Detection of Benomyl-Tolerant Strains of *Elsinoë fawcetti* in Florida Citrus Groves and Nurseries

J. O. WHITESIDE, Plant Pathologist, University of Florida, Agricultural Research and Education Center, Lake Alfred, FL 33850

ABSTRACT

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Elsinoë fawcetti is difficult to isolate from citrus scab pustules produced in the field because of contamination problems. Therefore, direct monitoring for tolerance to benomyl by transferring conidia or pieces of the stroma to benomyl-amended agar was impracticable. Instead, conidia were dispersed from scab pustules on fruit and leaves into water, and the resulting inoculum was applied to the apices of emerging shoots on greenhouse-grown rough lemon trap seedlings. Tolerance in the surviving population of *E. fawcetti* was based on the number of active pustules that developed on benomyl-soil-drenched plants relative to untreated plants. The fungus was readily isolated from young pustules produced in the greenhouse, and tolerance was confirmed by growing the isolates on benomyl-amended potato-dextrose agar. Estimates of tolerance in the surviving populations of *E. fawcetti* in seven 'Temple' orange groves and two citrus nurseries, in which scab was poorly controlled despite timely spraying with benomyl, varied from 27% to about 100%.

When a facultative pathogen is suspected of tolerance to benomyl, the usual confirmatory practice is to transfer spores singly and aseptically from the host substrate and to observe the resulting growth on benomyl-amended agar media (2,6). This procedure is impractical with the citrus scab fungus, *Elsinoë fawcetti* Bitanc. & Jenkins, which produces small, hyaline conidia of variable size that are difficult to distinguish from those of contaminating organisms present on naturally produced pustules. Thus, it is difficult to make accurate germination counts. Colonies of *E. fawcetti* on agar media are distinctive but grow slowly and are frequently inhibited or overrun by other organisms. Furthermore, the water needed to induce production of conidia and to ensure their survival greatly increases contamination by bacteria and other organisms. The previously described (4) time-consuming procedure for single-conidium isolation of this fungus was impracticable for testing the large population of conidia required to estimate tolerance.

This report describes a technique to detect benomyl-tolerant strains of *E. fawcetti* and gives estimates of the percentage of tolerance in surviving populations of the pathogen in some citrus groves and nurseries in which benomyl failed to provide acceptable scab control despite timely treatment.

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MATERIALS AND METHODS

Rough lemon (*Citrus jambhiri*) seedlings were grown in the greenhouse in a soil-peat mixture (1:1 v/v) in 15-cm-diameter plastic pots until the stem diameter 2 cm above the medium exceeded 1.5 cm. One week after pruning the plants to force new shoots, 200 ml of a suspension of 600 mg benomyl in 1 L of water was applied as a soil drench to three of the six plants to be inoculated with each test sample. The new shoots were inoculated before the last leaf had unfolded from the bud, usually about 2 wk after pruning.

Test samples consisted of 20–30, 3- to 6-mo-old diseased fruit from Temple (*C. temple*) groves and about 20 diseased Marsh grapefruit (*C. paradisi*) shoots from citrus nurseries. To induce production of conidia, the samples were kept continuously wet in a moist chamber (modified desiccator) for 2 days with periodic sprays of water. Conidia were swept from the pustules into water with a camel's hair brush, and drops of the resulting inoculum were applied to the apex of each expanding rough lemon shoot. After inoculation, the plants were atomized with water and covered with polyethylene bags for 2 days.

Benomyl-sensitive as well as benomyl-tolerant strains of *E. fawcetti* infected the shoots of benomyl-treated plants. However, pustules produced by sensitive strains of the fungus eventually became inactive, and the stromatic portion of the pustule disintegrated (5). Active pustules were easily identified by the white, aerial growth of *E. fawcetti* that appeared on the stroma (5). The active pustules on each inoculated shoot were counted 3–4 wk after inoculation.

Tests to confirm tolerance to benomyl were made on at least five active pustules from each trap plant. To isolate the fungus, thin tangential sections were cut from the stroma surface and chopped into small fragments in a sterile petri dish. The fragments were dispersed in water agar cooled to 40 C. After 3–4 days, fragments were viewed at ×100 magnification, and transfers were made from the periphery of contaminant-free mycelium to Difco potato-dextrose agar (PDA), on which the fungus was identified by its characteristic dense stromatic growth. Transfers were then made to four-compartment 90-mm petri dishes containing 0, 1, 10, and 100 μg/ml benomyl in PDA. The benomyl was added to PDA as an aqueous suspension before autoclaving.

RESULTS AND DISCUSSION

The amount of benomyl applied as a soil drench to the rough lemon trap plants was sufficient to inactivate 95–100% of the pustules produced by benomyl-sensitive isolates of *E. fawcetti*. Sensitive isolates did not grow on PDA containing 1 μg/ml benomyl, whereas tolerant isolates grew as rapidly as on non-amended PDA. All tolerant isolates grew at 10 μg/ml, but some were slightly suppressed at this concentration. Most tolerant isolates grew on PDA containing 100 μg/ml benomyl.

All 24 single-conidium isolates of *E. fawcetti* used in a previous study (4) proved to be benomyl-sensitive. These isolates were obtained between 1972 and 1976 from different Florida citrus groves and nurseries, mostly before any benomyl was applied.

Commercial use of benomyl to control scab in Florida plantings of susceptible citrus cultivars began in 1974. In early years, benomyl usually provided excellent control of scab, particularly when applied twice—just before budbreak and again at bloom. Failures that did occur were mostly due to applying benomyl too long before petal fall in groves that received only one treatment (3).

Monitoring for strains of *E. fawcetti* tolerant to benomyl began in 1978 in a Temple grove used experimentally since 1972 to test benomyl and other fungicides for scab control. Although scab was still well controlled on trees sprayed twice in 1978 with benomyl, some of the single-conidium isolates from the relatively few pustules produced proved to be benomyl-tolerant. These benomyl-tolerant isolates