Evaluation of 19 American Elm Clones for Tolerance to Dutch Elm Disease[1]

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Abstract

Rooted stem cuttings of 19 American elm (*Ulmus americana* L.) cultivars and selections, and rooted cuttings of two non-American elm selections, *U. carpinifolia* Gleditsch 51 and 970 (*U. glabra* Huds. x (*U. wallichiana* Planch. x *U. carpinifolia*)), along with a group of American elm seedlings, were planted in a randomized block design. When the trees were nine years old, they were inoculated with a mixed spore suspension of *Ophiostoma ulmi* (Buisman) C. Nannf. and *Ophiostoma novo-ulmi* Brasier, the causal fungi for Dutch elm disease (DED). Analyses of variance showed highly significant variation among clones in foliar symptoms 4 weeks after inoculation and in crown dieback one and two years after inoculation. After two years, 13 of the American clones showed significantly less dieback than the American elm seedlings, and 18 American clones showed significantly less injury than a randomly chosen, unselected American elm clone, 57845. The American clones with the most DED-tolerance were cultivars 'Valley Forge,' 'Princeton,' 'Delaware,' and 'New Harmony,' and selections N3487, R18-2, 290, 190, and GDH. The non-American selections 51 and 970 also exhibited high levels of disease tolerance. Most susceptible were American clones 57845, 'Augustine,' Crandall, W590, and the American elm seedlings. The most disease-tolerant American elm selections identified in this study are being evaluated further for possible naming and release to the nursery industry.

Index words: *Ulmus*, *Ulmus americana*, Dutch elm disease, disease tolerance, disease susceptibility, plant pathology.

Significance to the Nursery Industry

Dutch elm disease (DED), caused by the fungi *Ophiostoma ulmi* and *Ophiostoma novo-ulmi*, continues to threaten newly planted and established trees of American elm (*Ulmus americana* L.). In this study, we inoculated 526 nine-year-old trees representing 19 American elm selections and named cultivars, American elm seedlings, and two non-American elm selections, to determine their relative DED tolerance. Two years after inoculation, the American elm clones varied from 0 to 42% in average crown dieback. Several cultivars and selections showed superior disease tolerance. Results will be of interest to those nurserymen interested in evaluating, growing, and selling a diverse selection of American elm clones with high levels of tolerance to DED.

Introduction

Although elms (*Ulmus*) are threatened by Dutch elm disease (DED), they are well known for their exceptional vigor and tolerance to many environmental stresses (3, 15, 19). Breeding and selection of European and Asiatic elms for Dutch elm disease tolerance have been successfully carried out at the Morton Arboretum, Lisle, IL (22), the University of Wisconsin, Madison, WI (5, 12), the USDA-ARS Nursery Crops Research Laboratory in Delaware, OH (9, 14, 20), and the USDA-ARS-U.S. National Arboretum in Washington, DC, and Glenn Dale, MD (6, 15, 16, 17, 18, 19), with many disease-tolerant non-American elm cultivars developed and released. The nursery industry has been quite progressive in propagating, growing, and marketing these new elm cultivars (23).

The American elm (*Ulmus americana* L.) at one time was the dominant landscape tree species in eastern North America (3). It was prized for its adaptability, vigor, growth rate, ease of transplanting, and tall, vase-shaped crown which provided a beautiful arching canopy along many American streets. Much emphasis in the last 30 years has been given to the improvement of this species (3, 4, 5, 10, 11, 12, 15, 16, 18, 19, 20). The U.S. Department of Agriculture (USDA) screened American elm seedlings for DED-tolerance starting in Morristown, NJ, in 1937 (12, 13, 24). This work was transferred in 1946 to Columbus, OH, and then in 1961, to Delaware, OH (19, 20, 24). American elm breeding and selection research resumed in 1970 at the USDA Shade Tree and Ornamental Plants Laboratory (later named the Nursery Crops Research Laboratory), Delaware, OH, and continued until 1984, when the program was transferred to the U.S. National Arboretum at Glenn Dale, MD (15). A well-replicated study conducted during the 1990s at Glenn Dale identified several USDA American elm selections with highly significant levels of DED tolerance (16, 18). The two most disease-tolerant selections subsequently were named 'Valley Forge' and 'New Harmony' and released to the nursery industry (15). The release of these trees has generated much interest and enthusiasm for the return of this species to the American landscape (3, 15).

Because of the renewed interest in American elms, we saw a need for a large-scale, comparative evaluation of the disease tolerance of American elm clones, including six named cultivars, two selections from our breeding program (15), 10 selections that had survived in various locations where DED was prevalent, and one randomly selected American elm clone, which was used as a control. In addition to these 19 clones, we also included one group of American elm seedlings and two non-American selections. The purpose of the

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study was to identify the most DED-tolerant American elm cultivars and selections.

Materials and Methods

The six cultivars used were 'Augustine,' 'Delaware,' 'Independence,' 'New Harmony,' 'Princeton,' and 'Valley Forge.' 'Augustine' (formerly 'Augustine Ascending') is a fastigiate tree selected in 1927 in Bloomington, IL (7). 'Delaware' originally was selected by USDA researcher Curtis May as tree number 218, one of the two most DED-tolerant trees from a population of about 35,000 seedlings inoculated in Morristown, NJ (7, 12, 13). Follow-up research on this tree first at the USDA-ARS Nursery Crops Research Laboratory, Delaware, OH, and later at the U.S. National Arboretum, showed this clone to have a significant level of tolerance to DED (16, 18, 20). Although this tree was subsequently propagated and distributed for evaluation as Delaware #2 but never officially released, it was named 'Delaware' in a checklist of elm cultivars published in 1995 (7). 'Independence' was developed by Eugene B. Smalley and Donald T. Lester at the University of Wisconsin as one of the six clones that make up the 'American Liberty' elm (5, 12). 'Princeton' was originally selected in 1922 by Princeton Nurseries, Princeton, NJ, for its vase-shaped crown and vigorous growth (7). 'New Harmony' and 'Valley Forge' are DED-tolerant cultivars released by the U.S. National Arboretum to wholesale nurserymen in 1995 (15).

Selections 190 (NA 63507) and 290 (NA 63508) resulted from controlled crosses made in 1980 between American 3 (now 'Valley Forge') and Delaware #2, now considered as 'Delaware.' These two selections, 190 and 290, showed the best disease tolerance, after inoculation in 1984, of seedlings derived from this cross (15). Clone R18-2 (NA 57846) was one of 11 survivors out of 21,000 American elm seedlings screened for DED tolerance by Cornell University and the Boyce Thompson Institute for Plant Research from 1933 to 1947 at Yonkers, NY, and from 1947 to 1965 at Ithaca, NY (11, 12). Selection N3487 (NA 62001) is one of two clones selected and evaluated for disease tolerance by H.V. Wester and J.L. Sherald of the National Park Service (10). The following selections are survivors of disease epiphytotics in various locations: GDH (Glenn Dale Hospital) (NA 64256), Glenn Dale, MD; 180 (NA 55342), from near Findlay, OH; McNorth (NA 64254) and 11 (NA 57841), from Delaware, OH; W590 (NA 63501), selected by Donald Willeke in Aplington, IA; Crandall (NA 58328), from Annapolis, MD; Russ 3 (NA 64255), from Michigan State University's Fred Russ Forest Experiment Station, Decatur, MI; and Maine (NA 63495), from Yarmouth, ME. A group of American elm seedlings grown from seed collected in Ohio; a randomly selected American elm clone, 57845 (NA 57845); and two non-American elm clones, selection 51 (NA 64253) (*Ulmus carpinifolia* Gleditsch), and selection 970 (NA 55394) (*Ulmus glabra* Huds.) x (*U. wallichiana* Planch. x *U. carpinifolia*)) served as control trees.

Trees were planted into a field plot at Glenn Dale, MD, in April 1993, in a randomized block design with 7 blocks and, when available, 4 trees per block per clone in each block. Heights of trees were measured in February of 1996 and 2002, and caliper size 6 in above the root collar was measured in February 2002. Relative degree of growth initiation, or 'flushing,' was estimated for each tree by one observer (A.M.T.) on May 10, 2002, and was based on a visual index of leaf development from 0 = no budbreak or leaf development to 100 = leaves fully expanded.

Inoculations were made on May 21, 2002, into a 2.4 mm (0.1 in) hole in the bottom one third of the main trunk of each tree with an aqueous spore suspension containing 3.72×10^6 spores/ml of a mixture of two strains of *Ophiostoma novo-ulmi* Brasier (1, 2, 9) and two strains of *Ophiostoma ulmi* (Buisman) C. Nannf. (2) in a 2:1 ratio of *O. novo-ulmi* to *O. ulmi* spores.

The percentage of the crown showing wilting or death of foliage was estimated on all trees 4 weeks after inoculation. The percentage of the crown showing dieback (lack of foliage) was estimated one and two years after inoculation. For all data collected on foliar symptoms and crown dieback, estimates were made by two observers (A.M.T. and S.E.B.) examining a given tree at the same time, after which a consensus score was recorded.

Data were analyzed using the mixed procedure of the Statistical Analysis System (SAS), Version 8.2 (8). Foliar symptoms and crown dieback data were square root-transformed for statistical analysis; whereas height, caliper, and flushing data were not transformed. The fixed portion of the model included the effect of clone, while the random portion of the model included block, block by clone (experimental variance), and the residual variance (tree variance). Features of the mixed procedure were used to examine the possibility of variance heterogeneity, and when heterogeneity was indicated, the residual variance was partitioned to account for differences in the residual variance of different treatments. The Corrected Akaike's Information Criterion for goodness-of-fit was used to select an appropriate random effects model. Differences in least-square means between clones for each dependent variable were tested by the LSD procedure at the 0.05 significance level.

For attempts at fungal recovery, two samples from one tree of the American elm seedlings, and two samples from one tree of each of the following clones, were collected and plated out on potato dextrose agar (PDA) on June 11 and 12, 2002: 'Augustine,' 'Delaware,' 'Independence,' 'New Harmony,' Crandall, GDH, McNorth, Russ 3, Maine, W590, 290, 57845, and 51. These same trees were sampled again on June 11 and 12, 2003, along with samples of 'Princeton,' 'Valley Forge,' N3487, R18-2, 11, 180, and 190.

Results and Discussion

Analyses of variance showed highly significant ($P < 0.0001$) differences among American elm clones in symptom expression, including foliar symptoms four weeks after inoculation, and crown dieback one and two years post-inoculation (Table 1). Foliar symptoms were greatest for Russ 3, 'Augustine,' American elm seedlings, and Maine, and least for 'Valley Forge,' N3487, R18-2, 190, 290, 'Princeton,' 180, 'Delaware,' 11, and 57845, with these last 10 clones showing only 0 to 3% foliar symptoms at four weeks (Table 1).

Crown dieback generally is a more important measure of disease tolerance and susceptibility, and wide variation among clones was apparent in this trait as well. Clones varied from 0 to 58% in dieback, a range sufficient to identify cultivars or selections with good disease tolerance. Clones showing the least one-year dieback were 'Valley Forge,' N3487, R18-2, 'Princeton,' and 290 (Table 1). Those clones showing 8% or less two-year dieback included 'Valley Forge,' N3487, 'Princeton,' R18-2, 290, 190, 'Delaware,' GDH, 'New Har-

Table 1. Characteristics and disease responses of elm clones inoculated with *Ophiostoma*.

Cultivar or selection	No. of trees	Height (cm) Feb. 1996	Height (cm) Feb. 2002	Caliper (mm) Feb. 2002	Index of Flushing (0–100) May 2002	Foliar Symptoms (%) 4 weeks	Crown dieback (%) one year	Crown dieback (%) two years
American elm cultivars:								
'Augustine'	27	87cd[z]	489b–f	62cd	43b	33b	36bc	26b
'Delaware'	28	81e–i	397gh	44fgh	66ef	1f	10g	5i
'Independence'	28	85defg	529bc	72bc	89a	16d	25de	13e
'New Harmony'	27	82d–h	402gh	42f–j	43g	4ef	17ef	8gh
'Princeton'	27	86de	549b	65c	86a	0f	3h	0k
'Valley Forge'	28	83defg	465cdef	49defg	86ab	0f	0j	0k
American elm selections:								
N3487 (NA 62001)	26	98bc	443c–h	58cde	75bcde	0f	1ij	0k
R18-2 (NA 57846)	28	79f–j	478cdef	48def	78abcd	0f	2hi	1k
290 (NA 63508)	14	63l	295j	28k	80abcd	0f	3hi	1jk
190 (NA 63507)	8	66kl	377hi	38g–k	74cdef	0f	10fg	3ij
GDH (NA 64256)	24	70jkl	479cdef	43fghi	77abcd	6e	11g	6hi
180 (NA 55342)	16	77g–k	302j	33jk	83abc	0f	18ef	8fghi
11 (NA 57841)	26	83d–h	430fgh	44fgh	77abcd	3e	23de	15de
McNorth (NA 64254)	28	85defg	497bcd	59cd	81abc	6e	28cd	12ef
Maine (NA 63495)	19	74ijk	339ij	35hijk	41g	19cd	30bcd	16cde
W590 (NA 63501)	14	79c–l	440d–h	44e–j	75b–f	5e	33bcd	16cde
Crandall (NA 58328)	24	63l	384ghi	34ijk	68def	14d	37bc	20bcd
Russ 3 (NA64255)	27	73jk	437efg	45fgh	63f	45a	39b	11efg
57845 (NA57845)	28	86cef	410fgh	51def	89a	3ef	58a	42a
Amer. elm seedlings	25	102b	658a	97a	83abc	25bc	31bcd	21bc
Non-American elm selections:								
970 (NA 55394)	26	75hijk	377hi	34jk	75b–f	0f	0j	0k
51 (NA 64253)	28	152a	486cde	86ab	86ab	1f	0j	0k
Overall mean		83	439	50	73	8	19	10
Sig. clone ($P<$)		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

[z]Means within a column with any identical letters are not significantly different by LSD, 0.05 level. A sequence of 5 or more letters is abbreviated by the first letter followed by the last letter, e.g., f–j = fghij.

mony,' and 180. For all clones inoculated, average dieback was less (10%) after two years than after one year (19%); for many clones some recovery from DED occurred after the first year. Across both years, the most susceptible biotypes appeared to be 57845, 'Augustine,' Crandall, W590, and American elm seedlings (Table 1). The two non-American elm clones 51 and 970 showed no dieback one and two years after inoculation.

The *Ophiostom*a fungus was successfully recovered in June 2002 from all trees sampled. Recovery was successful a year later (2003) for all trees sampled, except for 'Valley Forge,' from which fungal recovery was unsuccessful.

Clones varied in height and caliper in February 2002, three months before fungal inoculations were made, but pre-inoculation size did not appear to be related to subsequent foliar symptoms or crown dieback (Table 1). Highly significant differences in degree of growth initiation occurred, but also were not consistently associated with disease symptomatology, with early flushing clones showing high (e.g., 57845, American elm seedlings) and low (e.g., 'Valley Forge' and 'Princeton') disease symptoms, similarly with late flushing clones (Table 1).

It is apparent that for many of the American elm clones evaluated in this study, selection for DED tolerance has been effective. This level of genetic differentiation is especially seen in the averages for two-year dieback, where 13 American clones showed significantly less dieback than the American elm seedlings, and 18 clones showed significantly less injury than a randomly-chosen American elm clone, 57845.

In a previous inoculation study (18), 'Valley Forge,' 'Princeton,' 'New Harmony,' R18-2, and 'Delaware' showed significantly less dieback and greater survival than 57845. The present study not only confirmed the superiority of these five clones, but identified other clones such as N3487, 290, 190, GDH, and possibly 180 as showing promising levels of DED tolerance (Table 1). The remaining selections and cultivars appeared too susceptible to consider using as parents for breeding purposes, or for direct use as landscape trees. Of these, 'Augustine,' Crandall, and W590 were most susceptible to DED.

Seven of the same clones ('Valley Forge,' R18-2, 'Princeton,' 'Delaware,' 'New Harmony,' 11, 57845) were included in both a previous (16, 18) and the present inoculation study. For these clones, crown dieback in the present study was not as great as in the previous study, possibly because of the older age (9 years vs. 3 years) and larger size of the trees used in this study. Results from inoculating these larger trees may be a more realistic measure of how well these American elm clones would perform in landscape situations.

This large-scale study enabled us to identify the best American elm cultivars and selections for tolerance to DED. This information can be used for choosing specific trees for nursery production, landscaping, and tree breeding; for possible

naming and release of the best clones to the nursery industry; and for ultimately increasing the genetic diversity of American elms planted in the future.

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