Resistance of Hybrid Elm Progenies to Ceratocystis ulmi

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The technical assistance of W. O. Masters is gratefully acknowledged.

Accepted for publication 22 March 1976.

ABSTRACT

TOWNSEND, A. M., and L. R. SCHREIBER. 1976. Resistance of hybrid elm progenies to Ceratocystis ulmi. Phytopathology 66: 1107-1110.

Five clones of *Ulmus* from the Netherlands, and with resistance to *Ceratocystis ulmi,* when hybridized as male parents with *Ulmus pumila* varied in their ability to transmit genes for Dutch elm disease resistance and growth rate. Significant differences in disease symptoms among the progenies occurred 15 and 56 weeks but not 4 weeks after inoculation. The progeny from one Dutch clone, N248 *(U. wallichiana* × *U. carpinifolia),* was more susceptible than were progenies from other clones. *Ulmus rubra* × *U. pumila* as a female parent transmitted a slower growth rate and less disease resistance to its progeny than did *U. pumila.* Date of inoculation, interaction between inoculation date and progeny, and site factors rarely influenced symptom expression. One *Ceratocystis ulmi* isolate from Tennessee and labeled earlier as "nonaggressive" similarly caused less disease than did six other "aggressive" isolates when tested on *U. pumila* × N260 *(U. pumila* × *U. hollandica).*

Additional key words: genetic variation, elm selection, tree breeding.

Selection and breeding of elms resistant to *Ceratocystis ulmi* (Buism.) C. Moreau has been carried out in the Netherlands (3, 4) as well as in the United States (6, 7, 8, 13) to replace American elms (*Ulmus americana* L.) lost to Dutch elm disease. Many of the Netherlands clones are not adapted to the United States (5).

The first objective of this study was to determine inheritance of resistance to *C. ulmi* by hybridizing some of the Netherlands selections with parents selected at this laboratory for their resistance and/or shape. The second objective was to determine the effect of differences in sites and inoculation dates on development of Dutch elm disease in the various progeny. A final objective was to evaluate the aggressiveness of single *C. ulmi* isolates (11) in two hybrid progenies.

MATERIALS AND METHODS

Selections of *U. pumila* L. (Clone 13), *U. rubra* Muhl. × *U. pumila* (Clone 6-13), and *U. glabra* Huds. (Clone 19-22) were crossed with the following Netherlands clones: N148 = *U. hollandica* Mill. 'Vegeta' × *U. carpinifolia* Gleditsch.; N248 = *U. wallichiana* Planch. × *U. carpinifolia;* N260 = *U. pumila* × *U. hollandica* 'Vegeta'; N262 = origin unknown (H. Heybroek, *personal communication*); N274 × 215 = *(U. hollandica* 'Vegeta' × *U. carpinifolia)* × *(U. pumila* × *U. carpinifolia);* N339 = *U. carpinifolia* × *U. hollandica* 'Vegeta'.

Ulmus glabra 19-22, N148, and N248, have never been inoculated at this location. However, *all* of the Netherlands clones used are final products of a rigorous selection program for *C. ulmi* resistance, and therefore N148 and N248 have proven themselves as resistant. The other parents have been inoculated in June at least once, and in all inoculations showed less than 20% symptoms. Following are the clones, years of inoculation, number of ramets inoculated (in parentheses), and average percentage with symptoms 6 weeks after inoculation: N274 × 215: 1964 (two), 0%; 1968 (two), 12%; 1969 (six), 0%; N339: 1964 (one), 0%; 1969 (one), 0%; N262: 1964 (two), 2%; 1967 (two), 15%; 1968 (two), 15%; N260: 1964 (six), 0%; 1968 (six), 4%; 1969 (six), 1%; *U. pumila* 13: 1970 (one), 0%; *U. rubra* × *U. pumila* 6-13: 1967 (one) < 20%; 1968 (one), < 20% (more exact data on *U. rubra* × *U. pumila* 6-13 is not available). According to Hans Heybroek *(personal communication)* clones N339, N260, N148, and N248 all have the same level of resistance.

Controlled pollinations were made in the spring of 1970 according to methods described elsewhere (12). Seedlings from pollinations were transplanted on 30 June 1970, directly from greenhouse flats to two field sites in a randomized block design. The two sites were within 0.81 km (one-half mile) of each other. Site 1 was somewhat more poorly drained than site 2. Both sites were Morley silt loam. At least five replicates were planted per hybrid combination on each site, with one to five trees per plot.

Heights of all seedlings were measured in October 1972. All seedlings were inoculated on 17 May 1973 with a mixed spore suspension of aggressive (N. Dakota and Colorado) and nonaggressive (Ohio, Massachusetts, and N. Carolina) isolates of *C. ulmi* (11). Inoculum was introduced into the base of the main stem of each tree by the technique of Schreiber and Stipes (10). Seedlings from some of the progenies were inoculated either on 17 May 1973 or on 1 June 1973 to determine the effect of date of inoculation on symptom expression. By 17 May, all seedlings had foliated completely.

Seedlings of the parentage *U. pumila* 13 × N260 were inoculated on 17 May 1973 with a standardized (10^6

spores/ml) spore suspension of the following individual *C. ulmi* isolates: North Dakota (ND), Alabama (AL), Maine (ME), Colorado (CO), Tennessee (TE), Wisconsin (WI), and Illinois (IL). For each isolate six to ten trees were inoculated. Seedlings of the parentage *U. pumila* 13 × N262 were inoculated with the first four isolates listed above. For each isolate five to eight trees were inoculated.

Percentage of crown with dieback and wilting was estimated on all trees 4 weeks after inoculation. Percentage dieback of the total length of the central leader was estimated on 4 September 1973, and again on 11 June 1974.

RESULTS

All *U. pumila* progenies showed appreciable and similar symptom expression 4 weeks after inoculation, but by 15 weeks and 56 weeks, differences among progenies were significantly large (Table 1). Many seedlings showed additional dieback in 1974, compared to dieback in 1973. Progeny from clone N248 generally were more susceptible than all the other progenies. The progeny of the male parent (N274 × 215), whether hybridized with *U. pumila* 13 or *U. rubra* × *U. pumila* 6-13 grew faster than progeny from other males, yet was as resistant as the slowest-growing progenies, namely, those from N260 and N262.

Of the two female parents, *U. rubra* × *U. pumila* 6-13 transmitted a slower growth rate and less disease resistance than did *U. pumila* 13 (Table 1), as indicated by a two-way analysis of variance. There were no differences among pollen parents, N274 × 215, N260, and N248, in their ability to transmit resistance when these were mated to *U. rubra* × *U. pumila* 6-13.

There were no significant differences in symptom expression between the two inoculation dates with the exception that progenies of *U. pumila* 13 × N262, and *(U. rubra* × *U. pumila)* 6-13 × (N274 × 215) showed the greatest symptoms after 56 weeks when inoculated 1 June, and *U. glabra* 19-22 × N248 progeny showed greatest symptoms after 15 and 56 weeks when inoculated on 17 May (Table 2).

Growth rate on site 2 for all but one progeny was significantly greater than on site 1 (Table 3). There was also a tendency for greater expression of disease on site 2 than on site 1, but statistically the differences were significant only on *U. pumila* 13 × N260 4 weeks after inoculation (Table 3).

The TE isolate caused less disease on *U. pumila* × N260 than did the other six isolates. Average percentages of

TABLE 1. Variation in response to *Ceratocystis ulmi* in *Ulmus* hybrids

Female parent	Male parent	No. of trees	Avg. height (cm) Oct. 1972	Avg. symptoms[a] (%) at intervals after inoculation		
				4 weeks[b]	15 weeks[b]	56 weeks[b]
U. pumila 13	(N274 × 215)	41	231 x	37 x	20 xy	17 y
U. pumila 13	N260	21	136 z	53 x	15 xy	12 y
U. pumila 13	N248	10	181 y	42 x	39 x	60 x
U. pumila 13	N262	38	133 z	37 x	15 xy	17 y
U. pumila 13	N148	15	160 y	34 x	7 y	1 y
(U. rubra × *U. pumila)* 6-13	(N274 × 215)	12	184 x	39 x	46 x	56 x
(U. rubra × *U. pumila)* 6-13	N260	11	101 z	74 x	70 x	66 x
(U. rubra × *U. pumila)* 6-13	N248	14	138 y	62 x	70 x	75 x

[a]Values followed by the same letter in each column for progeny of each female parent are not significantly different, $P = 0.05$, by Duncan's multiple range test. Data are for trees growing on site 2.

[b]Trees were inoculated on 17 May 1973. Foliar symptoms were estimated in June 1973. Extent of dieback of the central leader was estimated in September 1973 and June 1974.

TABLE 2. Effect of date of *Ceratocystis ulmi* inoculation on disease symptoms of *Ulmus* hybrids

Female parent	Male parent	Planting site	Avg. symptoms[a]					
			4 weeks after		15 weeks after		56 weeks after	
			17 May	1 June	17 May	1 June	17 May	1 June
U. pumila 13	(N274 × 215)	2	37 (41)	33 (10)	20	17	17	13
U. pumila 13	N260	1	20 (29)	27 (10)	11	7	7	2
U. pumila 13	N262	2	37 (38)	45 (9)	15	29	17 *	38
(U. rubra × *U. pumila)* 6-13	(N274 × 215)	1	23 (15)	52 (6)	20	32	24 *	67
(U. rubra × *U. pumila)* 6-13	N339	1	7 (17)	17 (8)	12	13	14	9
U. glabra 19-22	N248	2	47 (7)	36 (6)	56 *	13	66 *	39

[a]An asterisk indicates a significant difference at the 95% probability level between average symptoms after two inoculation dates. The number of trees used for each date are in parentheses under the means.

foliage with symptoms on 14 June 1973 of *U. pumila* 13× N260 seedlings inoculated with the various isolates were: TE, 1%; ND, 70%; AL, 71%; ME, 51%; CO, 67%; WI, 42%; and IL, 62%. Corresponding percentages for *U. pumila* 13 × N262 seedlings were: ND, 64%; AL, 46%; ME, 49%; and CO, 64%.

Analyses of variance indicated no significant differences among the ND, AL, ME, CO, WI, and IL isolates in their ability to cause symptom expression on both progenies. The interaction component between the first four isolates and the two progenies also was nonsignificant.

DISCUSSION

The Netherlands clones varied greatly in their ability to transmit disease resistance when used as male parents. This variability among clones in transmission of resistant genes when used as males has also been expressed when they were used as females (Townsend and Schreiber, *unpublished*). Thus, N262 transmits more resistance to its progeny than does N248, regardless of whether used as a male (Table 1) or female (Townsend and Schreiber, *unpublished*).

Resistance to Dutch elm disease is probably polygenic, otherwise it is difficult to explain why equally resistant phenotypes transmit varying degrees of resistance to their progeny. Such resistance is desirable especially when used in combination with different genes for resistance provided by different cultivars. Other evidence of polygenic resistance to Dutch elm disease has been presented elsewhere (1, 5, 6, 7).

Seedlings from *U. pumila* 13 × (N274 × 215) have shown rapid height growth, and good form and leaf characteristics (12). Vegetative propagation of symptomless individuals from this group should yield some disease-resistant, horticulturally-marketable cultivars with suitable resistance to *C. ulmi*. Clone N248 transmitted the least resistance when crossed with *U. pumila* 13. The progeny from this particular hybridization have longer and wider leaves than the other Dutch progenies (12). This positive relationship between leaf size and susceptibility also has been found in *U. pumila* and *U. rubra* hybrids (8).

The results of this study confirm the importance of using several evaluation times to estimate symptom expression. The resistance of some progenies was not expressed until 15 weeks after inoculation, and with others, not until a year or more after inoculation.

Ulmus pumila 13 as a female parent was more effective than *U. rubra* × *U. pumila* 6-13 in transmitting resistance, or more specifically, in the production of progeny with ability to recover from initially high symptoms. For example, the respective progenies from *U. pumila* 13 × (N274 × 215) and *(U. rubra* × *U. pumila)* 6-13 × (N274 × 215) showed the same percentage of foliar symptoms 4 weeks after inoculation, but by 15 and especially by 56 weeks symptom expression had declined in the former, and increased in the latter. In related research, Lester and Smalley (7) found that intensity of foliar symptoms in *U. pumila* × *U. rubra* progenies almost doubled from 6 to 56 weeks, yet stayed constant in *U. pumila* × *U. pumila* progenies.

For some progeny choice of date of inoculation was important in the expression of symptoms 1 year following inoculation. Significant interaction between date of inoculation and progeny indicates the importance of using two inoculation dates if sufficient seedlings are available for screening.

Most progeny grew faster on site 2 than on the wetter site 1, but expressions of symptoms on both sites generally were not statistically different because of large variation in symptom development among the trees. Symptom expression is influenced by environment (2), but the amounts of influence depends more upon which progenies are tested since in this study, symptom development of *U. pumila* 13 × N260 at least, was greater on site 2 than on site 1.

Previous investigations (1, 2, 6, 7, 9) on the resistance of seedling elms to *C. ulmi* have not used individual aggressive and nonaggressive isolates on the same elm progeny. In this study, the one nonaggressive isolate used (Tennessee) proved nonaggressive no matter which progeny was inoculated with it. The other isolates, all of which showed aggressiveness in this study, also showed aggressive characteristics in previous studies (11). The consistent ability of these isolates to create the same relative degree of symptoms on a variety of *Ulmus* genotypes is good evidence for the presence and

TABLE 3. Variation in height and response to *Ceratocystis ulmi* of *Ulmus* progenies on two planting sites

		Avg. height[a] (cm)		Avg. symptoms[b] (%) at intervals after inoculation					
		Oct. 1972		4 weeks[c]		15 weeks[c]		56 weeks[c]	
Female parent	Male parent	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
U. pumila 13	N262	101 * (5)	133 (41)	27	37	1	15	10	17
U. pumila 13	N148	174 (13)	160 (15)	34	34	5	7	2	1
U. pumila 13	N260	122 * (29)	136 (21)	20 *	53	11	15	7	12
(U. rubra × *U. pumila)* 6-13	(N274 × 215)	114 * (15)	184 (12)	23	39	20	46	24	56

[a]The number of trees used for each progeny and each site are in parentheses under the means.

[b]Trees were inoculated on 17 May 1973. Foliar symptoms were estimated in June 1973. Dieback of the central leader as a percentage of total height was observed in September 1973 and in June 1974.

[c]An asterisk indicates a significant difference, $P = 0.05$, between average values for site 1 and site 2.

continued effectiveness of horizontal resistance to Dutch elm disease in *Ulmus*.

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