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Variation in Response of Selected American Elm Clones to *Ophiostoma ulmi*[1]

A.M. Townsend[2], S.E. Bentz[3], and G.R. Johnson[4]
U.S. Department of Agriculture, Agricultural Research Service
U.S. National Arboretum, Washington, DC 20002

Abstract

Ramets of nine American elm (*Ulmus americana* L.) clones or cultivars were planted with ramets of *Ulmus* 'Frontier', *Ulmus* 'Prospector', and American elm seedlings in a randomized block, split-plot design. When they were three years old, the trees were inoculated in the main trunk on either one of two selected dates in May with a spore suspension of *Ophiostoma ulmi*, the causal fungus for Dutch elm disease (DED). Analyses of variance showed significant variation among clones and between inoculation dates in disease symptoms four weeks and one year after inoculation. Inoculations made on May 18 generally created significantly more symptoms than inoculations made only nine days later. Four-week symptom expression was influenced also by a significant interaction between clonal or seedling group and inoculation date. When data from both inoculation dates were combined, six American elm clones ('American Liberty', 'Princeton', 680, R18–2, 180, and 3) showed significantly fewer foliar symptoms after four weeks than the American elm seedlings and three other American elm clones. Five of these same six more tolerant American clones averaged significantly less crown dieback after one year than the other American clones or seedlings tested. One of the American elm clones (clone 3) showed a level of disease tolerance equal statistically to 'Frontier' and 'Prospector', two cultivars which have shown a high degree of tolerance to DED in other studies.

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

Significance to the Nursery Industry

Dutch elm disease (DED), caused by the fungus *Ophiostoma ulmi*, remains a threat to American elms (*Ulmus americana* L.) in the forest and in the landscape. If disease-tolerant American elm clones were identified, the nursery industry could once again consider growing and selling this adaptable and beautiful tree. This study examined the relative susceptibility to *Ophiostoma ulmi* of American elm clones that previously had been selected for their DED tolerance. Several clones showed levels of disease tolerance much higher than average American elm seedlings and, although not immune, offer promise for the return of this species to the landscape.

Introduction

Since its arrival on the North American continent in 1930, *Ophiostoma ulmi* (Buism.) C. Nannf., the causal fungus of Dutch elm disease (DED), has destroyed millions of American elms (*Ulmus americana* L.) (3, 4, 11). In order to overcome this serious disease problem, several research programs were developed to increase tolerance of elms to this fungus. Until recently, most of the breeding and selection and cultivar release efforts have concentrated on the European and Asiatic elms (3, 4, 5, 6, 7, 8, 11). Only recently has greater emphasis been given to the breeding and selection of American elms (3, 4, 11).

This paper reports on an extensively replicated field experiment, in which the differential response of nine American elm clones to *Ophiostoma ulmi* was measured. Promising variation in disease susceptibility and tolerance exists within the American elm.

Materials and Methods

One-year-old ramets (trees which result from vegetative propagation of a single clone) from each of nine selected American elm clones or cultivars and two non-American cultivars, 'Frontier' (9) and 'Prospector' (10), were planted with American elm seedlings (Ohio seed source) from March 1989 through May 1990, into a field plot at Glenn Dale, MD. Table 1 lists the groups planted. Some of the larger trees were planted in 1989, but planting of most of the trees was delayed until 1990. Five (clones 3, 11, 180, 680, and R18–2) of the nine clones had shown the least disease symptoms and best symptom recovery and survival of hundreds of clones inoculated with *O. ulmi* in Delaware, Ohio (5, 11). Clone R18–2 was one of 17 survivors out of 21,000 American elm seedlings screened cooperatively for DED tolerance by Cornell University and the Boyce Thompson Institute for Plant Research (4). In the 1960s, budwood of R18–2 was sent from Dr. Wayne Sinclair of Cornell University to the USDA laboratory in Delaware, OH, where it was grafted onto American elm understock, and later inoculated with *O. ulmi*. Clone 2, otherwise known as 'Delaware 2', or more simply, 'Delaware', was one of the two most disease-tolerant trees selected during the 1940s by the Bureau of Plant Industry from a population of about 35,000 seedlings (4). 'Delaware' was grown to maturity at the U.S. National Arboretum, where it eventually developed a vase-shaped crown and moderate size (4, 5). This clone has shown good DED tolerance in seasonal susceptibility and field trials over the years (3, 5, 11). One of the nine American clones, 57845, was previously unselected and served in the present test as a control clone. Two American elm cultivars, 'Princeton' and

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[2]Research Geneticist.

[3]Horticulturist.

[4]Research Geneticist. *Present address*: USDA Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, OR 97331.

Table 1. Relative susceptibility of elm genotypes to *Ophiostoma ulmi* inoculation.

Clonal or seedling group[z]	No. of trees	Height (cm) March 1992	Foliar symptoms (%) 4 weeks after inoculation			Crown dieback (%) one year after inoculation		
			Inoculation date (1992)		Both dates combined	Inoculation date (1992)		Both dates combined
			May 18	May 27		May 18	May 27	
Amer. 57845	28	394b[y]	87a	89a	88a	83ab	95a	86a
Amer. seedlings	25	159fg	85a	78a	82ab	88ab	89a	89a
Amer. 11	27	129g	75ab	73ab	74ab	87ab	89a	91a
Amer. 2 ('Delaware')	20	197ef	84a	55bc	70bc	81abc	76ab	78a
'American Liberty'	28	396b	73abc	41cde	57cd	99a	87a	93a
Amer. 680	28	202ef	74abc	34cdef	54cd	73abc	53cd	63b
'Princeton'	16	278d	54cd	49cd	53d	63bc	41d	57b
Amer. R18–2	25	206e	58bcd	34cdef	47de	71bc	49cd	60b
Amer. 180	6	210e	39d	45cd	41def	55c	62bc	57b
Amer. 3	28	337c	41d	16f	28f	16d	9e	13c
'Prospector'	28	293cd	48d	20f	34ef	9d	5e	7c
'Frontier'	28	443a	37d	26def	32ef	18d	6e	12c

[z]"Amer." = American elm.

[y]Mean separation within columns by Duncan's new multiple range test, 0.05 level.

'American Liberty' (4), were also included in the experiment.

Trees were planted in a randomized block, split-plot design with seven blocks and, when available, four trees per block per clonal or seedling group. Each four-tree plot was further subdivided into two two-tree subplots, each designated for inoculation on either May 18, 1992 (inoculation date 1) or May 27, 1992 (inoculation date 2). 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 (10) with an aqueous spore suspension containing 3×10^6 spores/ml of a mixture of two aggressive and two nonaggressive (2) isolates of *O. ulmi*. This inoculation was designed to be extremely severe in order to insure symptom expression on even the most disease-tolerant clones. The percentage of the crown showing wilting or death of the foliage, and the percentage of the crown's branches showing dieback (a lack of foliage), were visually estimated four weeks and one year, respectively, after each inoculation date. Data analysis was carried out using the Statistical Analysis System (SAS) (1).

Recovery of *Ophiostoma ulmi* was attempted from small branch segments collected from six symptomatic trees (one tree each of 'American Liberty', 'Princeton', 'Frontier', and clone 680; and two trees of clone 3) on June 14, 1994. Samples were plated out on potato dextrose agar (PDA) or acidified PDA.

Results and Discussion

Analyses of variance showed clone and inoculation date as highly significant sources of variation for disease response at four weeks and one year after inoculation (Table 2). The significant interaction between clone and inoculation date for symptoms after four weeks indicates that clones varied in their degree of response on different inoculation dates, with some clones showing similar symptoms after both inoculation dates, and other clones showing markedly different symptoms four weeks after the two inoculation dates. However, this same clone × date interaction was not significant for the one-year dieback data (Table 2).

Inoculation of trees on the earlier date generally evoked greater symptom expression (Table 1). Elms have been shown to be more susceptible earlier in the growing season than later (4, 5, 7, 11), but the highly significant difference between two inoculation dates only nine days apart was unexpected. Symptom expression was very pronounced on several clones and cultivars, and is a reflection of the highly severe inoculation technique used, which was meant to create maximum symptom expression. Inoculation in twig crotches would have simulated more closely natural inoculation, and caused fewer symptoms. The symptoms observed in this study can be considered unnaturally high, and probably underestimate the degree of inherent disease tolerance in these trees.

The American elm clones showed less variability in disease response four weeks after the first inoculation than after the second (Table 1). When four-week data were combined over inoculation dates, the more susceptible biotypes

Table 2. Summary of analyses of variance for elms inoculated with *Ophiostoma ulmi*.

Source of variation	df	Mean squares for each trait and time after inoculation[z]	
		Foliar symptoms 4 weeks	Crown dieback 1 year
Block (B)	6	0.121	0.089
Clone (C)[y]	11	1.484**[x]	4.987**
B × C	59	0.104**	0.135
Inoculation date (ID)	1	2.296**	0.744**
C × ID	11	0.179**	0.125
C × B × ID	60	0.048	0.106**
Error	138	0.037	0.056

[z]Data were transformed by inverse sine before analysis.

[y]American elm seedlings were considered as 'clonal ramets' for purposes of these analyses.

[x]Significant at the 0.01 (**) level.

were the American elm seedlings and clones 57845, 11, and 'Delaware'. It should be noted that 'Delaware' has been shown in previous studies to express significant tolerance (5, 11). In the present study, this clone did show fewer symptoms than the American elm seedlings four weeks after the second inoculation date. 'American Liberty', 680, 'Princeton', R18–2, 180, and 3 showed significantly fewer four-week combined symptoms than the American elm seedlings and clones 57845 and 11. In fact, American clones R18–2, 180, and 3 showed four-week combined symptom expression equal statistically to the two non-American cultivars, 'Frontier' and 'Prospector,' which have been shown previously to be highly tolerant to *Ophiostoma ulmi* (9, 10). It may be worthwhile to do more testing of clone 180 because of the low replication (n = 6) of this clone in the present study.

Crown dieback one year after inoculation was generally greater for those trees inoculated earlier than for those inoculated nine days later; but the differences between these two groups were not as pronounced as they were for the four-week foliar symptoms (Table 1). When dieback data from both inoculation times were combined, several clones (57845, 11, 'Delaware', and 'American Liberty') were similar to American elm seedlings in the degree of dieback sustained (Table 1). However, American clones 680, 'Princeton', R18–2, 180, and 3 all showed significantly less dieback than the American seedlings. In fact, clone 3 had only 13 per cent dieback averaged over both inoculation dates, equal statistically to 'Frontier' and 'Prospector' and markedly lower than the 89 per cent dieback sustained by the disease-susceptible American seedlings (Table 1).

Recovery of *Ophiostoma ulmi* two years after inoculation was successful from four of the six trees sampled; it was not recovered from one ramet of clone 680 and one ramet of clone 3. This successful recovery may be a result of the highly effective inoculation technique used, but is also evidence of the potential longevity of this organism in elm trees, and signifies more of a 'tolerance' than a 'resistance' mechanism, with some elms surviving and retaining vigor even in the presence of the fungus.

Although 'American Liberty' expressed significantly lower four-week symptoms than several other American clones and the seedling controls, dieback for this cultivar one year later was not significantly less than these other biotypes. Two possible explanations can be offered for the high degree of dieback of this cultivar, which has repeatedly shown significant disease tolerance in several studies (4). First, 'American Liberty' is comprised of six different clones (4), and it could be that the most disease-susceptible of these clones was more frequently represented in our experimental plot. Second, 'American Liberty' ramets were significantly taller at the time of inoculation than all but one of the other nine American clonal or seedling groups (Table 1). The large size combined with the unusually potent inoculation technique may have produced a synergistic effect, causing a reputedly disease-tolerant cultivar to express extensive crown dieback. Regardless, 'American Liberty' did reveal some disease tolerance, and each of the six clones (such as 'Independence') should be identified and tested separately in future experiments comparing clonal variation in disease response.

Results of this study show a wide and promising degree of tolerance to *O. ulmi* among American elm clones. American clone 3 appeared to show the highest tolerance to *O. ulmi*, followed as a group by clones 180, R18–2, 680, 'Princeton', and perhaps 'American Liberty'. 'Delaware' has expressed significant tolerance in past trials (5, 11) and in Wisconsin has shown pronounced ability to transmit DED tolerance to its progeny. In fact, it is a parent for two of the six clones that collectively make up 'American Liberty' (4). Its tolerance was not pronounced in this study, perhaps because of the severity of the inoculation. It is important to recognize that many of these selected clones may show, in areas of DED incidence, high 'field tolerance,' because inoculation of trees under natural conditions will unlikely ever be as severe as in this study (3, 4, 11).

The evidence presented here offers new hope for the reestablishment of the American elm. Even under the most strenuous of tests, several clones showed a significant tolerance to DED. This level of tolerance will allow for the future release of American elm clones to the public. At the same time, these clones have potential for use as parents in breeding programs designed to maximize disease tolerance in American elm.

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