Variation among American Elm Clones in Long-term Dieback, Growth, and Survival following *Ophiostoma* Inoculation[1]

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Abstract

Rooted stem cuttings of eight American elm (*Ulmus americana* L.) cultivars or selections, in addition to rooted stem cuttings of two non-American elm cultivars, 'Prospector' (*U. wilsoniana* Schneid.) and 'Frontier' (*U. carpinifolia* Gleditsch x *U. parvifolia* Jacq.), all planted in a randomized block, split-plot design, were inoculated on either one of two dates in May 1992 with a mixed spore suspension of *Ophiostoma ulmi* (Buisman) C. Nannf. and *Ophiostoma novo-ulmi* Brasier, the causal fungi for Dutch elm disease (DED). Crown dieback and survival were recorded once yearly for 7 years following inoculations, and height growth was measured after the sixth growing season. Analyses of variance and regression showed significant differences in disease severity among all clones tested. Among the American elms, crown dieback and mortality over time were least for 'Valley Forge,' 'Princeton,' and 'New Harmony;' intermediate for 'Delaware' and selection R18-2; and greatest for selections 57845 and 11 and the cultivar American Liberty. Dieback and survival of 'Prospector' and 'Frontier,' the non-American elms, were comparable to that of the more disease-tolerant American elm clones. Height growth on surviving American elms for six years after inoculation was greatest for 'Valley Forge' and 'Princeton' and least for 57845, 11, and 'American Liberty.' Results demonstrate the ability of certain American elm cultivars and selections to respond and then recover from heavy doses of the two fungi which cause DED.

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

Significance to the Nursery Industry

A study was conducted to determine long-term response of eight American elm cultivars or selections to inoculation with the fungi that cause Dutch elm disease (DED). Several susceptible cultivars or selections showed high levels of crown dieback and mortality throughout the 7-year period following inoculation. Other disease-tolerant cultivars and selections showed the ability to recover from DED, with lower degrees of mortality, crown dieback, and growth reduction compared to the susceptible clones. Results will enable selection of the most disease-tolerant, resilient American elm cultivars for nursery production.

Introduction

Elms (*Ulmus* sp.) generally make superior urban trees because they are able to withstand the many kinds of environmental stresses associated with city conditions. Their use, however, is hindered by the presence of Dutch elm disease (DED), which is caused by the less aggressive (8) or more aggressive strain of *Ophiostoma ulmi* (Buisman) C. Nannf. The more aggressive strain is now widely accepted as a separate species, *Ophiostoma novo-ulmi* Brasier (2, 3, 12). Fortunately, many breeding programs have been successful in increasing DED tolerance to acceptable levels, especially in the diploid elms, and many new elm cultivars have been released (4, 5, 6, 9, 10, 13, 14, 16, 17, 18, 21).

Emphasis in several breeding programs in recent years has been given to increasing DED tolerance in the tetraploid American elm, *Ulmus americana* L. (4, 9, 10, 14, 15, 16). The U.S. Department of Agriculture (USDA) began screening American elms for disease tolerance in 1937 (11, 16), but that effort was interrupted by World War II. Screening of American elms resumed in 1970 at Delaware, OH, and continued until 1984, when the program was transferred to Glenn Dale, MD (16, 17). A recent, well-replicated study at Glenn Dale showed several USDA American elm selections with highly significant levels of DED tolerance (15). The two most tolerant subsequently were named 'Valley Forge' and 'New Harmony' and released to the nursery industry (14).

This paper reports on a follow-up study to the one reported earlier on American elm (15). The present investigation examined variability among eight American elm cultivars or selections in their relative rates of survival and recovery for 7 years after inoculation with *O. ulmi* and *O. novo-ulmi.*

Materials and Methods

In 1989 and 1990, rooted stem cuttings representing eight American elm cultivars or selections were planted along with rooted stem cuttings of two non-American cultivars, 'Prospector' (*U. wilsoniana* Schneid.) (19), and 'Frontier' (*Ulmus carpinifolia* Gleditsch x *U. parvifolia* Jacq.) (20), into a field plot at Glenn Dale, MD. 'Frontier' and 'Prospector,' which are tolerant of DED, served as controls for comparison with the American clones. Table 1 lists the cultivars and selections planted. It should be noted that selection R18–2 is a clone selected by Cornell University for DED tolerance; USDA selection 11 had expressed field tolerance in Ohio (15); and selection 57845 was previously unselected and served as a susceptible control. The statistical design and inoculation techniques have been described previously (15), but are presented here briefly.

Trees were planted in a randomized block, split-plot design with seven blocks, and depending on the number of trees available, four trees per clone in each whole block. Half of

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Table 1. Long-term crown dieback of elm clones after *Ophiostoma* inoculation.

Cultivar or selection[z]	No. of trees	Height (cm) March 1992	Crown dieback (%) Years after inoculation 1	2	3	4	5	6	7
'Valley Forge'	28	337bc[y]	13d	9d	6e	5de	5de	4d	4cd
'Princeton'	16	186de	52c	29cd	20de	15cde	14de	12cd	10cd
'New Harmony'	28	202d	63c	41c	31cd	23c	20cd	18cd	16bcd
Amer. R18–2	25	194de	60c	64b	59b	51b	49b	47b	43b
'Delaware'	20	184de	79b	55bc	52bc	45b	41bc	40bc	41b
Amer. 57845	28	394ab	86ab	93a	86a	85a	84a	81a	81a
Amer. 11	26	128e	91a	91a	86a	81a	81a	81a	81a
'American Liberty'	28	396ab	93a	94a	91a	90a	90a	89a	89a
'Prospector'	28	293c	7d	0e	0e	0e	0e	0d	0d
'Frontier'	28	444a	12d	12de	12de	15cd	15d	19cd	22bc
Overall mean		276	55	49	44	41	40	39	39
Sig. clone ($P <$)		0.0004	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

[z]Amer. = American elm selection.

[y]Means within a column with any identical letters are not significantly different by LSD, 0.05 level of significance.

the trees in each whole plot were inoculated on May 18, 1992, and the other half (sub-plot treatment) were inoculated on May 27, 1992. The inoculation procedure involved injecting a mixture of spores of *O. novo-ulmi* and *O. ulmi* into the basal trunk of each tree (15).

The percentage of the crown showing dieback (lack of foliage) was estimated yearly from 1 to 7 years after inoculation. Height of all trees was recorded on dormant trees in March 1992 (before inoculation), and height of surviving trees was measured again in September 1997 to the highest leaf on the tree. Survival of all trees was recorded yearly from 1993 through 1999; trees that died back to below 1 m (1.1 yd) in height were considered dead, even if stump sprouts eventually arose from the base of the tree. Analyses of variance were carried out using the mixed model procedure of the Statistical Analysis System (SAS) (7).

Results and Discussion

Analyses of variance showed cultivar or selection as a highly significant source of variation in all years for crown dieback ($P < 0.0001$) and survival ($P < 0.0001$ to 0.0003). Effects due to inoculation date on dieback and survival were nonsignificant, except for dieback after 1 year ($P<0.0032$), and interaction between inoculation date and clone also was nonsignificant. Analyses of variance of height growth for the 6 years following inoculation showed significant effects from clone ($P < 0.0208$), from inoculation date ($P < 0.0042$), and from interaction between clone and inoculation date ($P < 0.0291$).

American elm clones differed significantly in their 7-year response to *Ophiostoma* inoculation. Crown dieback was greatest for 57845, 11, and 'American Liberty,' and least for 'Valley Forge,' 'Princeton,' and 'New Harmony,' with the latter three showing decreasing dieback throughout the 7 years following inoculation (Table 1). 'Delaware' and R18–2 generally were intermediate in response, with some decreased dieback over time. Dieback of 'Prospector' and 'Frontier,' the non-American diploid elms, was statistically equal 7 years after inoculation to that of 'Valley Forge,' 'Princeton,' and 'New Harmony' (Table 1). Regression analyses also con-

Table 2. Long-term survival of elm clones after *Ophiostoma* inoculation.

Cultivar or selection[z]	Survival (%) Years after inoculation 1	2	3	4	5	6	7
'Valley Forge'	100a[y]	100a	96ab	96a	96a	96ab	96a
'Princeton'	96ab	96ab	96ab	96a	96a	96ab	96a
'New Harmony'	89ab	86ab	86abc	86ab	86ab	86abc	86ab
'Amer. R18–2'	76b	68b	60cd	60c	60c	60cd	60bcd
'Delaware'	80ab	75b	70bc	70bc	70bc	70bc	65bc
Amer. 57845	50c	36c	36de	32d	32d	32de	32cde
Amer. 11	31cd	31c	31de	31d	31d	31de	31de
'American Liberty'	25d	21c	21e	18d	18d	18e	18e
'Prospector'	100a	100a	100a	100a	100a	100a	100a
'Frontier'	96ab	93ab	89abc	89ab	89ab	86abc	82ab
Overall mean	74	71	69	68	68	67	67
Sig. clone ($P <$)	0.0001	0.0002	0.0003	0.0001	0.0001	0.0002	0.0003

[z]Amer. = American elm selection.

[y]Means within a column with any identical letters are not significantly different by LSD, 0.05 level of significance.

Table 3. Six-year height growth of surviving trees after *Ophiostoma* inoculation.

Cultivar or selection[z]	Height growth (cm) for six growing seasons after inoculation		
	Trees inoculated May 18, 1992	Trees inoculated May 27, 1992	All trees combined
'Valley Forge'	218ab[y]	262ab	240ab
'Princeton'	148abc	338ab	243ab
'New Harmony'	105bc	164bc	135bcd
Amer. R18–2	153abc	146bcd	150bcd
'Delaware'	54bc	64cd	59cde
Amer. 57845	–108cd[x]	–72d	–90e
Amer. 11	61bc	–15cd	23de
'Amer. Liberty'	–323d	188abc	–67de
'Prospector'	307a	382a	344a
'Frontier'	175abc	107bcd	141bcd
Overall mean	79	156	118
Sig. clone ($P <$)	0.0444	0.0288	0.0208

[z]Amer. = American elm selection.

[y]Means within a column with any identical letters are not significantly different by LSD, 0.05 level of significance.

[x]Where growth is negative, average height of trees 6 years after inoculation was less than average preinoculation height. This 'negative' growth was due to extensive crown dieback sustained after inoculation, followed by an insufficient amount of compensating regrowth.

firmed significant clonal effects on dieback over time, with linear, quadratic, and cubic regression coefficients showing significant differences among clones.

Differences in survival rates among the American elm clones were pronounced 1 year after inoculation, and varied from 100% for 'Valley Forge' to 25% for 'American Liberty' (Table 2). Significant variability continued for 6 more years. The cultivars 'Valley Forge,' 'Princeton,' and 'New Harmony' showed the best American elm survival seven years after inoculation, equal statistically to that of 'Frontier' and 'Prospector'; whereas 57845, 11, and 'American Liberty' showed the poorest survival rate (Table 2). Survival of 'Delaware' and R18–2 after 7 years was intermediate, but not statistically different from that of the control clone, 57845.

Average height growth on trees inoculated on the earlier date was less than that for trees inoculated on the later date (Table 3). With the American elms, growth on surviving trees after inoculation was greatest on 'Valley Forge' and 'Princeton,' and least on 57845, 'American Liberty,' and 11 (Table 3). When data from both inoculation dates were combined, average height of 57845 and 'American Liberty' 6 years after inoculation was less than preinoculation height (Table 3). This 'negative' growth most likely was due to severe crown dieback sustained by these two biotypes after inoculation. Growth on inoculated 'Prospector' and 'Frontier' was comparable to that of the faster-growing American elms.

Correlations between pre-inoculation heights and subsequent crown dieback are shown by selection or cultivar in Table 4. There were significant correlations for susceptible clones such as 'American Liberty,' 11, and 57845, as well as for tolerant clones such as 'Princeton.' Taller rooted stem cuttings of 'American Liberty,' 'Princeton,' and 57845 were associated with greater crown dieback after inoculation than shorter trees (Table 4). In contrast, taller rooted stem cuttings of selection 11 tended to show less dieback (Table 4). The biological basis for these clonal differences warrants further investigation. Except for year one ($r = –0.18$; $P < 0.01$), correlations between pre-inoculation height and subsequent dieback, for individual tree data across all clones, were nonsignificant.

Most elm breeding programs have identified disease-tolerant selections based principally on their short-term response to *Ophiostoma* infection (1, 4, 5, 6, 10, 12, 17, 21). The wide degree of differences among American elm clones in their ability to recover from fungal inoculation in this study demonstrates the importance of assessing disease responses over a long period of time. Rooted stem cuttings of particular cultivars such as 'Valley Forge,' 'Princeton,' and 'New Harmony' showed appreciable dieback 1 year after inoculation, but in succeeding years these trees were able to maintain a high survival and reasonable growth rate, equivalent to trees representing the two well-known, disease tolerant, non-American elm cultivars 'Prospector' and 'Frontier.' Rooted stem cuttings of other, more disease-susceptible cultivars or se-

Table 4. Individual tree correlations by clone between preinoculation height and crown dieback. Trees were subjected to *Ophiostoma* inoculation in May 1992.

Cultivar or selection[z]	Avg height (cm) March 1992	Correlations between height and crown dieback by year						
		Year						
		1	2	3	4	5	6	7
'Valley Forge'	337bc[y]	NS[x]	NS	NS	NS	NS	NS	NS
'Princeton'	186de	0.50*	0.66*	0.61*	0.52*	0.51*	NS	NS
'New Harmony'	202d	NS	NS	NS	NS	NS	NS	NS
Amer. R18–2	194de	NS	0.42*	NS	NS	NS	NS	NS
'Delaware'	184de	NS	NS	NS	NS	NS	NS	NS
Amer. 57845	394ab	NS	NS	NS	NS	0.41*	0.42*	0.46*
Amer. 11	128e	–0.50**	NS	–0.40*	–0.50*	–0.48*	–0.49*	–0.48*
'American Liberty'	396ab	0.55**	0.52**	0.61**	0.60**	0.61**	0.61**	0.60**
'Prospector'	293c	0.39*	—[w]	NS	NS	NS	NS	—[w]
'Frontier'	444a	NS	NS	NS	NS	NS	NS	NS

[z]Amer. = American elm selection.

[y]Means within this column with any identical letters are not significantly different by LSD, 0.05 level of significance.

[x]NS, *, ** Nonsignificant or significant at $P < 0.05$ or 0.01, respectively.

[w]Crown dieback was zero for all trees in these years.

lections, such as 'American Liberty,' 57845, and 11, showed extensive dieback the first year, and subsequently sustained high mortality, with fewer trees showing a vigorous return to pre-inoculation vigor after 7 years.

Results of this study confirm the promising level of tolerance to *Ophiostoma* shown in an earlier, 1-year evaluation of American elm clones (15). Based on the present study, 'Valley Forge,' 'Princeton,' and 'New Harmony' appeared able to respond and recover over time from fungal inoculation, expressing a true tolerance. In contrast, other selections such as 57845, 11, and the cultivar 'American Liberty' did not express such resilience. Thus, the ability to recover from severe *Ophiostoma* inoculation appears to be very clone-dependent. Such variation will enable geneticists, plant breeders, and plant pathologists to further increase levels of DED tolerance in American elm.

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