resolved, the relationships among clones and hybrids of American elm were less distinct. Our data provides some evidence to support the hybrid origin of two previously unverified *U. parvifolia* x *U. americana* clones, and provides evidence that the new clone N3487 ('Jefferson'), an elm whose origin has been questioned, is an American elm.

Index words: DNA; genetic diversity; Jefferson elm; *Ulmus americana*.

Significance to the Nursery Industry

Elms have been widely used as urban trees because they can withstand numerous environmental stresses, including air pollution, deicing salts, soil compaction, drought, and flooding. In the past several decades, Eurasian hybrids have largely replaced American elms due to the susceptibility of American elm to Dutch elm disease. Recent breeding efforts using American elm and other species have resulted in the release of several disease-tolerant selections that have fueled a renewed interest in elms among nursery professionals and the general public. However, the parentage and authenticity of some hybrid elms have been questioned in the nursery trade. This study provides data on the genetic relationships among clones of American elm and information on identity of other popular accessions. The DNA fingerprinting techniques presented here will be useful for authenticating cultivars, clones, and hybrids as more selections enter commerce.

[1]Received for publication January 10, 2005, in revised form April 25, 2005. This research was supported by a grant from the J. Frank Schmidt Family Charitable Trust. The authors thank A. Berger for skilled technical assistance and R. Guries and S. Wiegrefe for critical review of the manuscript.

[2]Research Geneticists.

Introduction

The elms (*Ulmus* L.) are represented by approximately 35 species distributed throughout the temperate regions of the Northern Hemisphere and into the subtropics of Central America and Southeast Asia, including six species in eastern North America (2, 30). They are valued not only as timber trees, but also as trees for urban and suburban landscapes, as they can withstand numerous environmental stresses, including air pollution, deicing salts, soil compaction, drought, and flooding (22). In the United States, the best-known elm species is the American elm (*Ulmus americana* L.). Known for

The *Journal of Environmental Horticulture* (ISSN 0738-2898) is published quarterly in March, June, September, and December by the Horticultural Research Institute, 1000 Vermont Avenue, NW, Suite 300, Washington, DC 20005. Subscription rate is $65.00 per year for scientists, educators and ANLA members; $95.00 per year for libraries and all others; add $25.00 for international (including Canada and Mexico) orders. Periodical postage paid at Washington, DC, and at additional mailing offices. POSTMASTER: Send address changes to Journal of Environmental Horticulture, 1000 Vermont Avenue, NW, Suite 300, Washington, DC 20005.

its broad, vase-shaped habit and gracefully arching branches, American elm was once the dominant street tree in the U.S. until it was decimated by Dutch elm disease caused by the fungus *Ophiostoma ulmi* (Buisman) C. Nannf. In response to this epidemic, numerous disease-tolerant elm cultivars were generated from breeding programs involving the hybridization of less-susceptible Asiatic and European elms (6, 7, 20, 21, 24, 26), or from extensive testing and selection among clones of American elm (23, 25).

As more American elms succumbed to Dutch elm disease, the few trees that remained healthy were easy to recognize. One clone, N3487 (recently released as 'Jefferson'), was selected from approximately 600 elms that were planted on the National Mall in Washington, DC, in the mid 1930s. This selection is distinguished by its high tolerance to *O. ulmi*, as well as outstanding horticultural characteristics. Although N3487 was planted among American elms, its identity has been questioned due to its disease tolerance, broad U-shaped branch unions, and bark, branch, and leaf characteristics (18).

The successes of the elm breeding programs in the United States have led to a renewed interest in elms, particularly American elms, among nursery professionals and the general public (16, 20, 28). Therefore, an understanding of the genetic resources in elm has become a research priority. This research, conducted in response to the increasing use of and demand for elms in the landscape, had two objectives. The first objective was to determine the extent of genetic diversity and genetic relatedness among clones, cultivars, and hybrids of several popular American elms to assist in cultivar identification and the identification of new sources of tolerance to Dutch elm disease. The second objective was to analyze the utility of Amplified Fragment Length Polymorphism (AFLP) markers in determining the origin or parentage of selected clones, including interspecific hybrids and a clone of unknown origin, N3487.

Materials and Methods

Accessions used. A total of 43 elm accessions representing eight species was used for analysis (Table 1). The choice of species to use was based on prior taxonomic knowledge (30) of those species that would likely prove most informative in the identification of parents of N3487. Leaves were collected from plants in early summer and freeze-dried.

Table 1. Accession information for 43 *Ulmus* clones and species used in a study of DNA fingerprinting using AFLP markers.

Accession name	Species	Location of plant and origin, if known
NA57845	*U. americana* L.	Glenn Dale, MD; selection from Delaware, OH
'Augustine'	*U. americana* L.	Glenn Dale, MD; cultivar selected in Bloomington, IL
R18-2 (NA57846)	*U. americana* L.	Glenn Dale, MD; selection from Cornell University
Crandall (NA58328)	*U. americana* L.	Glenn Dale, MD; selection from Maryland
'Independence'	*U. americana* L.	Glenn Dale, MD; cultivar from Univ. of Wisconsin
'New Harmony'	*U. americana* L.	Glenn Dale, MD; cultivar from U.S. National Arboretum
Russ 3 (NA64255)	*U. americana* L.	Glenn Dale, MD; selection from Michigan
McNorth (NA64254)	*U. americana* L.	Glenn Dale, MD; selection from Delaware, OH
'Princeton'	*U. americana* L.	Glenn Dale, MD; cultivar selected in Princeton, NJ.
Maine	*U. americana* L.	Glenn Dale, MD; selection from Maine
W590 (NA635001)	*U. americana* L.	Glenn Dale, MD; selection from Iowa
GDH (NA64256)	*U. americana* L.	Glenn Dale, MD; selection from Glenn Dale, MD
'Valley Forge'	*U. americana* L.	Glenn Dale, MD; cultivar from U.S. National Arboretum
'Delaware' (NA57839)	*U. americana* L.	Glenn Dale, MD; selection from Delaware, OH
11 (NA57841)	*U. americana* L.	Glenn Dale, MD; selection from Delaware, OH
180 (NA55342)	*U. americana* L.	Glenn Dale, MD; selection from near Findlay, OH
NA68988-181	*U. americana* L.	Glenn Dale, MD; selection from George Ware, Morton Arboretum
NPS3-178	*U. americana* L.	Washington, DC; selection from National Park Service
N3487	*U. americana* L.	Washington, DC; selection from National Park Service
MOR446-48	*U. laevis* Pall.	Morton Arboretum; originally from Arnold Arboretum
MOR27-98-1	*U. laevis* Pall.	Morton Arboretum; original source was seed from Kuibyshev Botanic Garden
MOR27-98-2	*U. laevis* Pall.	Morton Arboretum; original source was seed from Kuibyshev Botanic Garden
'Dynasty'	*U. parvifolia* Jacq.	Washington, DC; cultivar from U.S. National Arboretum
'Ohio'	*U. parvifolia* Jacq.	Glenn Dale, MD; cultivar from U.S. National Arboretum
'Pathfinder'	*U. parvifolia* Jacq.	Glenn Dale, MD; cultivar from U.S. National Arboretum
W2233-1 (NA69142)	*U. parvifolia* x *U. americana* (W182-5)	Glenn Dale, MD; selection from University of Wisconsin - Madison
W2245-5 (NA69143)	*U. parvifolia* x *U. americana* (C4xC18)	Glenn Dale, MD; selection from University of Wisconsin - Madison
W2245-9 (NA69144)	*U. parvifolia* x *U. americana* (C4xC18)	Glenn Dale, MD; selectioin from University of Wisconsin - Madison
NA64253	*U. carpinifolia* Gled.	Glenn Dale, MD; selection from Delaware, OH
MOR1463-24	*U. carpinifolia* var. *suberosa* (Moench) Rehder	Morton Arboretum; original source was seed collected in Hungary
MOR649-62	*U. carpinifolia* 'Pendula'	Morton Arboretum; originally from Arnold Arboretum
PI341750	*U. carpinifolia* 'Hoerscholmiensis'	North Central Regional Plant Introduction (NCRPI) Station, Ames, IA; originally from the Netherlands
'Frontier'	*U. carpinifolia* x *U. parvifolia*	Glenn Dale, MD; cultivar from U.S. National Arboretum
MOR184-66	*U. rubra* Muhl.	Morton Arboretum; originally wild-collected seed from Kentucky
MOR325-70	*U. pumila* L.	Morton Arboretum; originally collected along Du Page River, IL
MOR53-74	*U. pumila* L.	Morton Arboretum; originally from Royal Botanic Gardens (Kew)
PI310432	*U. pumila* L.	NCRPI Station, Ames, IA; originally from USSR.
NA68983-475	*U. bergmanniana* C.K. Schneid.	Glenn Dale, MD; originally from seed from Yunnan Province, China
NA68997-190	*U. bergmanniana* C.K. Schneid.	Glenn Dale, MD; originally from seed from Yunnan Province, China
NA68977-470	*U. bergmanniana* C.K. Schneid.	Glenn Dale, MD; originally from seed from Yunnan Province, China
NA68986	*U. szechuanica* W.P. Fang	Glenn Dale, MD; originally from seed collected in China
NA68987	*U. szechuanica* W.P. Fang	Glenn Dale, MD; originally from seed collected in China
NA68991	*U. szechuanica* W.P. Fang	Glenn Dale, MD; originally from seed collected in China

Table 2. List of AFLP selective primer extensions used and number of polymorphic markers per primer pair detected for 40 *Ulmus* accessions.

*Eco*RI selective primer extension with dye name	*Mse*I selective primer extension	Number of polymorphic markers detected
ACC (FAM)	CTG	28
AAG (FAM)	CTC	18
AAC (NED)	CAC	22
AAC (NED)	CTG	38
AGG (HEX)	CTC	29

DNA extraction and AFLP analysis. DNA was extracted from freeze-dried leaf tissue using a CTAB buffer and the QIAamp Tissue Kit (Qiagen, Inc., Valencia, CA) following previously published methods (8). AFLP analysis was performed as described Vos et al. (27) and Invitrogen Corporation (4), with slight modifications, noted below, to prepare samples for analysis on an ABI 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA). DNA restriction digestion and ligation were carried out sequentially using approximately 0.25 µg of genomic DNA. Restriction digestion was carried out at 37C for three hours, and ligations were carried out at 20C for three hours. Preselective reactions were carried out in 20 µl volumes containing PCR buffer (Invitrogen Corp., Carlsbad, CA), plus 3 mM $MgCl_2$, 100 µM dNTP, 0.125 µM each preselective primer,2.0 U of Taq DNA polymerase (Invitrogen Corp.), and 3 µl diluted restriction/ligation reaction. Completed preselective reactions were diluted 1:50 with TE, and 5 µl were used as template for all selective reactions. Selective amplification reactions were carried out in 20 µl volumes containing the same reagents as for preselective amplification, except that 0.25 µM *Mse*I primer and 0.1 µM *Eco*RI primers (Table 2) were used instead of preselective primers and only 0.5 U Taq DNA polymerase was used. The *Eco*RI selective primers had fluorescently labeled 5' ends and were purchased from the Applied Biosystems Custom Oligonucleotide Synthesis Service (Foster City, CA). Completed selective reactions were analyzed on an ABI310 automated DNA sequencer with POP4 polymer. The sample was prepared by mixing 1.0 µl of selective reaction, 0.07 µl ROX size standard, and 10.93 µl deionized formamide. The experiments were repeated with five of the accessions (from DNA extraction through AFLP analysis) in order to test repeatability of results.

Data analysis. Markers were scored initially with Genotyper® 2.5 software (Applied Biosystems) with visual verification of each peak identified by the software. Markers were assigned a value of '1' (presence of a marker) or '0' (absence of a marker) for each sample. Similarity coefficients between each accession were calculated using the SIMQUAL program in NTSYS-pc, version 1.70 (11), by using the Dice similarity coefficient [2a / (2a + b + c), where a = total number of bands shared by both individuals, b = bands unique to one individual, and c = bands unique to the other individual]. These data were subjected to cluster analysis using the Unweighted Pair Group Method, Arithmetic average (UPGMA) clustering algorithm in the SAHN program of NTSYS to generate a dendrogram. This method generates a phenetic (as opposed to a phylogenetic) tree – that is, one that makes no evolutionary assumptions but rather is based entirely on the molecular genetic distances among accessions as they stand now. A cophenetic matrix was constructed and compared with the similarity matrix by using the MXCOMP program to test the goodness of fit of a cluster (11). Bootstrap analysis of 1000 replications was performed using WinBoot (31) to estimate support for individual clusters (3).

Results and Discussion

Our AFLP analysis using five selective primer pairs (Table 2) generated 135 polymorphic marker bands, ranging in size from roughly 100 to 500 base pairs. Markers were highly reproducible between the five duplicate control samples. A UPGMA-derived dendrogram of all accessions had a cophenetic correlation coefficient (*r*) of 0.902 (Fig. 1) with bootstrap confidence values ranging from 55 to 99% for the species-defined clusters. The bootstrap value is a statistical computation representing the percentage of times that individuals to the right of that fork occurred together in the consensus tree, providing an indication of the degree of support for that group (3, 31). The American elm cluster had a relatively low bootstrap value (58%), but when combined with *U. laevis*, the cluster was more robust (72%, Fig. 1). When the three putative *U. parvifolia* x *U. americana* accessions were omitted from the analysis (see section below), the *U. americana* and *U. laevis* cluster was well resolved with a bootstrap value of 96%, thereby indicating that, at the molecular level, these two species may be less distinct, but together form a larger, more robust cluster. Although the American elms as a whole formed a distinct and well-resolved cluster, the clones within the group had relatively low bootstrap values, indicating that this group is highly variable and that the true genetic relationships among these clones may be more complicated than the clustering indicated in Fig. 1. Prior studies using RAPD markers in American elm (5) also indicate a high degree of polymorphism in this group. The fact that American elm is tetraploid (12, 17) could partially explain the high degree of genetic variability in this group. Despite the fact that the molecular genetic relationships among the American elm clones are not well resolved, the AFLP technique was able to distinguish each genotype, and therefore could be useful in DNA fingerprinting or clonal identity studies of American elm.

In addition to describing the genetic variability present in these clones of American elm, the American elm cluster also provides information about two clones that have been of particular interest to elm researchers. One clone, NA68988-181, which was clearly distinguished from other American elm accessions (Fig. 1), is reported to be a diploid by George Ware of the Morton Arboretum, whereas all other American elms are tetraploid (12, 17). Preliminary (unpublished) data from flow cytometry indicate that the DNA content of this accession is roughly half that of other American elm clones, although the species appears to be highly variable in terms of DNA content also. Further research on the DNA content and associated ploidy of various clones is needed.

Another clone, N3487, was selected by the National Park Service for its tolerance to Dutch elm disease, but was of uncertain species origin. The position of this clone embedded in the American elm cluster in Fig. 1 provides evidence that it is a pure American elm, and not a hybrid. In addition, there were no unique markers present in N3487 that were not found in other American elms. This molecular evidence,

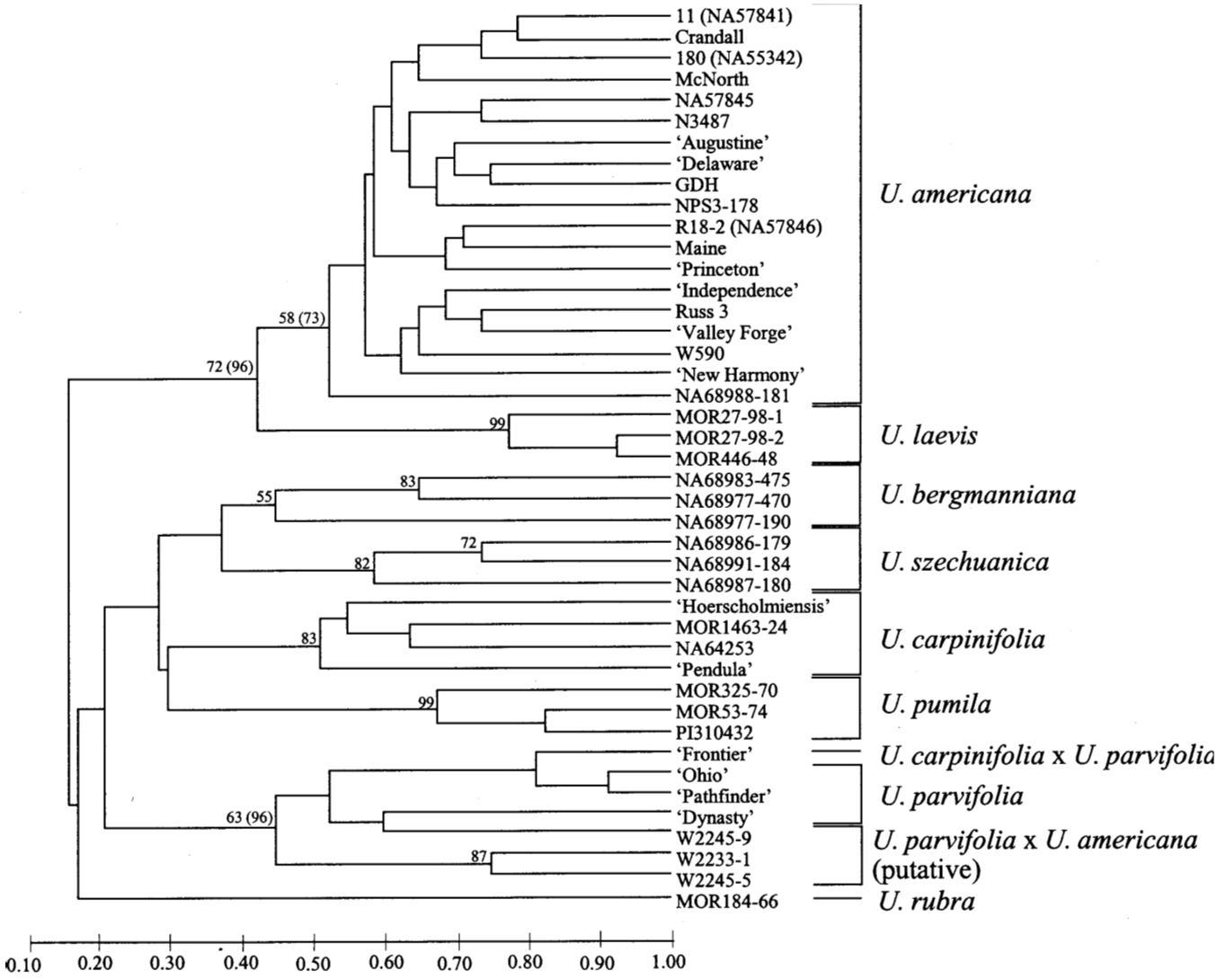

Fig. 1. UPGMA-derived dendrogram of genetic similarity based on the Dice similarity coefficient among elm species based on 135 characters from five AFLP primer pairs. Cophenetic correlation coefficient (*r*) = 0.902 for all accessions, and 0.957 when the three putative Wisconsin hybrids were removed (see text). Bootstrap confidence values for species clusters are indicated to the left of each node and represent the percentage of times that cluster appeared in the consensus tree. Bootstrap values in parentheses are the values when the three hybrid accessions were removed.

combined with the fact that there have been no reports of interspecific hybridizations involving American elm other than with *U. parvifolia*, make it unlikely that this clone is derived from species other than *U. americana*.

The three clones labeled W2233-1, W2245-5, and W2245-9 (Wisconsin clones) are putative hybrids between *U. parvifolia* and *U. americana*, but have never been verified by molecular data (Guries, personal communication). Anecdotal field observations indicate that two of the clones, W2233-1 and W2245-5, have leaf sizes intermediate between *U. parvifolia* and *U. americana*, while the third clone, W2245-9, has smaller leaves typical of *U. parvifolia*, but with a plant architecture atypical for that species. While DNA from the parent trees that contributed to these clones was not analyzed specifically, and the small sample size of *U. parvifolia* accessions limits the genetic assumptions that can be made, comparison of markers in the parental species and the hybrids provides some support for the hybrid origin of these clones. Specifically, there were 16 Wisconsin clone markers that were present in all or some of the American elm clones but in none of the *U. parvifolia*; 13 Wisconsin clone markers that were present in some of the *U. parvifolia* clones but in none of the American elm clones; and eight markers that were unique to the Wisconsin clone group. Clone W2245-9 had a molecular profile that was closer to *U. parvifolia* than the other two Wisconsin clones, although it did have three markers that were present in at least one American elm but in none of the tested *U. parvifolia*. Because *U. parvifolia* is diploid and *U. americana* is tetraploid, the progeny of this cross might be expected to have a closer genetic profile to *U. americana* since two-thirds of the genome would have come from this species. However, based on average similarity values and the derived dendrogram (Fig. 1), the Wisconsin clones are more closely related to *U. parvifolia*. While it is clear that further studies are necessary to provide conclusive evidence, the presence of American elm-specific markers in at

least two of these hybrids lends support to the interspecific hybrid origin of these clones.

Additional support for the hybrid nature of the Wisconsin clones comes from analysis of the bootstrap values in Fig. 1. If these accessions are hybrids, they would be expected to contain markers present in both parental species. Therefore, these individuals would not cluster tightly with either the *U. americana* or the *U. parvifolia* group. The low bootstrap values for the *U. americana* and *U. parvifolia* clusters in the dendrogram in Fig. 1 may be caused by these hybrids 'flip-flopping' between the two clusters. To test the hypothesis that the relatively poorly resolved species clusters for *U. americana* and *U. parvifolia* could be due to common markers from these putative hybrids, the data from the three hybrids was omitted and a new dendrogram was generated. This new dendrogram was identical to the original, but had a higher cophenetic correlation coefficient of 0.957 and significantly higher bootstrap values for the affected clusters (Fig. 1).

The diverse species used in this study were originally selected to maximize the likelihood of finding a species with genetic similarity to, and therefore a possible parent of, N3487. While the data from this study cannot be used to construct phylogenies and was limited in terms of the number of species and samples examined, our data do support the taxonomic classification proposed by Wiegrefe et al. (30) based on chloroplast DNA restriction site variation data in *Ulmus*. The two most closely related species in our study were *U. americana* and *U. laevis*, which occur in Section *Blepharocarpus*. Section *Ulmus* contains, among others, *U. rubra* and *U. carpinifolia*, while *U. parvifolia* falls into its own section, *Microptelea*, but under the same subgenus (*Ulmus*) as Section *Ulmus*.

Although the genus *Ulmus* is well defined in relation to other genera in the *Ulmaceae* (2), delimitation of species is confused by interspecific compatibility within a section and even across sections (1, 9, 13, 14), as well as variable vegetative traits and simple, inconspicuous flowers which can make it difficult to classify species based on morphological traits alone (10). Biochemical markers, including isozymes (1), flavonoids (15, 19), and DNA markers (5, 29, 30), have proven useful to classify species and to determine genetic identity. Our study shows that AFLP markers are another useful tool to distinguish among elm clones and hybrids, and can also help to determine origins of clones and genetic distances among species.

Literature Cited

1. Cogolludo-Agustin, M.A., D. Agundez, and L. Gil. 2000. Identification of native and hybrid elms in Spain using isozyme gene markers. Heredity 85:157–166.

2. Elias, T.S. 1970. The genera of *Ulmaceae* in the southeastern United States. J. Arnold Arboretum 51:18–40.

3. Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

4. Invitrogen Life Technologies. 2003. AFLP Analysis System I/AFLP Starter Primer Kit Instruction Manual, Version B. Invitrogen Corporation, Carlsbad, CA.

5. Kamalay, J.C. and D.W. Carey. 1995. Application of RAPD-PCR markers for identification and genetic analysis of American elm (*Ulmus americana* L.) selections. J. Environ. Hort. 13:155–159.

6. Kuser, J. and N. Polanin. 2001. Comparative test of six DED-tolerant elms: a preliminary report on nursery performance. J. Arboriculture 27:281–282.

7. Mittempergher, L. and N. LaPorta. 1991. Hybridization studies in the Eurasian species of elm (*Ulmus* spp.). Silvae Genetica 40:237–243.

8. Pooler, M.R. 2003. Molecular genetic diversity among 12 clones of *Lagerstroemia fauriei* revealed by AFLP and RAPD markers. HortScience 38:256–259.

9. Richens, R.H. 1980. On fine distinctions in *Ulmus* L. Taxon 29:305–320.

10. Richens, R.H. 1983. Elm. Cambridge University Press: Cambridge, England.

11. Rohlf, F.J. 1992. NTSYS-pc. Numerical taxonomy and multivariate analysis system, version 1.70. Exeter Publishing, Ltd, Setauket, NY.

12. Santamour, F.S. 1969. New chromosome counts in *Ulmus* and *Platanus*. Rhodora 71:544–547.

13. Santamour, F.S. 1970. A natural hybrid between American and Siberian elms. Forest Science 16:149–153.

14. Santamour, F.S. 1971. A triploid elm (*Ulmus pumila* x *U. rubra*) and its aneuploid progeny. Bull. Torrey Bot. Club 98:310–314.

15. Santamour, F.S. 1972. Flavonoid distribution in *Ulmus*. Bull. Torrey Bot. Club 99:217–131.

16. Santamour F.S. and S.E. Bentz. 1995. Updated checklist of elm (*Ulmus*) cultivars for use in North America. J. Arboriculture 21:122–131.

17. Sax, K. 1933. Chromosome numbers in *Ulmus* and related genera. J. Arnold Arboretum 14:82–84.

18. Sherald, J.L., F.S. Santamour, R.K. Hajela, N. Hajela, and M.B Sticklen. 1994. A Dutch elm disease resistant triploid elm. Can. J. For. Res. 24:647–653.

19. Sherman, S.L. and D.E. Giannasi. 1988. Foliar flavonoids of *Ulmus* in Eastern North America. Biochemical Systematics and Ecology 16:51–56.

20. Smalley, E.B. and R.P. Guries. 1993. Breeding elms for resistance to Dutch elm disease. Annu. Rev. Phytopathol. 31:325–352.

21. Townsend, A.M. 1975. Crossability patterns and morphological variation among elm species and hybrids. Silvae Genetica 24:18–23.

22. Townsend, A.M. 2000. USDA genetic research on elms. p. 271–278 *In*: C.P. Dunn (Ed.). The Elms: Breeding, Conservation, and Disease Management. Kluwer Academic Publishers, Boston, MA.

23. Townsend, A.M., S.E. Bentz, and G.R. Johnson. 1995. Variation in response of selected American elm clones to *Ophiostoma ulmi*. J. Environ. Hort. 13:126–128.

24. Townsend, A.M. and L.W. Douglass. 1996. Variation in growth and response to *Ophiostoma ulmi* among advanced-generation progenies and clones of elms. J. Environ. Hort. 14:150–154.

25. Townsend, A.M. and L.W. Douglass. 2001. Variation among American elm clones in long-term dieback, growth, and survival following *Ophiostoma* inoculation. J. Environ. Hort 19:100–103.

26. Townsend, A.M. and L.W. Douglass. 2004. Evaluation of elm clones for tolerance to Dutch elm disease. J. Arboriculture 30:175–184.

27. Vos, P, R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407–4414.

28. Warren, K. 2000. The return of the elm: The status of elms in the nursery industry in 2000. Proceedings of the Eleventh Conference of the Metropolitan Tree Improvement Alliance. Gresham, OR, Aug. 23–24, 2000. http://www.ces.ncsu.edu/fletcher/programs/nursery/metria/metria11/

29. Whiteley, R.E., S. Black-Samuelsson, and D. Clapham. 2003. Development of microsatellite markers for the European white elm (*Ulmus laevis* Pall.) and cross-species amplification within the genus *Ulmus*. Molecular Ecology Notes 3:598–600.

30. Wiegrefe, S.J., K.J. Sytsma, and R.P. Guries. 1994. Phylogeny of elms (*Ulmus*, Ulmaceae): Molecular evidence for a sectional classification. Systematic Botany 19:590–612.

31. Yap I.V. and R.J. Nelson. 1996. WinBoot: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. International Rice Research Institute, Manila, Philippines.