Response of Backcross Hybrids and Three-Species Combinations of Ulmus pumila, U. japonica, and U. rubra to Inoculation with Ceratocystis ulmi

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ABSTRACT

Thirty-two elm progenies, including half-sibs and full-sibs from parental, F_2, and backcrosses were tested for response to artificial inoculation with *Ceratocystis ulmi*. Time of inoculation and species combination were the main sources of variation. Crosses among *Ulmus pumila* and a putative natural hybrid of *U. pumila* × *japonica* had low disease susceptibility. The introduction of germ plasm from *U. rubra* markedly increased disease susceptibility.

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Screening for resistance to Dutch elm disease *Ceratocystis ulmi* (Buis.) C. Moreau in worldwide collections of elms has revealed that the northern Asiatic species *Ulmus pumila* L. and *U. japonica* (Rehd.) Sarg. are potential sources of disease-resistant germ plasm. The generally low susceptibility level of *U. pumila* is well established (1), but general levels of susceptibility in *U. japonica* are unknown. Resistance screening of *U. japonica* in Holland indicated high susceptibility (4), whereas screening in seed collections forwarded to us by Japanese cooperators suggests low to moderate levels of susceptibility (E. B. Smalley, *unpublished data*). Moreover, one collection (W44), sent as open-pollinated seed from one tree of *U. pumila*, produced individuals with very low levels of susceptibility plus ornamental traits of much greater attractiveness than the typical *U. pumila*. Trees inoculated at ages 3, 6, and 8 years were symptomless, and 70% of seedlings from wind pollination of W44 were symptomless after inoculation at 3 years of age (2). Leaf and bud morphology suggest that this outstanding progeny is a natural hybrid between *U. pumila* and *U. japonica*. The crossing studies described here were initiated to estimate the value of the putative hybrid in transmitting disease resistance.

MATERIALS AND METHODS.–The methods of pollination, hybrid verification, inoculation, and evaluation were identical to those described earlier (3), except that crosses using pollen from the putative hybrid required the mixture of pollen from several trees to obtain an adequate quantity. Some of the progenies studied were thus half-sibs, but the majority were full-sibs. No reciprocal crosses were included.

RESULTS.–There were two major sources of variation as illustrated by results from analyses of variance (Table 1). Time of inoculation had a highly significant effect for all variables except mortality. For example, the frequency of trees with dead branches 56 weeks after inoculation declined from 32% for trees inoculated on 1 June to 5% for trees inoculated 2 weeks later. Species combination was the second major source of variation, and gave highly significant effects for all variables. Frequency of trees with dead branches at 56 weeks ranged from 3% in progenies of *U. pumila* to 97% in wind-pollinated progenies of *U. americana*.

There were two minor sources of variation for some variables (Table 1). Variation among progenies within species combination was relatively small though statistically significant for three of six variables. Progenies in two combinations were especially heterogeneous for certain variables. Crown damage scored 56 weeks after inoculation in backcrosses to *U. pumila* ranged from 1.0 to 3.1. Mortality in the three-species combination (*U. pumila* X *japonica*) X *U. rubra* ranged from 15 to 45%. Variation attributable to interaction between inoculation time and progeny was minor, and occurred only in measures of early response.

The summary of mean response for different species combinations reveals the results of combining different germ plasms in different proportions (Table 2). Hybrids between *U. pumila* (p) and *U. japonica* (j) showed low susceptibility in combinations containing up to 50% germ plasm from *U. japonica*. In three-species combinations, the addition of genes from *U. rubra* (r) resulted in a marked increase in disease response. Genes from *U. japonica* contributed relatively little to increased susceptibility in three-species combinations, as illustrated by comparing p X r hybrids with or without j genes (Table 3).

The relative ranking of each species combination was similar for all variables, although mean response changed with time of evaluation (Table 2). Determination of symptom frequency 3 weeks after inoculation underestimated disease response, whereas determination at 56 weeks was somewhat confounded by winter damage and by recovery from disease. For species combinations including genes from *U. rubra*, crown damage substantially increased between 6 and 56 weeks, although the latter estimate

TABLE 1. Results of analysis of variance for six measures of response by elm progenies to artificial inoculation with *Ceratocystis ulmi*

Disease variables	Sources of variation[a]			
	Time of inoculation	Species combination	Progeny within combination	Time-progeny interaction
Frequency of symptoms				
Dead or wilted leaves, 3 weeks	342**[b,c]	54**	1	1.8*
Dead or wilted leaves, 6 weeks	753**	79**	1	1.3
Dead branches, 56 weeks	358**	97**	1.5	1.4
Dead trees, 56 weeks	2	50**	3.2**	1.0
Intensity of symptoms				
Crown damage, 6 weeks	187**	43**	2.2*	1.9
Crown damage, 56 weeks	1,248**	38**	2.8**	1.4

[a] A split-plot design was used with two blocks and two inoculation times in the main plot, and 32 progenies representing six species combinations in the subplot.

[b] Numerical values are variance ratios (F).

[c] Means differ at a probability of 95% (*) or 99% (**).

TABLE 2. Sample size, mean response, and significance tests for six measures of response by six species combinations of elms artificially inoculated with *Ceratocystis ulmi*

Disease variables	Species combination[a]						Approximate[b] $w_{.05}$
	p X p	p X pj	pj X pj	pj X pr	r X pj	a X wind	
Sample size (no.)							
Progenies	8	8	2	5	7	2	
Individuals	350	337	62	178	220	91	
Frequency of symptoms (%)[c]							
Dead or wilted leaves, 3 weeks	7	8	14	24	66	81	14
Dead or wilted leaves, 6 weeks	7	10	6	38	79	98	15
Dead branches, 56 weeks	3	7	4	30	71	97	12
Dead trees, 56 weeks	0	0	0	2	26	40	10
Intensity of symptoms (score)[d]							
Crown damage, 6 weeks	1.1	1.3	1.0	1.7	1.9	3.1	.4
Crown damage, 56 weeks	1.2	1.5	1.2	2.4	3.4	4.6	.8

[a] p = *Ulmus pumila*; j = *U. japonica*; r = *U. rubra*; a = *U. americana*.
[b] In 95% of similar experiments, means differing by more than the indicated amount are not elements in a homogeneous set. Values for testing percentage data are approximate because significance testing must be accomplished on means of transformed data.
[c] Data are percentages of trees with symptoms when observed after the indicated period following inoculation.
[d] Data are average scores for trees with visible crown damage. Scores represent five equal classes of 20% crown damage; e.g., score 1 = 0 to 20%; score 5 = 80 to 100%.

may reflect some winter damage as well as disease effects.

Associations of the six disease response variables, as measured by simple linear correlation, were closely comparable to associations presented for crosses between *U. pumila* and *U. rubra* (3). The moderate to high correlation coefficients (.7 to .8) for the experiment as a whole indicate that disease development was fairly constant over time for most of the progenies. Simple linear correlation coefficients calculated for variables within each species combination, however, ranged from 0 to 0.6.

DISCUSSION.—The importance of inoculation time in evaluating disease response was demonstrated for five of six disease response variables. A sharp decline in susceptibility occurred between inoculation dates, and all progenies responded similarly. In the current study, effects of inoculation time were more consistently evident than in an earlier study involving hybrids between *U. pumila* and *U. rubra* (3). The difference in results may be due to effects of *U. japonica* genes in producing progenies with narrower peaks of seasonal susceptibility. Both studies illustrate the importance of recognizing local patterns of seasonal susceptibility in testing elms for response to *C. ulmi*.

U. japonica was shown to have definite potential in developing progenies of low susceptibility. In crosses with *U. pumila* and in three-species combinations, genes from *U. japonica* had relatively little effect on susceptibility. Genes from *U. rubra*, by contrast, markedly increased susceptibility. Ornamentally desirable features such as large leaves and spreading crown form, thus, will be more easily combined with low susceptibility to *C. ulmi* in seedling progenies containing germ plasm mostly from *U. pumila* and *U. japonica*. Clonal propagation of selected individuals of low susceptibility in hybrid progenies could be used to avoid the increased susceptibility associated with genes from *U. rubra*.

The wide range of variation among species combinations suggests that identification of the most useful combinations will be an important step in developing ornamental elms with low disease susceptibility. Two species combinations are leading candidates. The backcross pj X j may prove suitable for immediate production of elms with adequately low susceptibility. Progenies from that combination will be inoculated in 1973. A combination of p and j with 10% genes from r may also prove useful for seedling propagation. After the most suitable species combinations are identified, utilization of variation

TABLE 3. Mean response of four elm combinations to artificial inoculation with *Ceratocystis ulmi*

Disease variables	Species combination[a]			
	p X pr	pj X pr	p X r	pj X r
Frequency of symptoms (%)[b]				
Dead or wilted leaves, 3 weeks	16	24	59	66
Dead or wilted leaves, 6 weeks	31	38	73	79
Dead branches, 56 weeks	21	30	65	71
Dead trees, 56 weeks	2	2	22	26
Intensity of symptoms (score)[c]				
Crown damage, 6 weeks	1.3	1.7	1.9	1.9
Crown damage, 56 weeks	1.9	2.4	3.5	3.4

[a] p = *Ulmus pumila*; j = *U. japonica*; r = *U. rubra*.
[b] Data are percentages of trees with symptoms when observed after the indicated period following inoculation.
[c] Data are average scores for trees with visible crown damage. Scores represent five equal classes of 20% crown damage; e.g., score 1 = 0 to 20%; score 5 = 80 to 100%.

among individual parents can further improve the ornamental value of elms.

The patterns of change in disease response over time suggest that choice of evaluation times in screening studies must be related to study objectives. As in the early study (3), evaluation about 6 weeks after inoculation seems adequate for large-scale screening studies, including a wide range of genetic materials. In our area, evaluation 6 weeks after inoculation will eliminate the confounding effects of summer leaf diseases and winter injury, although disease recovery mechanisms may be overlooked. As the range of genetic materials narrows and evaluation becomes more intensive, however, measures of disease at different times probably will be necessary.

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