Cryptosporiopsis Canker of *Acer rubrum*: Some Relationships Among Host, Pathogen, and Vector

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ABSTRACT

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Individual wild red maples (*Acer rubrum*) differed in susceptibility to isolates of the fungus *Cryptosporiopsis* sp. obtained from cankers associated with oviposition sites of the narrow-winged tree cricket (*Oecanthus angustipennis*). Isolates of the fungus differed in growth rate and appearance on bark extract and other culture media and in size of cankers produced on inoculated red maples. Observations of the insects' behavior indicate that inoculum arrives via infested bark used to plug oviposition wounds. Exposure to normal winter environment enhances canker development and incidence of cankers varies from year to year. The tree cricket succumbs to common insecticides.

Additional key words: buffalo treehopper (*Stictocephala bubalus*)

Canker of red maple (*Acer rubrum* L.) associated with oviposition wounds made by the narrow-winged tree cricket was described in 1979 by Taylor and Moore (5). Growers of cloned red maples had experienced economic losses and the disease was found on wild red maple trees throughout Connecticut in 1979. In this paper, I report on further studies with the pathogen and its relation to the host and the manner in which wounds are made by the tree cricket during oviposition.

MATERIALS AND METHODS

Inoculations. Four isolates of *Cryptosporiopsis* were obtained from cankers on red maples in Connecticut and designated B, C, D, and F. One isolate (A) from a canker produced by inoculation of a cut stem of red maple and one isolate of *C. curvispora* (ATCC 16502 from apple) were grown on malt-extract agar slants for 3 wk. On 15 December 1978, about 1 mm^3 of agar and mycelium of each isolate was placed into separate 1-mm-diameter holes freshly drilled 1–1.5 m above the ground into each of four 6- to 8-cm-diameter wild red maple trees growing within 3 m of each other. A seventh hole was plugged with sterile agar and an eighth hole was left untreated. The eight holes per tree were evenly spaced vertically within 50 cm of bark; each hole was placed 90° from the preceding one to produce a spiral arrangement around the tree. The sequence of the eight treatments was at random for each tree. The bark surface was not disinfested and the drill holes were not covered. The lengths and widths of cankers that developed around the inoculation points were measured on 25 April 1979. Four red maples and four sugar maples 2–4 cm in diameter transplanted to the greenhouse on 24 October 1978 were inoculated on 18 December 1978 with the same inoculants and arrangements used for the four trees growing in the field.

Growth of fungi on culture media. Media were prepared from fresh bark of red maple, sugar maple, or McIntosh apple as follows: 50 g of bark was blended in 250 ml of distilled water for 1 min. Each blend was filtered through eight layers of cheesecloth and rinsed with 150 ml of tap water. Agar was added to 300 ml of each filtrate to make 2% agar and the mixture was autoclaved 20 min at 2.68 kg/cm^2 of pressure. Culture dishes (Falcon 60 mm) were prepared with each dish containing 10 ml of one of the bark-extract agars, fresh lima bean agar, or malt-extract agar (Difco 0186-01).

Isolates A, C, and F and *C. curvispora* were transferred to the center of each of three plates for each medium. Plates were held at room temperature and colony diameter was measured after 14 days.

Observation of cricket oviposition. Male and female adults of the narrow-winged tree cricket (*Oecanthus angustipennis* Fitch) were captured from dogwood, lilac, and apple trees in October 1978. Five pairs of male and female crickets were kept from 1 to 4 days, each pair in a separate 1,000-ml Erlenmeyer flask containing a freshly cut segment of red maple stem about 12 cm long and 1.5 cm in diameter. Mating and oviposition behavior were observed from darkness to midnight by periodically providing low light.

Infested crickets. One captured cricket was confined to each of two sporulating cultures of *Cryptosporiopsis* on malt-extract agar in 100-mm culture dishes for about 10 min. The insects were then transferred at about 10-min intervals to a succession of dishes containing sterile agar. Dishes were observed for development of *Cryptosporiopsis*. On 11 September 1979, four captured female crickets were placed in separate 15-mm-diameter culture tubes with *Cryptosporiopsis* isolate C sporulating on the slant surface of malt-extract agar. Four other females were placed in similar tubes containing sterile malt-extract agar. Tubes containing the crickets were taken to the field within 0.5 hr. Four wild red maple trees with stem diameters of 3–4 cm were each fitted with two waxed cup containers (15 × 11 × 8 cm, 1,000 cc). By slitting one side, the cup and lid could be placed to form a sealed space around the stem. No attempt was made to disinfest the bark before placing the container. A cricket was transferred from a *Cryptosporiopsis* culture tube to one cup and a cricket from a sterile agar tube to the other cup on each tree. The relative position of each cup was determined at random for each tree. The slits in the cups and any other openings were sealed with masking tape. One infested and one uninfested cricket on a different tree died within 48 hr, and all were removed after 2 wk. In the area where each insect had been confined, the number of oviposition sites and the number plugged was noted.

On 19 October 1979, three other trees were each inoculated by placing about 1 mm^3 of isolate C into a 1-mm hole drilled into each tree. The seven trees were examined during spring and summer of 1980 for canker development. On 19 October 1979, infested and uninfested tree crickets were similarly caged on four wild red maple trees 2–3 cm in diameter growing in a greenhouse.

Insecticides. Detached red maple branch ends with three or four leaves were sprayed to runoff on 8 August 1979 with (a.i. $\mu g/g$) malathion 595, carbaryl 1,190, endosulfan 890, diazinon 580, or water. Two snowy tree crickets (*O. niveus*), male or female, were caged in a waxed paper cup (1,000-cc) that had a screen window of 3 × 4 cm and contained a leaf sprayed with one of the materials described. There were four replicates. The number of dead individuals was recorded at 2 and 24 hr. On 16 August 1979, two more snowy tree crickets were added to each of the cages containing the

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leaves that had been sprayed either with malathion or water and a narrow-winged tree cricket was added to two of the replicates. The number of dead individuals was recorded after 24 hr.

In a second test, twigs on living red maples in the greenhouse were sprayed on 21 August 1979 with the same treatments. Plastic shields protected neighboring branches from spray. Sprayed branches were covered with paper bags and an individual narrow-winged tree cricket was introduced into each sealed bag. Insect mortality was noted for the ensuing 3 days.

RESULTS

Inoculations. On wild trees, cankers similar to those occurring naturally were evident in April 1979 only where the five isolates of *Cryptosporiopsis* from Connecticut had been introduced. One of the four trees had a small atypical canker where *C. curvispora* was introduced. No cankers developed at the other drill holes. Canker lengths in millimeters (means of four trees) were 0.5, 16.5, 19.5, 19.5, 27.0, and 35.2 for *C. curvispora* and isolates D, B, F, C, and A, respectively. Among the isolates from Connecticut, only isolates D and A differed significantly ($P = 0.05$, Duncan's multiple range test) from one another in lengths of cankers induced. Canker lengths for trees 1–4 (means of six isolates) were 12.8, 17.2, 19.8, and 29.0, respectively. Trees 1 and 4 differed significantly at the 5% level. Thus, isolates differed in virulence and trees differed in susceptibility.

Cankers developed at only four inoculated drill holes on red maple trees in the greenhouse. Three cankers were caused by isolate C and one by isolate F. No cankers developed on the four sugar maple trees.

Growth on culture media. Mean colony diameters (five media) 14 days after initiation were 4.6, 4.5, 4.4, and 2.5 for isolates F, C, A, and *C. curvispora*, respectively. Isolates F, C, and A did not differ significantly (Duncan's multiple range test, $P = 0.05$) from each other but did differ from *C. curvispora*. Mean colony diameters (four isolates) were 4.4 a, 4.1 b, 3.8 c, 3.9 bc, and 3.7 c for red maple, sugar maple, and apple bark extract and fresh lima bean and Difco malt agar, respectively. Colony appearance differed markedly on the various media. For example, growth on lima bean agar remained fluffy and no acervuli formed. Acervuli were most abundant on malt extract agar.

Cricket oviposition behavior. Pairs of narrow-winged tree crickets confined with red maple stem segments mated as described by Parrott and Fulton (2). Fertilized females began the egg-laying process at dusk by nipping with mouthparts at different areas of the bark. To oviposit, the insect positioned itself so that drilling with the ovipositor began at one of the points sampled by mouth. Nearly always, the insect faced downward and drilled the hole on an upward slant. Sometimes one, but usually two, channels were drilled at different lateral angles through the same external opening. After eggs were deposited, the insect maintained leg position and foraged over the bark surface, scooping up bits of material, then used this material to plug the hole made by the ovipositor. This activity was repeated five times for each hole. The whole process lasted about 45 min. Some females oviposited four times in one evening.

Infested crickets. Individuals confined on a sporulating culture of *Cryptosporiopsis* transferred the fungus to five successive agar plates. Colonies developed at points of foot and mouthpart contact. The number of ovipositions by each of the eight crickets in the tree-caging experiment varied from none to six. Typical cankers developed by June 1980 at two oviposition sites of the 15 made by the four crickets confined on a *Cryptosporiopsis* culture before being caged on wild red maples. One other oviposition site appeared swollen and different from the other sites but did not develop into a typical bleeding, black, sunken canker. A typical canker developed at only one of the five oviposition sites made by crickets previously caged on sterile agar. All of the crickets had been observed to feed, or at least use mouth parts, to manipulate the surface of both the culture and the sterile agar. No cankers developed where infested crickets had been caged on trees growing in a greenhouse.

Insecticides. Male or female crickets exposed to malathion died within 12 hr whether caged over leaves on the tree or in containers containing a leaf sprayed with the insecticide. All crickets exposed to carbaryl, endolsulfan, or diazinon were dead within 24 hr. Crickets exposed to unsprayed leaves in either situation were alive after 3 days.

DISCUSSION

The role of the narrow-winged tree cricket during inception of the Cryptosporiopsis canker on *A. rubrum* described by Taylor and Moore (5) remains unclear. In captivity, the insects chewed on fruiting bodies of the fungus growing on agar. Whether or not they feed on this fungus in the wild has not been determined. Fungal spores and mycelium have been found in the insect's gut (2). I confirmed earlier reports (2) that the narrow-winged tree cricket uses mouthparts to scrape up bits of bark and insert them into the freshly drilled oviposition hole. Thus, spores of *Cryptosporiopsis* washed or blown onto the bark from a nearby canker may be the source of the inoculum, which seems more likely than the ovipositor itself inserting the fungus from internal contamination of the insect's gut. Indeed, isolations I made from various parts of tree cricket bodies such as ovipositor, head, or gut yielded many fungi, yeasts, and bacteria but not *Cryptosporiopsis*. Possibly, infections exist in the cambium area of the tree from some other source and the tree cricket ovipositor becomes infested from oviposition in this area, then introduces the fungus to a fresh wound where it causes the typical canker. This view is supported by the fact that the fungus was recovered several times from discolored woody tissue as far as 10 cm from the externally observable canker area.

The experiment with infested crickets caged on trunks of red maple was inconclusive because a small canker developed where an uninfested cricket was caged and cankers developed in only two of the 15 oviposition holes made by infested crickets. It was observed that many holes were started but did not extend to the cambium and did not contain an egg. Few of such holes were plugged. Conversely, about 90% of the holes containing an egg had been plugged. The extension of the oviposition wound to the cambium and wood may be important for canker formation. On 19 January 1979, I observed a group of eight red maple trees with ovipositions by both the narrow-winged tree cricket and the buffalo treehopper (*Stictocephala bubalus* (Fabricius)) of the order Homoptera. The latter lays a series of five to seven adjacent eggs in both sides of a slit in the bark but in such a way that the wound seldom penetrates to the cambium. Cankers were associated with 5.3% of 189 cricket wounds and with none of 107 treehopper wounds.

Results of my experiments support the observation that a range of susceptibility exists among populations of wild red maples. The named selections Autumn Flame, October Glory, and Red Sunset, widely propagated by cloning, appear to be highly susceptible (5). There is also a range of virulence or aggressiveness among isolates of *Cryptosporiopsis*.

Since 1977, the year of discovery, there has been a general decline in the incidence of Cryptosporiopsis canker on wild red maples in Connecticut. Cankers become callused and change in appearance with time. These differences allowed me to assign year of formation to cankers on 10 trees in each of three geographically separate groups of red maples on 3 May 1979. Mean numbers of cankers per tree (30 trees) for 1977, 1978, and 1979 were 3.7, 2.2, and 0.8, respectively. New cankers were difficult to find in 1980 and 1981 but were at about the 1979 level in 1982.

The reasons for these reductions are not clear but they resemble yearly fluctuations reported for the Fusarium canker on sugar maples (6). Changes in fungus presence or numbers of crickets or in local climate may be responsible. Cold temperature after inoculation seems to be

important because, in my experiments, red maples in the greenhouse developed far fewer cankers than those grown in the open. This situation exists for other fungus-host relationships (1,3,4).

Control measures in commercial plantings seem straightforward. The insect is susceptible to any of the commonly used insecticides such as malathion, carbaryl, endosulfan, or diazinon. Applications in late August would allow the insectivorous narrow-winged tree cricket to help keep aphids under control but prevent widespread oviposition. For the long term, it would seem possible to select aesthetically desirable clones of *A. rubrum* that are either resistant to the canker fungus or avoided by the narrow-winged tree cricket.

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