Response of Elm Species and Clones to Inoculation with Verticillium albo-atrum

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ABSTRACT

Seedlings of three elm species, and two clones from each of five different elm species or hybrid families, were inoculated with *Verticillium albo-atrum*. Response was measured as reduction in stem elongation.

For seedlings, growth reduction was greatest in American elm and least in Siberian elm. Patterns of response to different concns of inoculum were curvilinear, with apparent threshold values at about 2×10^4 propagules/ml for American elm and 2×10^5 propagules/ml for Siberian elm. In most treatments, one or more seedlings grew as rapidly as control seedlings. The possibility that absence of growth reduction following inoculation has a genetic basis is being investigated.

For clones, there was wide variation in the amount by which stem elongation was reduced. One pair of sibling hybrid clones showed little reduction but most variation was among clones irrespective of genetic similarity. Another experiment involving four clones inoculated and grown under three watering regimes substantiated clonal variation in response to the pathogen and illustrated additive effects of reduced moisture availability and the pathogen on stem elongation.

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Additional key words: host variation, wilt disease, disease resistance.

Verticillium wilt is a common problem in shade tree culture. The causal organism, *Verticillium albo-atrum* Reinke & Berth., is widely distributed as a soil-borne fungus and includes a host range of 270 species in 18 orders (2, 3). Elm species of North America, Europe, and Asia have been included as susceptible to this disease (4). Disease symptoms in elm include sharply reduced growth in seedlings and wilting of infected branches in older trees (7).

Impetus for a closer look at response of elms to *V. albo-atrum* was provided when an outstanding elm hybrid, a product of breeding for resistance to *Ceratocystis ulmi* (Buisman) C. Moreau, exhibited Verticillium wilt. The following experiments were conducted to estimate whether variation in response to *V. albo-atrum* was present within and among elm species.

MATERIALS AND METHODS.—*Seedlings.*—Host materials of American elm (*Ulmus americana* L.) were seedlings from wind-pollinated seed collected on city streets. For Siberian elm (*U. pumila* L.) wind-pollinated seed was obtained from plantation trees and for Japanese elm (*U. japonica* (Rehd.) Sarg.) one full-sib family produced by controlled pollination was used. Seeds were germinated and grown in vermiculite until inoculation.

For a preliminary experiment on American elm, the inoculum was prepared from pure cultures of *V. albo-atrum* isolated from Amur maple (*Acer ginnala* Maxim.) and maintained on potato-dextrose agar (PDA). For screening of the three elm species, a second isolate from eggplant (*Solanum melogena* var. *esculentum* Nees) was used. Inoculum was prepared on the day of inoculation by flooding PDA cultures with sterile water and scraping fungal material from the agar. Crude inoculum thus obtained was homogenized in a blender and filtered through cheesecloth. The resulting suspension was designated as maximum concn and a dilution series was prepared using successive one-tenth dilutions with sterile water. Inoculum concn was estimated by plating 1 ml of each dilution (further diluted 1/100,000) on PDA, and counting the number of viable propagules after 4 days. Concentrations ranged from 1.6×10^4 to 2.6×10^7 viable propagules/ml.

Seedlings to be inoculated were removed from vermiculite and immersed for 2 min in petri dishes containing the inoculum suspension. During immersion, about one-third of the root system was removed using a razor blade. This treatment was intended to fulfill the apparent requirement of root wounds for fungal invasion (1). A comparable treatment was applied in sterile water for control seedlings.

After inoculation, seedlings were transplanted in 10.2-cm (4-inch) diam plastic pots. The sterilized soil was an artificial mixture of Hancock sand and peat moss (1:1, v/v) plus calcium, phosphorus, potassium, and nitrogen fertilizers. Potted seedlings were watered daily for the first week after transplanting to insure establishment. Thereafter, watering was approximately weekly. Soil moisture, as evidenced by soil color, reached low levels between watering dates but few seedlings, including the more vigorous controls, wilted.

As the first experiment proceeded, it was evident that growth reduction was the principal treatment effect. Leaf discoloration, necrosis, and wilting were occasionally observed but were too infrequent to use in treatment evaluation. Stem length or seedling height was chosen as the variable by which to compare treatments. Treatments could be differentiated by eye after 30 days and final evaluation was made on seedlings harvested after 50 or 60 days. At harvest, stem lengths were measured and leaf traces were checked for vascular discoloration as evidence of infection. Vascular discoloration was present for most seedlings inoculated with the pathogen although attempts to reisolate the fungus gave erratic results. Data were summarized as treatment means and treatment differences were determined by analysis of variance and a multiple comparison method (8).

Clones.—Ten host clones representing diverse genetic backgrounds were chosen for testing. Each clone was represented by from 40 to 80 ramets produced through rooting of leaf-bud cuttings in a mist-propagation bed. Cuttings were transplanted to a potting mixture, grown to about 30 cm in height, and forced into dormancy in a cold room. The dormant cuttings were trimmed to a root length of about 40 mm and stem-length of 15 cm. After 3 mo in cold storage, the cuttings were transplanted in 10.2-cm (4-inch) diam plastic pots and moved to a greenhouse. The potting medium was as described for seedlings. The potted trees broke dormancy and were allowed to grow until stems reached heights of 30-45 cm. Growing conditions were natural daylength (13-15 h) and temp (15-30 C) typical for May and June.

The inoculum from Amur maple, was prepared as previously described. A three-level dilution series, as measured by viable propagules on PDA, gave concns of 1.6×10^5, $\times10^6$, and $\times10^7$ propagules/ml. Sterilized water was used for control inoculations.

All inoculations were made on the same day by flooding a razor incision on the lower stem with inoculum. Clone and inoculum level were chosen randomly for treatment. Clones containing 40 ramets were distributed randomly with 10 ramets per inoculum concn. Four clones for which 80 ramets were available were distributed to provide 6 ramets per concn for long and short watering cycles, and 8 ramets per concn for the medium water cycle used where watering regime was not an experimental variable. Short, medium, and long watering cycles were approximately 3, 5, and 7 days respectively. The long watering cycle occasionally resulted in wilting foliage between waterings.

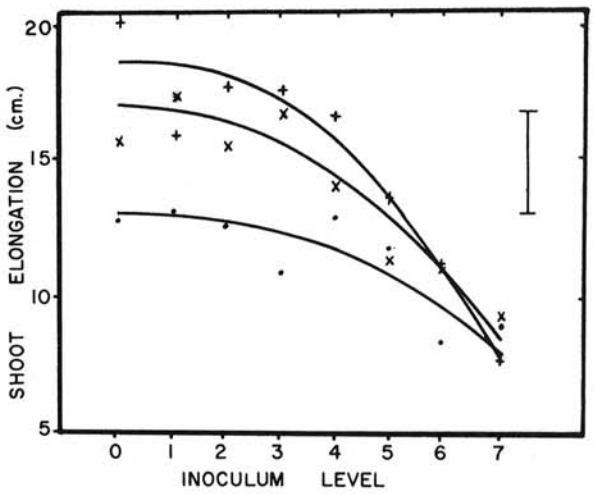

Fig. 1. Shoot elongation of *Ulmus americana* (+), *U. japonica* (x), and *U. pumila* (·) 60 days after inoculation with *Verticillium albo-atrum* at eight inoculum concns. Inoculum levels were a tenth-dilution series starting at 2.6×10^7 propagules/ml (level 7). Datum points separated by vertical distances greater than the length of the vertical line at the right differ significantly, $P=0.05$.

TABLE 1. Mean height (cm) of American elm seedlings 51 days after inoculation with *Verticillium albo-atrum* at five inoculum concns. Two seedling developmental stages were tested

Inoculum concn (propagules/ml)	Mean height (cm) at developmental stage	
	Two nodes	Six nodes
0	22.5	39.7
1.6×10^4	...	36.3
1.6×10^5	14.4	29.8
1.6×10^6	13.3	27.4
1.6×10^7	11.6	...
LSD[a]	2.4	3.2

[a]Means differing by at least the indicated amount represent statistically different treatment effects, $P = 0.05$.

TABLE 2. Results from analysis of variance for seedling height growth by three elm species 31 and 60 days after inoculation with *Verticillium albo-atrum* at eight inoculum concns

Source of variation	Degrees of freedom	F-ratio
Block	4	1.4
Species	2	29.3[a]
Inoculum concn	7	16.7[a]
Time of measurement	1	944.3[a]
Species × concn	14	2.4[a]
Species × time	2	5.6[a]
Concn × time	7	4.7[a]
Species × concn × time	14	1.0
Error	188	

[a]Means differ statistically, $P = 0.01$.

Response to the pathogen was measured as stem elongation 18 and 48 days after inoculation. At harvest, after 48 days, disease symptoms were confirmed by examining each tree for vascular discoloration. Presence of the causal organisms was verified by plating several tissue samples. Data on stem growth were summarized to provide mean responses, and differences among means were estimated by analysis of variance and multiple-range tests.

RESULTS.—*Seedlings.*—The preliminary experiment with American elm used four inoculum concns and two stages of seedling development with 30 seedlings per treatment. The results are summarized in Table 1. At 51 days after inoculation, growth reduction was a function of inoculum concn although the data suggest curvilinear response with little difference among means at concns above 1.6×10^5 propagules/ml. Response patterns were similar for the two developmental stages. Growth reduction, as a percentage of control seedlings, was somewhat less for seedlings inoculated at the six-node stage, probably due to greater vigor at the time of treatment.

The experiment to compare American, Siberian, and Japanese elm was evaluated 31 and 60 days after inoculation. Analysis was based on mean stem elongation of 15 seedlings for each treatment. The composite analysis of variance in Table 2 illustrates main effects and several interactions. Treatment means clearly differed for species, inoculum concn, and time of measurement. In Fig. 1, fitted curves of seedling growth response to inoculum concn show that the degree of growth inhibition by the fungus was in the following order: American elm >

TABLE 3. Stem elongation, expressed as a percentage of controls, by seedlings of three elm species 60 days after inoculation with *Verticillium albo-atrum* at eight inoculum concns

Ulmus spp.	Stem elongation (% of control) at inoculum concn (2.6 × propagules/ml)								
	0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	LSD[a]
American elm	100	78	88	87	82	67	52	36	19
Japanese elm	100	110	99	107	90	71	69	59	37
Siberian elm	100	101	98	90	110	100	68	77	47

[a]Within rows, means differing by at least the indicated amount are statistically different, $P = 0.05$.

TABLE 4. Stem elongation, expressed as a percentage of noninoculated controls, for each of ten elm clones inoculated with *Verticillium albo-atrum*

Clones	Parentage	Relative stem elongation (%)
5-2	(*U. glabra* × *carpinifolia*) × wind[a]	98
5-5	(*U. glabra* × *carpinifolia*) × wind[a]	69
7-4	(*U. pumila* × *rubra*) × wind	81
7-6	(*U. pumila* × *rubra*) × wind	74
43-5	(*U. japonica*)	90
408-6	(*U. japonica*)	67
44-11	*U. pumila* × *japonica*	89
44-26	*U. pumila* × *japonica*	99
185-1	*U. americana*	59
185-2	*U. americana*	78
LSD[b]		9

[a]*U. pumila* was the highly probable pollen parent.
[b]Means differing by at least the indicated amount differ statistically, $P = 0.05$.

Japanese elm > Siberian elm. Statistical interaction was the consequence of the different response pattern for Siberian elm.

Inoculum concn was a major effect and produced a curvilinear pattern of growth reduction. A concn threshold above which growth reduction was severe is suggested in Fig. 1 and Table 3. Approximate thresholds were 2.6×10^4 propagules/ml for American elm and Japanese elm, and 2.6×10^5 for Siberian elm.

Time of measurement similarly was a major effect but was biologically meaningful only as an illustration that the infected trees continued to grow. More important was the observation that although treatment effects were evident at 31 days, data collected at 60 days was much more clearly interpretable, especially for response at lower inoculum concns. The statistical interactions with time of measurement were interpreted as reflecting premature measurement at 31 days. For example, American elm seedlings inoculated at a concn of 2.6×10^4 propagules/ml were 102% as tall as noninoculated seedlings at 31 days and 82% as tall at 60 days.

To relieve the interpretative problems posed by interactions, 60-day data were considered in an additional way. Part of the difference among species in response to *V. albo-atrum* was inherent difference in rate of stem elongation. By transforming actual measurements of stem elongation to percentage of growth by control seedlings, data for different species were placed on a common base. The results of Table 3 substantiate points noted earlier about differential response of species both in terms of general severity of growth retardation, and pattern of reaction to different inoculum concns. Larger numbers of seedlings per treatment presumably would produce a less erratic pattern of response.

Clones.—Response following inoculation was rapid and stem elongation of ramets exposed to the pathogen was reduced to about 80% of the controls. The difference between infected and control trees was statistically significant ($P = 0.01$). Growth reduction increased as inoculum concn increased but the effect of concn was minor.

In the second growth period, stem elongation was sharply reduced in both infected and control ramets. Again, ramets inoculated with the fungus grew about 80% as fast as controls, but the difference was not statistically significant. Presumably the difference between growth periods reflected generally declining stem elongation

TABLE 5. Relative mean stem elongation of four elm clones 18 days after inoculation with *V. albo-atrum* at four inoculum concns and being grown under three watering regimes

Watering cycle[a]	Inoculum concn (propagules/ml)			
	Control	1.6×10^5	1.6×10^6	1.6×10^7
Short	100	83	86	91
Medium	98	86	87	76
Long	88	73	74	74

[a]Approximate number of days between waterings was 3, 5, and 7 for short, medium, and long cycles, respectively.

either as a consequence of root inhibition in small pots or the endogenous growth pattern. White flies (Aleyrodidae) were also numerous on leaves during the second growth period.

Clonal response to the pathogen is summarized for 18-day data in Table 4. Analyses of 18-day and 48-day data differed only in statistical precision. The principal feature of Table 4 is the range of variation in response to *V. albo-atrum*. The clones for testing were chosen in pairs within species or family to estimate the relative importance of similar genetic backgrounds. It is evident that the major variation was among clones rather than between species or families. Our studies with root inoculation of seedlings showed that American elm was much more susceptible to the pathogen than Siberian elm. The American elm clones tested here similarly showed large growth reductions, but clones containing Siberian elm genes were not necessarily resistant to the fungus.

The experiment utilizing four clones and three watering regimes resulted in significant differences among clones, inoculum concn, and watering cycle for both 18-day and 48-day data. The absence of interactions with watering cycle means that each clone responded similarly to moisture stress and the joint effects of moisture stress with inoculum concn. The clones tested, 5-5, 43-5, 44-11, and 44-26, followed the same order of response to the fungus as in the larger study, although the range of mean response was only between 90 and 102% of controls.

Individual and combined effects of inoculum concn and watering are summarized in Table 5. The data suggest that the pathogen accounted for approximately 10-15% growth reduction whereas the extended drying associated with a long watering cycle reduced shoot elongation an additional 10%.

DISCUSSION.—These experiments showed a wide range of host variation in response to *V. albo-atrum*. The basis for seedling variation could have been either resistance or escape. Marked differential response among clones supports an hypothesis of a genetic basis for host variation, but identification of individual resistant seedling genotypes probably will require clonal testing. Both seedling and clonal studies demonstrated species and individual-tree components in the variation pattern.

Our clonal data, unlike an earlier report (5), did not necessarily suggest common resistance mechanisms for *V. albo-atrum* and *C. ulmi*. Response to the two diseases corresponded only for one highly resistant, and two highly susceptible clones. Association between growth rate of controls and response to *V. albo-atrum* similarly was insignificant ($r = +0.1$) although vigor and response to *C. ulmi* were often correlated (6).

If the results reported here are verified under field conditions, resistance to *V. albo-atrum* and *C. ulmi* perhaps can be combined through vegetative propagation. Genotypes resistant to *V. albo-atrum* may be identified in seedling populations, then multiplied by rooting of cuttings and utilized as rootstocks for *C. ulmi* resistant scions. Disease control by genetic barriers in the form of resistant rootstocks has been an effective approach (9). If vegetative propagation proves unsatisfactory, Siberian elm amy provide genes useful for developing trees resistant to *V. albo-atrum* through selection and breeding.

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