TOXIN PRODUCTION AND PATHOGENICITY OF CERATOCYSTIS ULMI[1,2]

by Lawrence Pusey[3] and Charles L. Wilson[4]

Abstract. Highly pathogenic isolates of *Ceratocystis ulmi* produced more toxin (cerato-ulmin) than less pathogenic ones. Toxin production may be a useful criterion for distinguishing aggressiveness among *C. ulmi* strains.

A long standing controversy exists as to whether Dutch elm disease (DED) symptoms are induced by a toxin or by mechanical blockage of vessel elements. Some argue that wilting is the primary leaf symptom and is caused by the production of gums and tyloses in vessel elements. Others feel that wilting is not the primary leaf symptom, but that foliar necrosis, chlorosis and desiccation are. In support of the latter view, some severely diseased trees can be found with few occluded vessels. This has led to the conclusion that *C. ulmi* primarily affects the living parenchyma tissue of the sapwood and that plugging of vessels is a secondary development (1, 11, 12). Such effects could be caused by a toxin.

The purpose of this investigation was to test Takai's (7, 9) findings that pathogenicity of *C. ulmi* is correlated quantitatively with the production of a toxin named cerato-ulmin. If true, the hypothesis that *C. ulmi* primarily affects living tissue is strengthened, and the amount of cerato-ulmin produced may be a useful way to assay the aggressiveness of *C. ulmi* strains.

Materials and Methods

Ceratocystis ulmi isolates from the United States were obtained from L.R. Schreiber at the USDA Nursery Crops Research Laboratory, Delaware, Ohio. Pathogenicity of the isolates was determined in two experiments in June 1979. For each isolate in one group (MA2, MA4, TN4, and TN5), eight 5-year-old rooted cuttings of American elm were stem inoculated with 3 ml of a spore suspension (10^6 spores/ml) using the technique of Sterrett and Creager (6). Another group of isolates (TN1, TN2, and TN3) was tested in the same way except that, for each isolate, twelve 3-year-old rooted cuttings were inoculated with 0.5 ml of a spore suspension. Six weeks after inoculation, disease was assessed as the percentage of the crown showing leaf symptoms (chlorosis, necrosis, or wilting). After 12 weeks, the percentage of the main stem showing dieback (death of buds and bark) was recorded.

All *C. ulmi* isolates that were tested for pathogenicity were also assayed for toxin. Isolates AL and ND, which are highly pathogenic strains of *C. ulmi* (5), were included in the toxin assays for comparison. A liquid medium that favors cerato-ulmin production (10) was inoculated with *C. ulmi* mycelium from PDA cultures. After a one-week incubaton on a shaker at 24 C, one drop of the liquid culture was added to 25 ml of medium in 125 ml Erlenmeyer flasks. These cultures were incubated on a gyratory shaker (125 rpm) at 24 C in the dark for one week. Cerato-ulmin production was determined for four cultures of each isolate; each experiment was performed three times.

Determination of cerato-ulmin in culture filtrate was done by the turbidity measurement method of Takai and Richards (8) with some modification. Following centrifugation of the shake culture at 11,000 rpm in a Sorvall SS-34 rotor (Norwalk, CT 06470) for 30 min, the supernatant was filter-sterilized through a 0.45 µm membrane, then divided into two test tubes. Air was blown into one sample with a Pasteur pipet for 5 min, causing it to

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bubble vigorously (Fig. 1). If a sample contained cerato-ulmin, it turned milky as the "unit" form of cerato-ulmin transformed into "rods" and "fibrils" (4, 7). Using the untreated sample from the same culture filtrate as a reference, we measured optical density at 400 nm. Cerato-ulmin production was expressed as $A_{400} \times 1000$.

Results and Discussion

Isolates which caused relatively little damage to inoculated trees also produced the least amount of cerato-ulmin, and vice versa (Table 1). Cerato-ulmin production varied greatly, and with the small number of isolates used, a correlation between cerato-ulmin production and ability to damage trees could not be quantified.

As suggested by Takai (7, 9), the production of cerato-ulmin might be a useful criterion for determining fungal pathogenicity. It appears that cerato-ulmin production may be a more sensitive indicator of pathogenicity than any of the cultural characteristics associated with aggressiveness (2, 3). This is illustrated by the fact that all isolates, excluding AL and ND, fell into one category (less aggressive) based on growth rate and their inability to produce synnemata; however, when relative production of cerato-ulmin

Table 1. Comparison of pathogenicity with cerato-ulmin production in isolates of *Ceratocystis ulmi*.

Exp. no. and isolates	Pathogenicity: Crown symptoms (%) after 6 wk	Pathogenicity: Crown dieback (%) after 12 wk	Cerato-ulmin production index[a]
Experiment I:[b]			
AL[d]	—	—	637 x
MA 2	9 z[e]	12 z	186 yz
MA4	88 x	85 x	330 y
TN4	40 y	50 y	210 y
TN5	1 z	4 z	60 z
Experiment II:[c]			
AL[d]	—	—	628 x
ND[d]	—	—	865 w
TN1	27 z	16 z	3 z
TN2	60 y	46 y	87 z
TN3	62 y	49 y	299 y

[a]Cerato-ulmin production index = $A_{400} \times 1000$.

[b]Eight 5-year-old American elm trees were inoculated June 1979.

[c]Twelve 3-year-old American elm trees were inoculated June 1979.

[d]Highly pathogenic isolates AL and ND (5) were included for comparison.

[e]Within the same column and experiment, values followed by different letters are significantly different by Duncan's multiple range test ($P=0.05$).

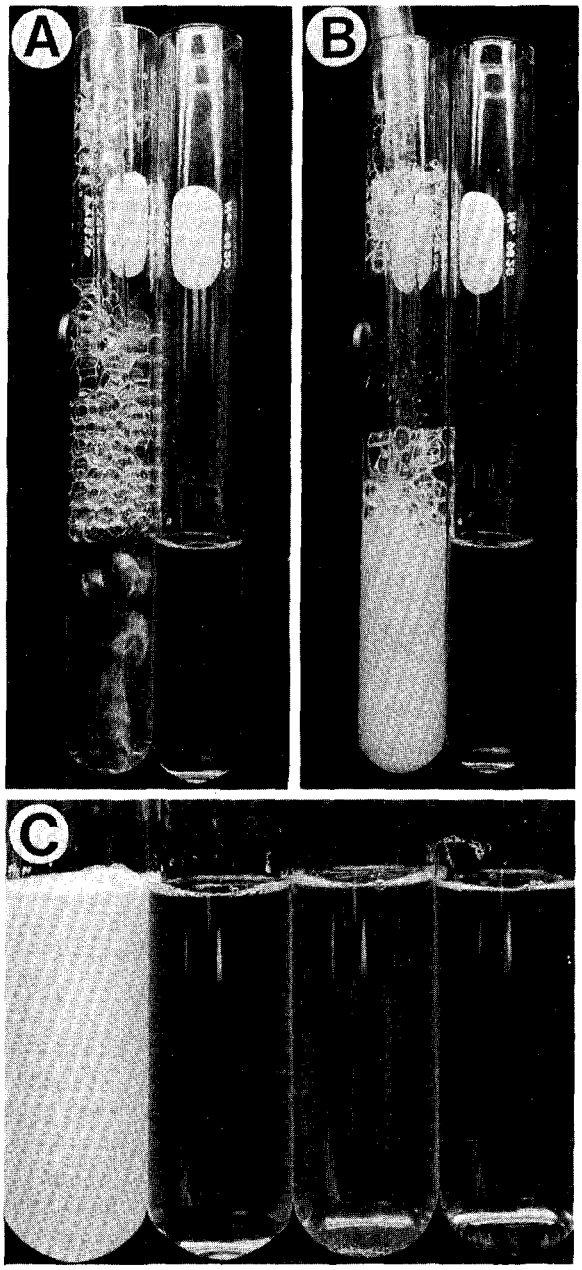

Fig. 1. *Cerato-ulmin* production by *Ceratocystis ulmi* evidenced by turbidity of centrifuged culture filtrate following aeration. A. With a Pasteur pipet, air was blown into the culture filtrate of isolate ND (left). Non-aerated filtrate is at right. B. Appearance of culture filtrate of isolate ND after several minutes of aeration. C. From left to right, aerated and non-aerated culture filtrate of isolate ND and aerated and non-aerated culture filtrate of isolate MA2. Isolate ND is highly pathogenic and isolate MA2 is weakly pathogenic (5).

was determined, differences were related to differences in disease expression.

Cerato-ulmin may be directly involved in the disease process, and its production may prove to be a good indicator of pathogenicity.

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ABSTRACT

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When I worked at a garden center during my undergraduate days at Ohio State, it was a standard joke to bet whether the customer could get his white-barked (usually *Betula pendula*) birch home and in the ground before the borers found it. Little has changed for the old-standard, borer-susceptible white birch. Some nurserymen have started to grow other white-barked birches that supposedly offer bronze-birch borer resistance. Before we progress too far, let's eliminate *B. pendula* (European white birch) and *B. populifolia* (gray birch) from consideration. Although gray birch is somewhat resistant to the borer, it suffers terribly from leaf miner and is short-lived — not exactly endearing traits for a landscape specimen. The search for the elusive, borer-free, white-barked birch continues but why not consider a native species, as a possible alternative. The river birch, *B. nigra,* has many significant advantages over the white-barked species. One is the resistance to bronze birch borer; the other relates to the excellent heat and cold tolerance. River birch, as a landscape tree, forms a graceful pyramidal to oval outine that with maturity becomes rounded. The average landscape size ranges from 40 to 60 feet. The foliage is lustrous dark green above, gray-green beneath, and triangular- to diamond-shaped in outline. The bark of river birch is fantastic, varies from gray-brown to cinnamon-brown, and exfoliates in papery curls and flakes. As plantsmen, nurserymen, landscape architects, and homeowners become more aware of this tree, it will assume a rightful place in the forefront of landscape plants.