

# EVALUATION OF ELM CLONES FOR TOLERANCE TO DUTCH ELM DISEASE

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**Abstract.** Rooted cuttings of ten hybrid elm (*Ulmus*) clones, including one named 'Patriot', and all derived from various crosses of 'Homestead', 'Prospector', 'Urban', and Selection 970, were established along with a randomly selected American elm clones and three disease-tolerant cultivars ('Frontier', 'Homestead', and 'Prospector') in a replicated field plot. When the rooted cuttings were 3 years old, they were inoculated with a mixed spore suspension of *Ophiostoma novo-ulmi* and *O. ulmi*, the fungi that cause Dutch elm disease. Analyses of variance and regression showed significant variation among clones in crown dieback, survival, and height growth over a 7-year time period following inoculation. The American elm clone and selection 15-87 (an 'Urban' × 'Prospector' clone) showed the most crown dieback and lowest survival rate and were among the slowest in growth rate, 7 years after inoculation. Selections and cultivars showing the least dieback and highest survival were most hybrids from parentages of 'Urban' × 'Prospector', those from 'Homestead' × 'Prospector', and 'Homestead' × 970; and cultivars 'Patriot', 'Homestead', and 'Prospector'. Many of the same disease-tolerant clones and cultivars also showed the greatest height growth after inoculation. Significant variation in disease symptoms occurred among clones from the 'Urban' × 'Prospector' cross. Results of this study emphasize the importance of selection and testing of specific clones within full-sib families.

**Key Words.** Disease tolerance; tree breeding and selection; clonal evaluation; *Ophiostoma novo-ulmi*; *Ophiostoma ulmi*; *Ulmus americana*; *Ulmus carpinifolia*; *Ulmus glabra*; *Ulmus hollandica*; *Ulmus parvifolia*; *Ulmus pumila*; *Ulmus wallichiana*; *Ulmus wilsoniana*.

Elms (*Ulmus*) have long been proven superior in withstanding the drought, soil compaction, de-icing salts, air pollution, and other rigors of the modern urban environment. Their inherent vigor, hardiness, and tolerance to stresses are unsurpassed by most tree genera. Over the last several decades, breeding and selection work on developing elms with tolerance to the fungi (*Ophiostoma novo-ulmi* Brasier and *O. ulmi* (Buisman) C. Nannf), which cause Dutch elm disease, has yielded much new scientific information and many new American and non-American elm cultivars (Lester and Smalley 1972; Santamour 1973; Townsend 1975; Townsend and Schreiber 1976; Townsend 1979; Townsend and Douglass 1996; Guries and Smalley 2000; Townsend 2000; Ware 2000).

Work also has been carried out on determining and exploiting the variation among elm species and clones in tolerance to the elm leaf beetle (*Xanthogaleruca luteola* (Muller)), with selections of *Ulmus wilsoniana* Schneid. and *U. parvifolia* Jacq. being used as parents capable of transmitting elm leaf beetle tolerance (Townsend 1979; Hall and Townsend 1987; Hall et al. 1987; Townsend and Douglass 1996; Miller 2000). The U.S. National Arboretum has carried out an advanced-generation crossing program, in which disease and insect tolerance from superior cultivars and selections have been combined through extensive use of controlled pollinations (Hall and Townsend 1987; Townsend and Douglass 1996). As a result of these crosses, 686 new hybrid seedlings were created, and these were screened for tolerance to the elm leaf beetle (Hall and Townsend 1987; Townsend et al. 1995) and to the fungi that cause Dutch elm disease (Townsend and Douglass 1996).

This paper reports on a 7-year comparison for Dutch elm disease tolerance of clonal selections created from these breeding efforts. The objective was to identify those hybrid clones showing the best disease tolerance.

## METHODS

Clonal selections used in this study were derived from various seedling progenies created in 1980 from controlled pollinations, and inoculated in May 1984 with a mixture of *Ophiostoma novo-ulmi* and *O. ulmi* (Townsend and Douglass 1996). Most of the parents, including 'Urban' (*U. hollandica* 'Vegeta' × *U. carpinifolia* Gleditsch) × *U. pumila* L., 'Homestead' (*U. pumila* × [*U. hollandica* 'Vegeta' × *U. carpinifolia*] × [*U. pumila* × *U. carpinifolia*]), and 'Prospector' (*U. wilsoniana* Schneid.), had been or were eventually named and released as cultivars (Schreiber and Main 1976; Townsend and Masters 1984; Townsend et al. 1991a). Another parent, Selection 970 (*U. glabra* × [*U. wallichiana* Planch × *U. carpinifolia*]), has shown good disease tolerance in several replicated tests (Townsend and Masters 1984; Townsend et al. 1991a; Townsend et al. 1991b).

Ten hybrid selections from the progeny test, a randomly selected American elm (*Ulmus americana* L.) clone (57845), and three cultivars ['Frontier' (= *U. carpinifolia* × *U. parvifolia*) (Townsend et al. 1991b), 'Homestead', and 'Prospector'] were evaluated. Eight of the hybrid selections were derived from the cross, 'Urban' × 'Prospector' [clone

numbers 1-85, 6-87, 14-87, 15-87, 16-87, 18-87, 24-87, and 17-87, the last of which was eventually named and released as 'Patriot' (Townsend et al. 1995)]. Selection 1-87 represented a 'Homestead' × 'Prospector' cross; and selection 5-87 resulted from crossing 'Homestead' with Selection 970. All ten of the numbered hybrid selections had shown disease symptom development of 5% or less 4, 8, and 55 weeks after the 1984 progeny test-inoculation (Townsend and Douglass 1996).

Trees were planted into a field plot at Glenn Dale, Maryland, U.S., in 1989 and 1990, in a randomized block, split-plot design with seven blocks and, when available, four trees per clone were planted in each whole plot (Townsend and Douglass 1996). Half of the trees in each whole plot were inoculated May 18, 1992; the other half were inoculated on May 27, 1992. Inoculations on each date were made into a 2.4 mm (0.1 in.) hole in the bottom one-third of the main trunk of each tree with 0.11 mL (0.003 fl oz) of an aqueous spore suspension containing  $3 \times 10^6$  spores/mL ( $1 \times 10^8$  spores/fl oz) of a mixture of two isolates each of *O. novo-ulmi* and *O. ulmi*. This inoculation was intentionally severe in order to induce symptom expression on even the most disease-tolerant clones.

The percentage of the crown showing dieback (lack of foliage) was estimated yearly from 1 to 7 years after inoculation. Height of all trees before inoculation was recorded in March 1992, 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, but trees that died back to below 1 m (3.3 ft) in height were considered dead, even if stump sprouts were present near the base of the tree. Analyses of variance and regression were carried out using the mixed model procedure of the Statistical Analysis System (SAS Institute 1996).

## RESULTS AND DISCUSSION

Analyses of variance showed selection or cultivar as a highly significant source of variation for crown dieback ( $P < 0.0001$ ), survival ( $P < 0.0001$ ), and height growth after inoculation ( $P < 0.0001$ ). Regression analyses also corroborated significant clonal effects on dieback and survival over time, with linear, quadratic, and cubic regression coefficients showing significant differences among clones. The earlier inoculation evoked significantly more crown dieback and lower survival and height growth than the second inoculation (Table 1). Averages of all clones across all years showed the greater dieback (18% vs. 11%), lower survival (88% vs. 94%), and reduced height growth [222 cm (88.8 in.) vs. 268 cm (107.2 in.)] after the May 18 inoculation compared to the May 27 inoculation (Table 1). The greater disease susceptibility of

**Table 1. Effect of inoculation date on crown dieback, survival, and height growth following *Ophiostoma* inoculation, for all 14 elm clones combined.**

Years after inoculation	Crown dieback (%)		Survival (%)		Height growth (cm)		
	Inoculation date <sup>z</sup>		Inoculation date		Inoculation date		
	1	2	1	2	1	2	
1	24	**y	16	92	96	—	—
2	19	**	13	89	* 94	—	—
3	17	**	10	88	* 94	—	—
4	17	**	10	88	* 94	—	—
5	16	*	10	88	* 94	—	—
6	16	*	10	87	* 94	222 <sup>x</sup>	** 268 <sup>x</sup>
7	16	*	10	87	* 93	—	—
Overall	18	*	11	88	* 94	—	—

<sup>z</sup>Inoculation date 1 = May 18, 1992; inoculation date 2 = May 27, 1992.

<sup>y</sup>Single and double asterisks indicate significant difference at 0.05 and 0.01 levels, respectively. The absence of asterisks indicates nonsignificance.

<sup>x</sup>Height growth for the 6 years following inoculation.

elms earlier in the growing season has been observed in other studies (Townsend and Masters 1984; Townsend et al. 1991a; Townsend et al. 1995). Analyses of variance showed no significant interaction between clone and inoculation date for dieback, survival, and height growth in all years, except for dieback after 3 years ( $P < 0.032$ ).

The 14 clones differed significantly in their 7-year response to fungal inoculation. Three years after inoculation, clones varied in severity from greatest to least crown dieback in the following order: American clone 57845, 15-87, 5-87, 'Frontier', 6-87, 'Homestead', 1-85, 16-87, 24-87, 18-87, 1-87, 14-87, 'Patriot', and 'Prospector' (Table 2). The corresponding ranking of clones from greatest to least dieback 7 years after inoculation was as follows: American 57845, 15-87, 'Frontier', 'Homestead', 5-87, 6-87, 1-85, 1-87, 16-87, 24-87, 18-87, 14-87, 'Patriot', and 'Prospector' (Table 2). 'Frontier' appeared intermediate in dieback after 7 years.

Those clones (American 57845 and 15-87) showing the most crown dieback also showed the lowest 7-year survival, 32% and 67%, respectively (Table 3). Survival after 7 years for 'Frontier' was intermediate at 82%, but not statistically different from 'Homestead' (90%) or 5-87 (95%). The other 9 clones showed 100% survival (Table 3).

Height growth on surviving trees after inoculation was greatest for 'Patriot', 'Prospector', 14-87, 1-85, 18-87, 24-87, and 'Homestead', and least for American 57845, 5-87, 'Frontier', and 15-87 (Table 4). The slower growth on American 57845 and 15-87 probably is a manifestation of the greater amount of crown dieback sustained by these clones after inoculation. Correlation analyses of individual tree data across all clones combined showed that height growth was negatively correlated ( $P < 0.01$ ) with crown dieback in all years; higher levels of dieback generally therefore were associated with lower growth rates.

**Table 2. Crown dieback on elm clones after inoculation with *Ophiostoma*.**

Selection or cultivar <sup>2</sup>	No. of trees	Height (cm) March 1992	Crown dieback (%)						
			Years after inoculation						
			1	2	3	4	5	6	7
Am 57845	28	394 de <sup>y</sup>	86 a	93 a	86 a	85 a	84 a	81 a	81 a
15-87 (U × P)	24	292 fg	49 b	44 b	43 b	42 b	41 b	40 b	40 b
6-87 (U × P)	28	432 cd	30 c	16 c	11 cd	9 cde	8 c	7 cd	5 d
5-87 (H × 970)	16	224 g	26 cd	17 c	15 c	10 cde	9 c	8 cd	7 cd
1-85 (U × P)	28	504 ab	22 cd	12 cd	6 cde	5 cde	4 cd	3 d	3 d
16-87 (U × P)	26	371 ef	14 de	8 d	3 de	2 de	3 cd	3 d	2 d
'Frontier'	28	444 bcd	12 ef	12 cd	12 cd	15 c	15 c	19 c	22 bc
18-87 (U × P)	27	392 de	12 ef	5 e	2 e	2 de	1 d	1 d	1 d
24-87 (U × P)	28	369 e	9 ef	5 e	2 e	1 de	1 d	1 d	1 d
1-87 (H × P)	28	382 de	8 efg	1 e	1 e	4 cde	4 cd	3 d	2 d
'Prospector'	28	293 fg	7 fg	0 e	0 e	1 de	0 d	0 d	0 d
14-87 (U × P)	28	458 bc	5 h	1 e	0 e	0 e	0 d	0 d	0 d
'Homestead'	19	531 a	1 h	10 d	10 cd	11 cd	11 c	10 cd	10 cd
'Patriot' (U × P)	24	302 f	1 h	0 e	0 e	2 de	2 cd	2 d	0 d
Overall mean			20	16	14	13	13	13	12
Sig. clone ( $P <$ )			0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

<sup>2</sup>Am = American; U = 'Urban', P = 'Prospector', H = 'Homestead'.

<sup>y</sup>Means within a column with any identical letters are not significantly different by LSD, 0.05 level.

**Table 3. Survival of elm clones after *Ophiostoma* inoculation.**

Selection or cultivar <sup>2</sup>	Survival (%)						
	Years after inoculation						
	1	2	3	4	5	6	7
Am 57845	50 b <sup>y</sup>	36 c	36 c	32 c	32 c	32 c	32 d
15-87 (U × P)	67 b	67 b	67 b	67 b	67 b	67 b	67 c
6-87 (U × P)	100 a	100 a	100 a	100 a	100 a	100 a	100 a
5-87 (H × 970)	100 a	100 a	95 a	95 a	95 a	95 a	95 ab
1-85 (U × P)	100 a	100 a	100 a	100 a	100 a	100 a	100 a
16-87 (U × P)	100 a	100 a	100 a	100 a	100 a	100 a	100 a
'Frontier'	96 a	93 a	89 ab	89 ab	89 ab	86 ab	82 bc
18-87 (U × P)	100 a	100 a	100 a	100 a	100 a	100 a	100 a
24-87 (U × P)	100 a	100 a	100 a	100 a	100 a	100 a	100 a
1-87 (H × P)	100 a	100 a	100 a	100 a	100 a	100 a	100 a
'Prospector'	100 a	100 a	100 a	100 a	100 a	100 a	100 a
14-87 (U × P)	100 a	100 a	100 a	100 a	100 a	100 a	100 a
'Homestead'	100 a	90 ab	90 ab	90 ab	90 ab	90 ab	90 abc
'Patriot' (U × P)	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Overall mean	94	92	91	91	91	91	90
Sig. clone ( $P <$ )	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

<sup>2</sup>Am = American; U = 'Urban', P = 'Prospector', H = 'Homestead'.

<sup>y</sup>Means within a column with any identical letters are not significantly different by LSD, 0.05 level.

**Table 4. Height growth of surviving elm trees after *Ophiostoma* inoculation.**

Selection or cultivar <sup>a</sup>	Pre-inoculation height (cm)	Six-year height growth after fungal inoculation (cm)
Am 57845	394d e <sup>b</sup>	-89 f <sup>b</sup>
15-87 (U × P)	292 fg	214 de
6-87 (U × P)	432 cd	266 bcd
5-87 (H × 970)	224 g	130 e
1-85 (U × P)	504 ab	315 bcd
16-87 (U × P)	371 ef	233 cde
'Frontier'	444 bcd	140 e
18-87 (U × P)	392 de	306 bcd
24-87 (U × P)	369 e	303 bcd
1-87 (H × P)	382 de	217 de
'Prospector'	293 fg	344 ab
14-87 (U × P)	458 bc	326 bc
'Homestead'	531 a	284 bcd
'Patriot' (U × P)	302 f	444 a
Overall mean	385	245
Sig. clone ( $P <$ )	0.0001	0.0001

<sup>a</sup>Am = American; U = 'Urban', P = 'Prospector', H = 'Homestead'.

<sup>b</sup>Means within a column with any identical letters are not significantly different by LSD, 0.05 level.

<sup>c</sup>Average height of trees 6 years after inoculation was less than average pre-inoculation height because of the high degree (81%) of dieback after inoculation during these 6 years. Trees did not grow in height enough to make up for the loss of crown due to Dutch elm disease.

Selection 5-87 showed the least growth after inoculation, despite intermediate dieback and high survival. The low amount of growth after inoculation by 5-87 may be a result not only of its unique parentage ('Homestead' × 970), but also of its inherently slow growth rate; it was the smallest of all clones just before inoculation (Table 4).

Clones from the same cross ('Urban' × 'Prospector') showed significant variability in dieback the first few years after inoculation, but in later years only one of these clones, number 15-87, showed more dieback and lower survival than the other full-sib clones (Tables 2 and 3). For example, selection 15-87 showed 40% dieback and 67% survival after 7 years; its seven full-sib sister seedlings averaged dieback of 5% or less and 100% survival after 7 years. This variation among full-sib seedlings in Dutch elm disease tolerance could be explained by specific combining ability, which in past studies (Townsend and Schreiber 1976; Townsend 1979; Townsend and Douglass 1996) has been found to be of equal importance to general combining ability. Specific combining ability is a result of nonadditive gene effects. Examples of such effects are dominance of one gene over an allelic gene, or epistasis, which is dominance exerted by nonallelic genes (Wright 1976).

Most of the unnamed clones included in this study show excellent tolerance to Dutch elm disease. However, these

clones need to be tested in different geographic regions for their environmental adaptability and horticultural desirability. Further assessment is also needed of their elm leaf beetle tolerance. Initial research indicates that the clones with *U. wilsoniana* as a parent have increased levels of tolerance to the elm leaf beetle, perhaps sufficient for field performance (Hall and Townsend 1987; unpublished data). However, expanded in-depth laboratory and field studies are needed to determine more accurately clonal differences in suitability to beetle feeding. Those clones showing insufficient tolerance could be backcrossed to Wilson elm (*U. wilsoniana*) or to lacebark elm (*U. parvifolia*) in order to increase the level of resistance to the elm leaf beetle in their progenies.

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**Acknowledgments.** We thank S.E. Bentz, T.E. Abell, and W.O. Masters for their assistance in propagation, planting, inoculating, and maintenance of the trees used in this study. We also acknowledge the support given by the Maryland Agricultural Experiment Station, College Park, Maryland.

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**Résumé.** Des coupes de racines sur 10 hybrides clonés d'ormes—incluant un appelé 'Patriot' et tous les autres dérivés de divers croisements entre 'Homestead', 'Prospector', 'Urban' et de la sélection 970—ont été faites avec d'autres arbres parmi une sélection aléatoire d'ormes d'Amérique clonés et de trois cultivars résistants à la maladie hollandaise de l'orme ('Frontier', 'Homestead' et 'Prospector') localisés dans un champ de multiplication. Lorsque les coupes de racines ont atteint trois ans d'âge, elles ont été inoculées avec un mélange de spores en suspension de *Ophiostoma novo-ulmi* et *O. ulmi*, le champignon causal de la maladie hollandaise de l'orme. Les analyses de variance et de régression ont montré une variation significative parmi les clones en ce qui a trait à la mortalité de la cime, la survie et la croissance en hauteur durant la période de sept années après l'inoculation. Le clone d'orme d'Amérique et la sélection 15-87 (un clone 'Urban' × 'Prospector') ont montré le plus haut taux de mortalité de la cime et le plus faible en regard de la survie, et ils ont aussi été ceux qui avaient les plus faibles taux de croissance sept ans après l'inoculation. Les sélections et les cultivars qui démontré les plus faibles taux de dépérissement et les plus haut de survie étaient la plupart des hybrides de 'Urban' × 'Prospector', ceux de 'Homestead × 'Prospector' et de 'Homestead × 970, et les cultivars 'Patriot', 'Homestead' et 'Prospector'. Plusieurs de ces mêmes clones et cultivars tolérants à la maladie ont aussi montré les taux de croissance les plus élevés après l'inoculation. Une variation significative dans les symptômes de la maladie s'est produite parmi les clones provenant des croisements 'Urban' × 'Prospector'. Les résultats de cette étude mettent l'emphase sur l'importance de la sélection et de l'essai de tous les clones spécifiques dans les sous-familles.

**Zusammenfassung.** Bewurzelte Stecklinge von 10 hybriden Ulmenklonen, einschließlich einem namens 'Patriot' und alle mit der Herkunft von verschiedenen Kreuzungen von 'Homestead', 'Prospector', 'Urban' und Selektion 970 wurden zusammen mit einem zufällig ausgewählten Klon von Amerikanischer Ulme und 3 krankheitsresistente Kultivare (Frontier, Homestead, Prospector) an einen Feldstandort gepflanzt. Als die bewurzelten Stecklinge 3 Jahre alt waren, wurden sie

inokuliert mit einem Sporecocktail aus *Ophiostoma novo-ulmi* und *O. ulmi*, dem Erreger von Ulmenkrankheit. Die Analysen von Unterschieden und Regression zeigen signifikante Variationen zwischen den Klonen bezüglich Kronenrücksterben, Überleben und Höhenwachstum über eine 7 jährige Periode nach der Inokulation. Der Klon der Amerikanischen Ulme und die Selektion 15-87 (Kreuzung aus 'Urban' und 'Prospector') zeigten das größte Rücksterben und die niedrigste Überlebensrate und waren sehr langsam in dem Zeitraum von 7 Jahren gewachsen. Die Selektionen und Kultivare mit dem geringsten Absterben und der höchsten Überlebensrate waren Hybriden mit der Herkunft von 'Urban' × 'Prospector', 'Homestead' × 'Prospector', und 'Homestead' × 970; und die Kultivare 'Patriot', 'Homestead', and 'Prospector.' Viele der krankheits-toleranten Klone und Kultivare zeigten das größte Höhenwachstum nach der Inokulation. Signifikante Variationen bei den Symptomen tauchten bei den Klonen aus 'Urban' × 'Prospector' auf. Die Ergebnisse dieser Studie betonen die Wichtigkeit der Selektion und der Erprobung bestimmter Klone innerhalb der sib-Familien.

**Resumen.** Los clones de diez olmos híbridos, incluyendo uno denominado 'Patriot' y todos derivados de varias cruzas de 'Homestead', 'Prospector', 'Urban' y selección 970, fueron establecidos en forma aleatoria con cultivares de clones de olmo americano tolerantes a la

enfermedad holandesa del olmo ('Frontier', 'Homestead' y 'Prospector') en parcelas de campo con repeticiones. Cuando los clones tenían tres años fueron inoculados con una mezcla en suspensión de esporas de *Ophiostoma novo-ulmi* y *O. ulmi*, el hongo causante de la enfermedad holandesa del olmo. Los análisis de varianza y de regresión mostraron variación significativa entre los clones, en muerte regresiva de la copa, supervivencia y crecimiento en altura en un período de 7 años después de la inoculación. El clon del olmo americano y la selección 15-87 (un clon 'Urban' × 'Prospector') mostraron la mayor muerte regresiva de la copa y la más baja tasa de supervivencia, y estuvieron entre los de más baja tasa de crecimiento, siete años después de la inoculación. Las selecciones y los cultivares que mostraron la menor muerte regresiva y las más alta tasa de supervivencia fueron en su mayoría híbridos de parentales de 'Urban' × 'Prospector', de 'Homestead' × 'Prospector' y 'Homestead' × 970; y cultivares 'Patriot', 'Homestead' y 'Prospector'. Muchos de los mismos clones y cultivares tolerantes a la enfermedad, también mostraron los crecimientos más altos después de la inoculación. Ocurrió variación significativa en los síntomas de la enfermedad entre clones de cruzas de 'Urban' y 'Prospector'. Los resultados de este estudio enfatizan la importancia de la selección y prueba de clones específicos dentro de familias.