Response of Ulmus pumila and U. pumila × rubra Hybrids to Inoculation with Ceratocystis ulmi

D. T. Lester and E. B. Smalley

Associate Professor of Forestry and Professor of Plant Pathology, respectively, University of Wisconsin, Madison 53706.

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ABSTRACT

Forty-six full-sib elm progenies including parental, F_1, F_2, and backcross combinations were tested for response to artificial inoculation with *Ceratocystis ulmi*. Time of inoculation and interaction of progeny with time were statistically significant for three of six measures of disease response. Species combination was the major source of variation for all variables, although variation among progenies within species combination was present for five of six variables. Disease response was a function of the proportion of genes present from *Ulmus rubra*. Despite high susceptibility of progenies containing more than 25% *U. rubra* genes, symptomless individuals occurred in most inoculated progenies. Selection and vegetative propagation of resistant individuals, irrespective of average progeny performance, may still allow the elm breeder access to the ornamentally desirable features of *U. rubra*.

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Widespread mortality of urban and forest elms in The Netherlands, USA, and Canada from infection by *Ceratocystis ulmi* (Buis.) C. Moreau has prompted studies of genetic variation in disease resistance among species, clones, and hybrids of elms. A relative ranking of species for susceptibility was developed, and commercially useful levels of resistance were produced in a few clones (2).

Our studies explored inheritance of disease resistance in two species, *Ulmus pumila* L. and *U. rubra* Mühl., and their hybrids. These were chosen for study for several reasons. *U. pumila* is rated as the most highly resistant elm species (2). As an ornamental, however, *U. pumila* has several features which compare unfavorably with the species it would be expected to replace (*U. americana* L.). Excessively fine branching, excurrent habit, small leaves, brittleness of branches, and twig mortality are generally undesirable features in urban ornamental planting. *U. rubra* by comparison is large-leaved, with wide-spreading, coarse branches and an uncertain, but probably high to moderate, level of disease susceptibility. Natural hybrids between the species are common, and several have been introduced as named cultivars in the commercial nursery trade (1).

MATERIALS AND METHODS.—Parental

materials of *U. pumila* (p) included three trees grown from commercial seed collections in Iowa and Michigan, and one tree of the Dropmore variety, an introduction from Harbin, Manchuria (1). The three *U. rubra* (r) parents were from two native populations in southern Wisconsin. Putative natural hybrids were the Fremont elm and a tree in a native Wisconsin population of *U. rubra*.

Pollination was achieved with traditional tree breeding techniques (5) using greenhouse-forced pollen and sausage casing isolation bags on field-grown trees. Isolation bags into which no pollen was sprayed served as checks on self-fertility, the elms being hermaphroditic. Isolation bags remained on the trees until all stigmas had withered and were then replaced by cheesecloth bags in which mature seed was collected. Wind-pollinated seeds were collected from two street trees of *U. americana* to provide susceptible materials for checks on inoculum virulence and suitability of the inoculation procedure.

Seed was sown in vermiculite, and 3-week-old seedlings were transplanted in a randomized complete-block design with four replicates. Hybridity was verified by comparing morphological traits of putative hybrids with *U. pumila* × *U. pumila* (p × p) progenies. Seeds from check isolation bags and a few clearly nonhybrid individuals in progenies from interspecific pollinations revealed a variable, although generally low, self-fertility, especially in *U. pumila*. Questionable individuals were omitted in all data. One-year-old dormant seedlings were field-planted at a spacing of 1.8 m in the same design, kept weed-free during the first field season, and maintained in mown Kentucky blue grass (*Poa pratensis* L.) thereafter.

The inoculum was prepared by the propagating of *C. ulmi* isolates from infected wood collected in 14 localities in Wisconsin. At the time of inoculation, the cultures were combined to give an approximate spore density of 5×10^3/ml. We achieved inoculation by drilling a 1.6-mm hole into the xylem of a 12-mm branch and filling the hole to overflowing with spore suspension from a hypodermic syringe. Two inoculation times were chosen to lessen the possibility that different progenies might have markedly different patterns of seasonal susceptibility. The first inoculation, on 1 June 1970, was at the estimated general peak of susceptibility, and the second inoculation time, 2 weeks later, was expected to come during a general decline in susceptibility.

The number of infected trees per plot was recorded at 3, 6, and 56 weeks after inoculation. Fifty-six weeks after inoculation, the number of dead trees was recorded. Per cent of crown damage was scored in equal classes from 1 to 5 (e.g., score 3 = 41-60% crown damage) at 6 and 56 weeks after inoculation. It was assumed that for trees of the size tested, infection would become systemic in moderately to highly susceptible individuals. Inoculation of one branch followed by evaluation of damage in the whole crown was thus an approximate measure of each tree's ability to isolate the initial infection. The experiment was analyzed as a split-plot design with inoculation time as the main plot. Prior to analysis, the angular transformation was applied to percentages, and crown damage scores were transformed to $\sqrt{X + 0.5}$ to normalize data including zeros. Linear correlation coefficients were calculated to express associations along the variables. Statistical procedures followed Steel & Torrie (4).

RESULTS.—Analyses of variance indicated several significant sources of variation (Table 1). Mean response of the two inoculation times differed significantly for three of six variables and produced substantial variance ratios for the other three variables. Variation associated with inoculation times was greatest in disease responses evaluated relatively soon after inoculation. The magnitude of inoculation-time effects was substantial as shown by a 60% frequency of symptoms (dead or wilted leaves—3 weeks) among trees inoculated on 1 June as compared with 30% frequency among trees inoculated on 15 June.

Species combination was the principal source of variation, with highly significant differences among means for each variable. Variance ratios were large and of similar magnitude for each variable.

TABLE 1. Results of analysis of variance for six measures of response by 46 elm progenies to artificial inoculation with *Ceratocystis ulmi*

Disease variables	Source of variation[a]			
	Time of inoculation	Species combination	Progeny within combination	Time-progeny interaction
Frequency of symptoms				
Dead or wilted leaves, 3 weeks	1,900[b]**[c]	52**	1.2	<1
Dead or wilted leaves, 6 weeks	43	123**	1.9**	1.7*
Dead branches, 56 weeks	284*	82**	1.7*	1.2
Dead trees, 56 weeks	62	64**	4.1**	1.2
Intensity of symptoms				
Crown damage, 6 weeks	425*	57**	2.4**	2.7**
Crown damage, 56 weeks	42	46**	1.6*	<1

[a] A split-plot design was used with two blocks and two inoculation times in the main plot, and 46 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 6 species combinations of elms artificially inoculated with *Ceratocystis ulmi*

Disease variables	Species combination[a]						Approximate[b] $w_{.05}$
	p × p	p × pr	p × r	pr × pr	r × pr	a × wind	
Sample size (number)							
Progenies	8	11	16	2	7	2	
Individuals	350	415	463	81	249	91	
Frequency of symptoms (%)[c]							
Dead or wilted leaves, 3 weeks	7	16	59	21	68	81	12
Dead or wilted leaves, 6 weeks	7	31	73	47	88	98	10
Dead branches, 56 weeks	3	21	65	59	85	97	12
Dead trees, 56 weeks	0	2	22	14	53	40	10
Intensity of symptoms (score)[d]							
Crown damage, 6 weeks	1.1	1.3	1.9	1.7	2.4	3.1	0.5
Crown damage, 56 weeks	1.2	1.9	3.5	2.7	4.4	4.1	0.7

[a] p = *Ulmus pumila*; 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 after 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%.

Variation among progenies within species combinations contributed relatively little to total variation, although statistically significant differences were present for five of six variables. Separate analyses of variance for progenies in each species combination revealed that the heterogeneity was mostly among F_1 hybrid progenies. For example, frequency of dead branches at 56 weeks varied from 40 to 80% among p × r crosses. In some combinations, the superiority or inferiority of specific parent trees was consistently reflected in crosses with other trees.

Interaction between time of inoculation and progeny was minor except in crown damage evaluated at 6 weeks after inoculation. The absence of substantial interaction or differential response of different progenies to different times of inoculation is reassuring because it allows a clear interpretation of the major source of variation; namely, species combination.

A summary of the disease responses of progenies from different species combinations is presented in Table 2. Comparison of symptom frequency, 3 and 6 weeks after inoculation, points out the influence of evaluation time on results. Although relative ranking of susceptibility did not change between 3 and 6 weeks, average frequency of symptoms increased for all species combinations except p × p. By 56 weeks after inoculation, recovery mechanisms had eliminated symptoms in some trees so that symptom frequency was generally lower.

Intensity of symptoms, as measured by crown damage score, was higher when measured at 56 weeks than at 6 weeks for all species combinations. To a minor extent, the crown damage scores at 56 weeks may include snow and cold damage as well as further disease development.

The most striking result, illustrated in Table 2, was the association between disease response and proportion of germ plasm from *U. rubra*. Susceptibility and disease intensity increased as the proportion of genes from *U. rubra* increased until, in backcrosses to *U. rubra* (r × pr), disease response was comparable to response in the highly susceptible *U. americana*.

One apparent anomaly is the disease response of p × r and pr × pr hybrid progenies, both of which should contain equal amounts of germ plasm from each species. The somewhat lower disease response of pr × pr hybrids may result from using natural hybrids as parents. One of the pr parents may contain more than 50% germ plasm from *U. pumila*.

Associations among the six disease response

TABLE 3. Simple linear correlation coefficients (r) among six measures of response by elm progenies to artificial inoculation with *Ceratocystis ulmi*

Disease variables	Disease variables				
	B	C	D	E	F
Frequency of symptoms (%)[a]					
Dead or wilted leaves, 3 weeks (A)	.87[b]	.76	.63	.69	.70
Dead or wilted leaves, 6 weeks (B)		.87	.63	.71	.75
Dead branches, 56 weeks (C)			.73	.73	.77
Dead trees, 56 weeks (D)				.72	.73
Intensity of symptoms (score)[c]					
Crown damage, 6 weeks (E)					.63
Crown damage, 56 weeks (F)					

[a] Data were plot-mean percentages of trees with symptoms when observed after the indicated period following inoculation.

[b] Coefficients greater than .20 are statistically significant at a probability of 99% ($r_{.01}$ = .20 with 182 degrees of freedom).

[c] Data were plot-mean 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%.

TABLE 4. Simple linear correlation coefficients among selected measures of response by selected elm progenies to artificial inoculation with *Ceratocystis ulmi*

Cross	No. observations	Pairs of disease variables			
		A-B[a]	B-D	B-E	E-F
p × p[b]	32	.55	0	−.07	0
p × pr	44	.82	.36	.49	.45
p × r	64	.80	.41	.40	.64
r × pr	28	.53	.39	.40	.61

[a] A = frequency of trees with dead leaves after 3 weeks; B = frequency of trees with dead leaves after 6 weeks; D = frequency of dead trees after 56 weeks; E = score of crown damage after 6 weeks; F = score of crown damage after 56 weeks.

[b] p = *Ulmus pumila*; r = *U. rubra*.

variables are presented in Table 3 for the experiment as a whole. Correlation coefficients, calculated using plot means, indicated a moderate to high degree of association among the different measures of response, suggesting that all disease response variables were fairly closely related. When associations were estimated within species combinations, however, the results were much less conclusive. In Table 4, correlation coefficients are generally lower, and vary widely depending on the disease response variable and species combination. For example, the association between foliar symptoms observed at 3 and 6 weeks is moderate to high for all species combinations, but far less association is apparent between frequency of foliar symptoms at 6 weeks and frequency of dead trees at 56 weeks. In the latter case, a correlation coefficient of 0 for p × p progenies reflects the absence of mortality.

DISCUSSION.—Results from analyses of variance illustrate important aspects of experimental design in testing elms for susceptibility to *Ceratocystis ulmi*. Of primary importance is the timing of inoculation to coincide with maximum host susceptibility. Seasonal variation in susceptibility is well documented (3) and the substantial variation attributable to different inoculation times in this study reflects a large decrease in susceptibility over a period of 2 weeks. In initial stages of breeding programs where many progenies are being studied, available plant materials and space probably will preclude screening with more than one inoculation per season. Knowledge of susceptibility patterns under local environments will thus be necessary if screening is to be efficient.

Seasonal susceptibility patterns also are central to the question of interaction between progenies and inoculation time. Interaction was minor in this study presumably because all progenies had similar susceptibility patterns. Studies in which host materials are highly diverse in terms of genetic and climatic origin may include important time-progeny interactions if susceptibility patterns differ.

One approach to eliminating uncertainties associated with variation in susceptibility would be to determine seasonal susceptibility patterns for each clone or seed-propagated progeny intended for commercial use. Single fungal inoculations at the presumed peak of susceptibility in two different years, selection of ornamentally desirable trees with negligible disease symptoms, clonal propagation, and periodic inoculation of different ramets from May to August should effectively insure reliable evaluation of disease response for elm clones to be released in commercial use. A similar scheme with selection of half- or full-sib progenies and omitting clonal propagation could be used for seed-propagated progenies.

Results from analyses of variance also illustrate important features of host variation in disease response. Variation among species combinations was much greater than within species combinations. Breeding efforts thus should concentrate on identifying the most promising species combinations before initiating intensive estimation of breeding value for individual trees. The presence of significant, though small, variation among progenies within species combinations demonstrates a potential for progress through selection of individual parents for crossing and emphasizes the need to base evaluation of species combinations on crosses among several individuals.

Arrays of disease response as measured by several variables, illustrate the polygenic nature of susceptibility in crosses between *U. pumila* and *U. rubra*. Even in the backcross p × pr, where r contributed only 25% of the germ plasm, disease response was too high to recommend general landscape use of such progenies. For seed-propagated materials, the genes conveying desirable ornamental traits of *U. rubra* seem inaccessible without advanced-generation breeding and selection to combine ornamental value with low susceptibility. The presence of symptomless individuals in all progenies, however, suggests that vegetative propagation may allow development of *U. rubra*-like individuals of very low susceptibility in most types of crosses. This possibility will be explored in the materials from the present study.

The importance of association among disease response variables depends on experimental objectives. The moderate to high correlation coefficient for the data as a whole suggests that disease development was fairly consistent over time and among the genetically diverse groups of host material. For example, determining the frequency of trees with dead leaves 6 weeks after inoculation would give a fairly good picture of relative disease response throughout the first year. For some studies, one response variable may be adequate for evaluating variation in susceptibility. Evaluation at 6 weeks avoids potential confounding effects of winter although disease recovery mechanisms may be overlooked.

Correlation coefficients calculated for much narrower ranges of variation within species combinations illustrate the need for matching data with objectives. Associations estimated over the whole experiment had little value in estimating association of variables within species combinations.

As a consequence, multiple evaluations may be necessary to achieve a comprehensive picture of disease response among progenies of similar genetic background. Selection of highly resistant individuals of *U. pumila*, for example, would require earlier evaluation than selection in other species combinations, whereas studies of disease recovery mechanisms would require materials for which both early and late evaluations were available.

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