Pathogenicity of *Ceratocystis ulmi* Isolates from Resistant Host Trees in California

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ABSTRACT

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California isolates of *Ceratocystis ulmi* from naturally infected *Ulmus americana, U. pumila, U. parvifolia*, and *Zelkova serrata* were tested for pathogenicity to *U. americana, U. parvifolia*, and *Z. serrata*. Field symptoms of naturally infected *U. parvifolia* and *Z. serrata* were very limited. All *C. ulmi* isolates tested, including those from the resistant hosts, proved to be severely pathogenic to the susceptible indicator *U. americana*.

The initial discovery of Dutch elm disease (DED) in California (6) was in an American elm (*Ulmus americana* L.). Since then, *Ceratocystis ulmi* (Buism.) C. Mor. has been commonly isolated from naturally infected American elms, Siberian elms (*U. pumila* L.), and various European species including their hybrids. In 1978, however, five Chinese elms (*U. parvifolia* Jacq.) and two Japanese Zelkovas (*Zelkova serrata* Thunb.), both considered resistant to DED (7,9), were discovered in California that were naturally infected with *C. ulmi*. A resistant tree is defined as one that is parasitized but does not develop severe symptoms (8). In 1979, three DED-infected Chinese elms were found, and in 1980, eight more Chinese elms were added to the list.

Isolates from naturally infected resistant California hosts (*U. parvifolia, U. pumila*, and *Z. serrata*), which were relatively symptomless in the field, were tested for pathogenicity on a sensitive "indicator," ie, American elm. Pathogenicity of these resistant host isolates was compared with that of an isolate from an American elm that exhibited typically severe field symptoms.

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MATERIALS AND METHODS

***C. ulmi* isolates.** The *C. ulmi* isolate from *Z. serrata* (P5791-78) was cultured from a naturally infected tree in Redwood City, CA, in 1978. The Chinese elm isolate (P5717-78) and the Siberian elm isolate (P6068-78) were obtained from trees in Palo Alto, CA, in 1978, and the American elm isolate (P5116-78) was obtained from a tree in Los Altos, CA, also in 1978. All four isolates of *C. ulmi* used in these tests were typical aggressive isolates (2) of the B mating type, as were all of the more than 200 California isolates we have tested. Control A and B mating type cultures were supplied by R. J. Campana. *C. ulmi* isolates were grown on sterilized American elm wood slices cut from DED-free American elms from outside the California DED area. The cultures were single-spored and subsequently hyphal-tipped to several plates of elm extract agar (EEA) (4). The plates were incubated at 24 C for 7 days.

Inoculum spore suspensions were prepared at the time of inoculation by flooding two plates with sterile distilled water. Spore suspensions were pooled, the concentration adjusted to 10^7 spores per milliliter, and the inoculum drawn up into a 2.5-ml hypodermic syringe that had a 20-gauge needle.

Host trees. Host trees used in these tests were standard nursery stock trees in 20-L cans. Five-year-old American elms were purchased from Orange County Nursery, Norwalk, CA 90650. Two-year-old Chinese elms (*U. parvifolia*) and Zelkovas (*Z. serrata*) were purchased from Oki Nursery, Sacramento, CA 95826.

Inoculations. Inoculations were done by making a tangential incision into the xylem with a 3-mm gouge, then immediately placing a drop of spore suspension in the wound and allowing the inoculum to be drawn up into the tree's vascular system by transpiration. For each isolate tested, nine American elms, six Chinese elms, and six Zelkovas were inoculated. Each tree was inoculated in five places along the trunk with 1 ml of inoculum per tree. Control trees were inoculated with sterile distilled water.

Pathogenicity tests for the Zelkova isolate were conducted in Palo Alto, CA, in a custom-built screenhouse 2 × 2 × 4 m covered with insectproof material (Saran screen #51032-00, Chicopee Manufacturing Co., Cornelia, GA 30531). Trees were inoculated on 9 May 1979 and harvested 37 days later for processing and reisolation of the pathogen. Tests using the *C. ulmi* isolates from Chinese elm and American elm were conducted in Sonoma, CA, in a screenhouse 4 × 4 × 4 m. Trees were inoculated on 13 May 1980 and harvested for processing 35 days later. Eight Chinese elms from 1980 tests were permitted to incubate until 10 December 1980 (212 days), at which time they were harvested and processed.

Reisolation of the pathogen. Recovery of the inoculated fungus to complete Koch's postulates was accomplished by aseptically

Table 1. Pathogenicity of isolates of *Ceratocystis ulmi* originating from various hosts

Hosts	Mean of symptom expression ratings														
	Ceratocystis ulmi isolate														
	Ulmus americana			*Ulmus pumila*			*Ulmus parvifolia*			*Zelkova serrata*[a]			Water control		
	Int.[b]	Ext.[c]	% Rec.[d]	Int.	Ext.	% Rec.	Int.	Ext.	% Rec.	Int.	Ext.	% Rec.	Int.	Ext.	% Rec.
Ulmus parvifolia	1.0	0	83	1.0	0	100	1.0	0	100	2.1	0	100	0	0	0
Zelkova serrata	3.0	1.0	100	3.0	1.1	100	3.0	1.8	100	1.7	0	100	0	0	0
Ulmus americana	2.2	2.2[e]	89	2.2[e]	2.2[e]	89	2.6	2.0	100	3.0	3.0	100	0	0	0

[a] All *Zelkova serrata* tests were conducted during the 1979 season; all other isolates were tested during the 1980 season.
[b] Internal symptom rating: 0 = no streaking, 1 = streaking involved less than one-fourth of the circumference of the trunk, 2 = streaking involved more than one-fourth but less than one-half of the circumference, and 3 = streaking involved more than one-half of the circumference.
[c] External symptom rating: 0 = no wilting, 1 = less than one-fourth of the canopy wilted, 2 = more than one-fourth but less than one-half of the canopy wilted, and 3 = more than one-half of the canopy wilted.
[d] Percent recovery of *C. ulmi* from the inoculated trees.
[e] Although 30 of 36 *U. americana* inoculations had a rating of 3 both internally and externally, some *U. americana* inoculations did not "take." The resulting 0 ratings are reflected in these mean figures.

plating out pieces of streaked wood on elm extract agar (EEA) (4). Septic cultures (1) were also made. After five days of incubation at 24 C, all plates were examined daily for the presence of *C. ulmi*.

Evaluation of pathogenicity. The method used to evaluate pathogenicity was similar in part to that of Santamour (7), who used an estimate of the percentage of canopy exhibiting wilt symptoms. External wilt symptoms were numerically rated as follows: 3 = more than one-half of the tree's canopy exhibited wilting, 2 = less than one-half but more than one-fourth of the tree's canopy exhibited wilting, and 1 = less than one-fourth of the tree's canopy exhibited wilting.

Internal streaking was rated as follows: 3 = streaking involved more than one-half the circumference of the trunk at midtrunk level (about breast height for most of the trees), 2 = streaking involved more than one-fourth but less than one-half of the circumference of the trunk, and 1 = streaking involved less than one-fourth of the circumference of the trunk (frequently, this type of discoloration appeared as just a few unconnected dots in cross section).

RESULTS

All four isolates of *C. ulmi* from Siberian elm, American elm, Chinese elm, and Zelkova were severly pathogenic to American elm with respect to internal and external symptoms (Table 1). The DED fungus was reisolated from all but two American elms inoculated with the various isolates of *C. ulmi*. Thirty of 36 inoculated American elms had ratings of 3 for both internal and external symptoms. Two of the American elm inoculations (one each with the American elm and Siberian elm isolate) failed to "take." Thus, 0 ratings are reflected in the mean symptom rating figures in Table 1 (footnote e). No water-inoculated control American elms developed symptoms and no fungus was isolated from them.

In the 1979 tests, Zelkovas inoculated with the Zelkova isolate of *C. ulmi* exhibited streaking in categories 1–2, with no external wilting symptoms. In the 1980 tests, limited to moderate wilting symptoms in categories 1–2 developed on each Zelkova inoculated with either the Siberian elm, American elm, or Chinese elm isolate of *C. ulmi*. Internally, however, the wood of each inoculated Zelkova exhibited extensive streaking.

Inoculated Chinese elms exhibited no external wilting symptoms but did exhibit limited to moderate streaking and in one case, extensive streaking. The DED fungus was reisolated from every Chinese elm inoculated with the Zelkova isolate.

In the 1980 tests using isolates of *C. ulmi* from Siberian elm, American elm, and Chinese elm, inoculated Chinese elms showed limited wood discoloration and no wilting symptoms after 2 mo of incubation. It was decided at that time to permit eight of the inoculated Chinese elms to incubate for a longer period to see if any wilting symptoms would develop. After five additional months, there were still no external symptoms and the trees' streaking symptoms remained in category 1. *C. ulmi* was reisolated from the inoculated Chinese elms in each case except one, which was inoculated with the American elm isolate, even though that one Chinese elm was indistinguishable from the others with respect to wood streaking. Repeated attempts to reisolate the fungus from several other parts of the discolored wood of the one tree were also unsuccessful.

All 1979 and 1980 control Chinese elms remained free of symptoms and *C. ulmi* was not isolated from them.

DISCUSSION

Internal and external field symptoms of naturally infected Chinese elms and Zelkovas were generally very limited. The results of these pathogenicity tests indicated that all the *C. ulmi* isolates were highly virulent as demonstrated by the severe internal and external symptoms expressed on inoculated American elms. This greater susceptibility of American elms to *C. ulmi* compared with other hosts is consistent with the observations of Smucker (10). Internal and external symptom expression in inoculated Zelkovas was variable (Table 1). External symptoms were consistently lacking in inoculated Chinese elms even after 6 mo, although internal symptoms were variable.

Naturally infected Chinese elms and Zelkovas as well as Siberian elms were difficult to detect in the field by observation of external symptoms. The two naturally infected Zelkovas in 1978 were also found to have very limited internal symptoms when the trees were removed and dissected. Naturally infected Chinese elms had internal discoloration but generally exhibited only subtle external symptoms. Only occasionally would one exhibit more severe wilting symptoms. Naturally infected Siberian elms also characteristically showed only limited external wilting symptoms. The only consistent symptom among the resistant hosts was the presence of one or more small twigs with brown wilted leaves and associated limited wood streaking. Thus, it is possible for these resistant hosts to harbor a highly virulent strain of *C. ulmi* while exhibiting few or no detectable field symptoms. Holmes (3) recommended that such resistant host species should be scrutinized frequently. He also suggested that those showing severe symptoms should be destroyed quickly to prevent them from serving as sources of inoculum for new pathogenic variants of *C. ulmi*.

Although reports of naturally infected *U. parvifolia* (5) and *Z. carpinifolia* (8) are known, this is the first report of pathogenicity tests of *C. ulmi* isolates from naturally infected *U. parvifolia* and *Z. serrata*. Our results indicate that such isolates are probably not new, mild, or unusual strains of DED but rather aggressive isolates as pathogenic as those isolated from severely wilted and streaked American elms. As Holmes (3) pointed out, resistant hosts such as Chinese elms and Zelkovas may be helpful in coping with DED but are certainly not a panacea. Under California conditions, these hosts may in fact prove

to be a severe hindrance in controlling DED because of the difficulty of detecting reservoirs of the pathogen that are highly virulent to the more susceptible elm species.

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