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SCANNING ELECTRONMICROGRAPHS OF CERATO-ULMIN (CU) AND *CERATOCYSTIS ULMI* INDUCED VASCULAR SYMPTOMS IN ELM

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Internal vascular (xylem vessel) symptoms induced by cerato-ulmin (CU) and Ceratocystis ulmi *were observed using SEM and compared. Symptoms caused by CU were indistinguishable from those caused by* C. ulmi. *They are: a) edema-like surface alteration of vessel wall, b) granular deposit, c) pit membrane heaving, d) smooth coating, e) bubble and/or tylosis, f) foam, g) droplet, and h) callus-like development. Internal symptoms appear earlier in the CU-treated samples (as early as 2 hours) than in* C. ulmi-*infected trees (later than 10 hours).*

INTRODUCTION

Cerato-ulmin (CU) is a protein toxin produced specifically by *Ceratocystis ulmi* (Buism.) Moreau, the pathogen of Dutch elm disease (DED) (8, 9, 10). At a relatively low concentration, the toxin produces external symptoms similar to those of DED (10). Further studies have revealed that the lowest concentration to cause significant toxicity, evaluated by reduction in transpiration of elm cuttings, is as low as 10^{-1} μg/ml (Takai, unpublished). In addition, physiological disorders such as increased cellular respiration and damage to cell membrane permeability appeared parallel to the host reaction to *C. ulmi* (9).

In vivo production of CU in diseased white elm has also been confirmed serologically by using the antiserum against the purified CU (2, 11). It has been known that various internal symptoms such as tyloses, wall alterations, and plugging occur in xylem vessels of DED-infected trees (1, 3, 4, 5, 6, 7).

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It is important, therefore, to examine if CU-treated material also produces these internal symptoms. This presentation deals with SEM observation of internal xylem vessel symptoms in white elm (*Ulmus americana* L.) caused by *C. ulmi* infection and by CU application. More-detailed accounts will be published in two papers submitted by Takai and Hiratsuka to a journal.

MATERIAL AND METHODS

White elm grown in a greenhouse was used for the experiments, and for the observation of *C. ulmi*-infected material naturally infected samples collected in several locations in Ontario were also used.

For treatment with CU, both intact seedlings and cuttings of detached seedlings were used. CU isolated from a culture filtrate was purified and dissolved in distilled water. The solution was centrifuged (1500 x for 30 minutes) before using in order to keep CU from assembling into spheres and rods. Concentrations were between 10-60 μg/ml. CU was administered in two ways: 1) for intact elm seedlings, CU was administered through a slit that was made below the surface of the CU solution held in a cup around the stem, and 2) with detached seedlings, cuttings were inserted into a CU solution to enable them to take up the solution. The end of the cutting was cut back 1 cm every 24 hours in order to keep the cut end fresh. The solution was changed every 12 hours to minimize microbiological contamination.

For fungal inoculations, an aggressive North American strain, CESS-16K, was used. One drop of spore suspension containing about 10^8 spores/ml was applied to a slit made in the seedling about 5 cm above soil level.

For both treatments, sections of stem were taken from different intervals and different parts of the stem, depending on the extent of discoloration in the xylem wood. Debarked sections were sliced tangentially with a sharp razor blade in order to cut open xylem vessels of the outermost xylem wood, where the symptoms usually occur. These sections were vapor fixed with 2% osmium tetraoxide (OsO_4) for about 24 hours and then vacuum dried for 3 days until the specimens were completely dry.

RESULTS AND CONCLUSIONS

In general, in both *C. ulmi*-infected samples and CU-treated samples symptom development was erratic in terms of time of initiation and place of development; however, all internal xylem vessel symptoms observed in *C. ulmi*-infected samples were observed also in CU-treated samples. Internal symptoms could be detected earlier in the CU-treated samples (as early as 2 hours) than in the *C. ulmi*-infected samples (about 10 hours). Internal vessel symptoms that were observed in both treatments were: 1) edema-like wall surface alteration, 2) granular deposit, 3) pit membrane heaving, 4) smooth coating, 5) bubble and/or tylosis, 6) muddy coating, and 7) droplet and callus-like development. These symptoms occurred in various combinations in a same sample. Often more than one symptom was observed in the same vessel (Figs. 1, 5, 7, 8).

Edema-like surface wall alteration: The vessel wall surface was altered often to exhibit irregular, edema- or vine-like lines. This is the first observable symptom and can be recognized as early as 2 hours after CU treatment or 10 hours after *C. ulmi* inoculation.

Granular deposit: Granules of irregular sizes (up to 0.8 μ in diameter) were distributed over the vessel wall surface including pit membrane (Fig. 2) and on the surface of bubbles and/or tyloses (Fig. 5).

Pit membrane heaving: Often the pit membrane heaved up uniformly or irregularly during the early stages of symptom development. This may be the initial stage of tylosis development.

Smooth coating: A smooth, uniform coating was often observed on the vessel surface, over bubbles (Fig. 1), or on fungal elements in the vessel.

Bubble and/or tylosis: ~~Many~~ balloon-like structures of various sizes were observed in the vessel lumens (Figs. 3, 4, 5). They were either thin walled (Figs. 3, 4) or thick walled (Fig. 5). They can be interpreted as true tyloses or as bubbles according to the definitions of Ouellette (1980). The surfaces of these structures were mostly smooth (Figs. 3, 4) but sometimes were covered by a smooth coating (Fig. 1) or granular deposit (Fig. 5). They were observed as early as after 5 hours in CU-treated samples and 15 hours after *C. ulmi* inoculation.

Muddy coating: A thick coating of muddy appearance was spread over vessel walls. This could be the early stage of callus-like development.

Droplet: Droplets of various sizes and shapes were observed over vessel walls (Fig. 6) and sometimes on the surface of bubbles (Fig. 7). Although they were solid bodies in the fixed and dried samples, originally they probably exist in liquid form.

Callus-like development: Often callus-like material developes in vessels and plugs the vessel lumens completely, frequently together with membraneous bubbles (Fig. 8). Plugging of vessel lumens takes place at an advanced stage of symptom development.

Obviously, much more work is needed to find out the true nature of these structures and the meaning of these xylem vessel symptoms in relation to stoppage of water transported through xylem vessels; we believe that the results presented here will support our contention that a compound named cerato-ulmin (CU), which is produced by *C. ulmi*, is involved in the disease syndrome expression of Dutch elm disease.

Fig. 1. Two bubbles with smooth coating. Cutting treated with CU for 2 days.

Fig. 2. Spherical granules on vessel walls. Cutting treated with CU for 3 days.

Fig. 3. Thin-walled tyloses or bubbles. Eight days after *C. ulmi* inoculation.

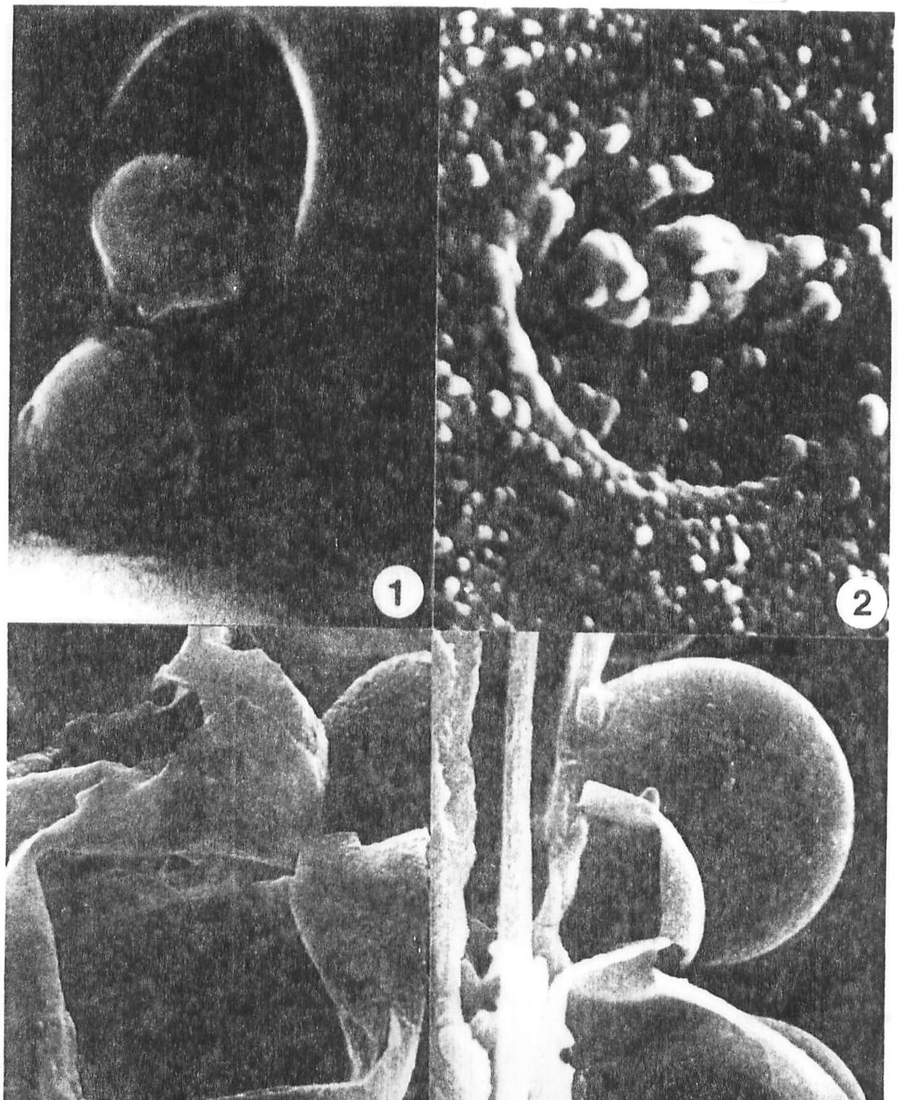
Fig. 4. Thin-walled and smooth-surfaced bubbles. Eight days after *C. ulmi* inoculation.

Fig. 5. Bubbles with granular coating. From a sample naturally infected with DED.

Fig. 6. Droplets covering the surface of vessel walls. Two and a half months after *C. ulmi* inoculation.

Fig. 7. Droplet produced with bubbles. Two and a half months after *C. ulmi* inoculation.

Fig. 8. Plugging of vessels with membraneous bubbles and callus-like development. Two and a half months after *C. ulmi* inoculation.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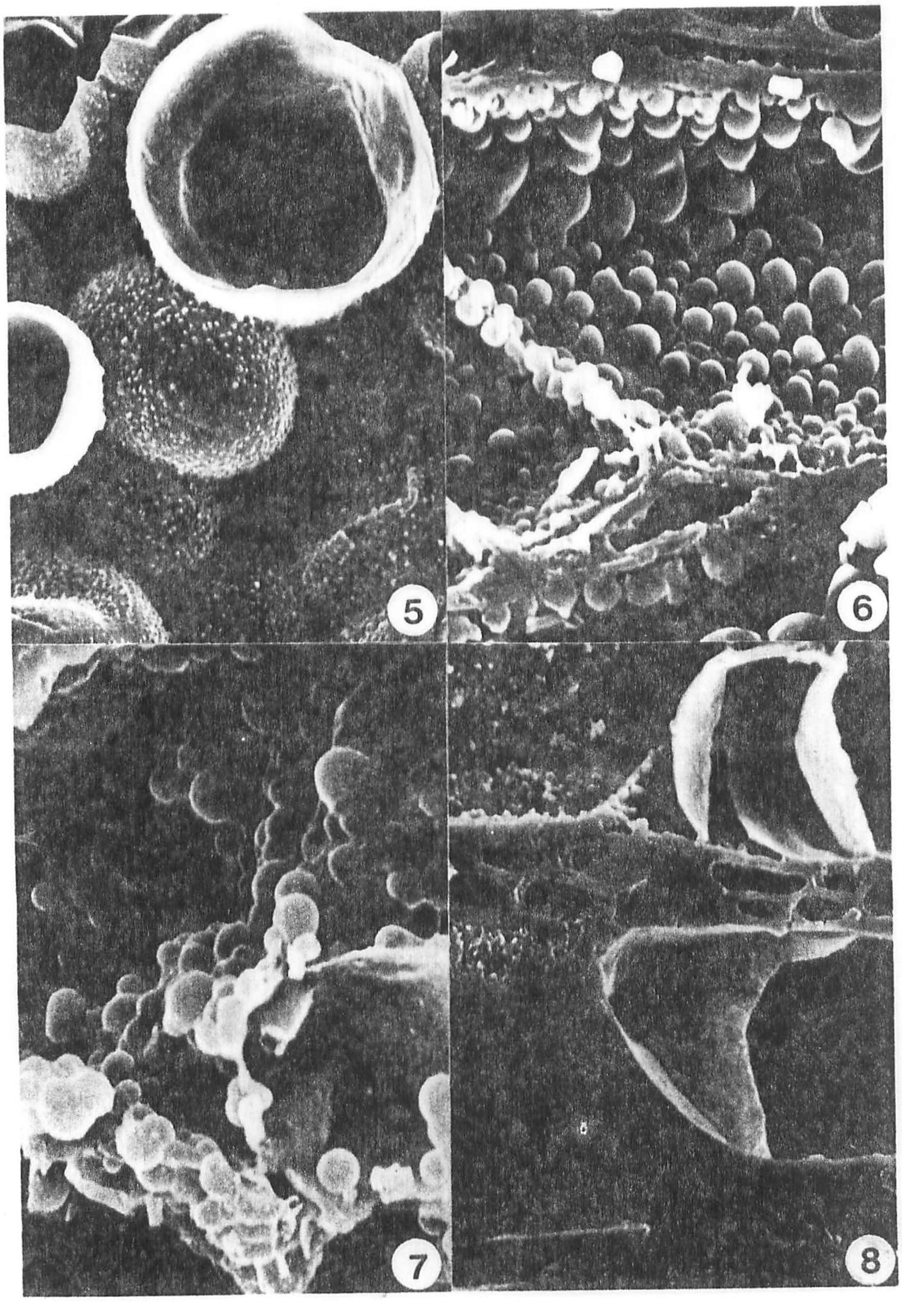

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