Canker of Japanese Maple Caused by *Colletotrichum acutatum*

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ABSTRACT

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Colletotrichum acutatum was identified as the pathogen causing a canker and dieback of Japanese maple (*Acer palmatum*) in propagation at a Connecticut nursery. Symptoms and signs included cankers frequently associated with wound sites, abundant acervuli, prominent masses of spores under conditions of high humidity, and death of cuttings. The fungus was consistently isolated from affected plants, and similar symptoms were expressed on inoculated seedlings in a greenhouse. One isolate from maple incited bitter rot of apple cv. Granny Smith fruit. This is the first report of *C. acutatum* causing a disease on a woody plant in North America.

Colletotrichum acutatum J.H. Simmonds is pathogenic on apple (4) and strawberry (5) in Connecticut, pine in New Zealand (1), and coffee in Kenya (3). It has not been reported to be a primary pathogen on a woody plant in North America.

This investigation was prompted by continuing (5–7 yr) problems in propagation of Japanese maple (*Acer palmatum* Thunb.) at a nursery in Madison, Connecticut. This popular landscape tree is frequently propagated by cuttings from stock trees showing desirable characteristics. Cuttings from apparently otherwise healthy stock trees failed to survive in a greenhouse maintained at 8 C. Affected cuttings showed cankers (Fig. 1), especially at wounds; abundant black acervuli on necrotic stems; and, under conditions of high humidity, copious salmon-colored spore masses. This problem caused the loss of over 50% of cuttings in 1 yr. *C. acutatum* was consistently isolated from affected cuttings.

C. acutatum causes bitter rot of apple fruit (4). Although there is evidence of host specialization in this fungus (1), it was not known if the isolate found on *A. palmatum* was capable of inciting bitter rot of apple or if the isolates from apple could cause the canker disease of *A. palmatum*.

The objectives of this study were to test the pathogenicity of *C. acutatum* to *A. palmatum* and to determine if an isolate from maple was capable of inciting bitter rot of apple. The ability of isolates of *C. acutatum* from apple to cause canker disease of Japanese maple was also determined. An attempt was made to produce the perithecial state in vitro. A preliminary report has been published (7).

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MATERIALS AND METHODS

Isolation of the fungus. Japanese maple cuttings with cankers were obtained from a commercial nursery in Madison, Connecticut. Bark from cankers bearing acervuli was washed with sterile distilled water and placed on 2% water agar. In addition, cuttings with cankers were placed in clean plastic boxes containing moist paper towels and incubated overnight at 18–20 C. Spores oozing from the acervuli were transferred to potato-dextrose agar (PDA). Growth of the fungus, sporulation, and formation of appressoria were observed after 7–10 days of incubation at 25 C. The fungus was identified on the basis of conidial morphology and cultural characteristics (2,6).

Cross-inoculation experiments. Half-sib Japanese maple seedlings were collected from beneath mature trees, potted in Promix BX (Premier Brands, Stamford, CT), and placed in a greenhouse maintained at 20 C. Stems at least 5 mm in diameter were wiped with 70% ethanol and allowed to dry. Slashes about 5–7 mm long were made in the bark of the stems with a flamed scalpel, and a flap of bark was left attached. A small (3 × 3 mm) plug of PDA containing growing mycelium of the fungus was inserted into the wound underneath the bark flap, and the wound was sealed with Parafilm. Four trees per isolate were inoculated, and four control trees were given PDA plugs. Seedlings were placed in a dew chamber at 28–30 C for 48 hr, then returned to the greenhouse. Canker formation was evaluated after 2 wk, and infected tissues were placed on PDA for recovery of the fungus from cankers. This test was performed three times.

To test the capability of the isolate of *A. palmatum* to cause bitter rot on apple (*Malus domestica* Borkh. 'Granny Smith'), unbruised fruit were washed in 70% ethanol, rinsed in sterile distilled water, and allowed to air-dry. Apples were wounded on three sides with a 1-mm-diameter dissecting needle to a depth of 5 mm. Wounds were swabbed with a water suspension of 10^5 conidia per milliliter from a 7-day-old culture on PDA using a cotton-tipped applicator. Two apples per isolate were inoculated. Wounds on two apples swabbed with sterile distilled water served as controls. Apples were incubated at room temperature in sealed plastic boxes containing moist paper towels and were evaluated for bitter rot development after 10 days. This test was performed three times.

Induction of perithecial formation. An attempt was made to induce the isolate of *C. acutatum* from *A. palmatum* to produce perithecia in vitro (6). Four cultures of the isolate were grown on each of the following media in petri dishes: PDA, cornmeal agar, nutrient agar, acidified PDA (PDA + 2.5 ml/L of 50% lactic acid), milk PDA (PDA + 5 g/L of powdered skim milk), PDA-oatmeal agar (1:1, v/v), and Czapek solution agar. Cultures were grown under continuous fluorescent light at 25 C and examined for perithecial production after 3 and 6 wk. In addition, the *A. palmatum* isolate was paired with six isolates of *C. acutatum* from apple fruit in all possible combinations. Duplicate culture dishes were started on each of the media named, and culture dishes were incubated as described above. Dishes were examined for perithecia after 6 wk and every week thereafter for 10 wk. This experiment was done once.

RESULTS

Characteristics of the fungus. Within 24–48 hr, masses of salmon-colored

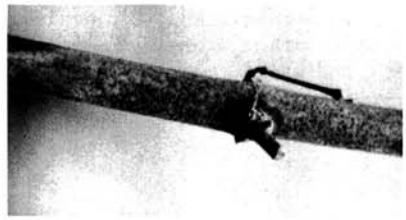

Fig. 1. Canker of Japanese maple caused by *Colletotrichum acutatum* after winter storage.

spores had oozed from acervuli in cankers both on bark placed on water agar and on cuttings in the moist chamber. Spores were fusiform, 16–18 μm long, and 4–6 μm wide. No setae were observed in culture or on any tissues. The upper side of the colonies was olive to brown on PDA, and the bottom side was bright rose to vermilion. Numerous dark brown to black lobate appressoria formed around the edges of the petri dish. Spore masses in vitro were salmon to orange. These characteristics conform to published descriptions of *C. acutatum* (2,6).

Cross-inoculations. Canker formation was evident on inoculated seedlings within 2 wk. The cankers were similar to those on specimens from the nursery, except that less sporulation occurred. No cankers developed on seedlings inoculated only with PDA. *C. acutatum* was consistently recovered from cankers.

Typical symptoms of bitter rot were evident on apple fruit within 3–5 days after inoculation. Caramel-colored, slightly sunken lesions developed at the inoculation sites. Abundant salmon to orange spore masses were evident after 7–8 days. Apples had collapsed and were completely brown and coated with conidia after 10 days. No bitter rot developed on apples inoculated with sterile distilled water. Symptoms induced by one isolate from *A. palmatum* and six isolates from apple fruit developed identically.

Perithecial formation. Perithecia were not found on diseased plant material from the nursery or on stock trees from which the cuttings had been taken. The isolate of *C. acutatum* from *A. palmatum* did not produce perithecia either alone or in any of the paired combinations on any of the media tested. Lack of production of perithecia is consistent with published descriptions of *C. acutatum* (2,6).

DISCUSSION

Although canker of Japanese maple caused by *C. acutatum* has not previously been reported as a problem on nursery stock, it may be a common but unrecognized malady. The disease develops slowly in cold storage. Losses of stored plants have been attributed to winter kill (in the case of *A. palmatum*) or to failure of a graft union (in other instances).

Host specificity has been reported for *C. acutatum* infecting pine (1). In the present study, however, the isolate from *A. palmatum* and apple caused identical symptoms. Conversely, isolates from apple fruit caused cankers to the same extent as did the maple isolate on *A. palmatum*. Lack of host specificity in this study indicates that *C. acutatum* may have more hosts than those now known.

Stock trees from which the cuttings were taken were examined for infection by *C. acutatum*, and none was found. The source of inoculum for the cuttings in storage remains unknown.

This is the first report of *C. acutatum* causing a canker disease on a woody nursery crop in North America. I propose the name of this disease to be Colletotrichum canker.

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