Baseline Sensitivity of Florida Isolates of *Penicillium digitatum* to Imazalil

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ABSTRACT

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Sensitivity to imazalil was determined for 47 single-spored isolates of *Penicillium digitatum* selected from imazalil-free environments. The ED_{50} values of imazalil ranged from 27 to 146 ng/ml, with a mean of 55. These values are less than those reported with biotypes collected in California citrus packinghouses, where ED_{50} values were near 1,000 ng/ml after intensive commercial use of imazalil.

Additional keywords: postharvest pathology, fungicides

Imazalil is an effective postharvest fungicide used commercially to control decay and soilage of citrus caused by *Penicillium digitatum* (5,7). The fungicide is effective against biotypes of the fungus that have developed resistance to the benzimidazole fungicides, thiabendazole and benomyl, both of which are registered to control postharvest decay on citrus. Recently, biotypes of *P. digitatum* with reduced sensitivity to imazalil were collected from commercial citrus packinghouses in California (4). A decrease in sensitivity of *P. digitatum* to imazalil has not been observed in the Florida citrus industry, where the fungicide has not been used intensively and where it is not normally applied in situations that enhance resistance, such as to fruit in long-term storage at the packinghouse.

Imazalil inhibits ergosterol biosynthesis, specifically by inhibiting the C-14 demethylation of lanosterol (6,9). Fungal resistance to imazalil in *Aspergillus nidulans* (10) and *P. italicum* (3) appears polygenic, and selection for resistance should follow a directional scheme (6). Resistance of the pathogen during continued use of the fungicide thus could increase gradually until a loss in disease control is observed with commercial treatments (3). To monitor the gradual shift toward resistance, the establishment of the baseline sensitivity distribution is of utmost importance. Therefore, single-spore isolates of *P. digitatum* were selected from imazalil-free environments. Sensitivity (ED_{50} values) of 47 isolates to imazalil was determined, and the individual isolates were preserved for long-term storage. Both the sensitivity data and the availability of representative isolates will provide the tools to monitor future developments. Buildup of resistance could be anticipated, and alternative or combination fungicide programs with good sanitation practices could be initiated before the commercial application of imazalil became ineffective.

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MATERIALS AND METHODS

Isolates 1–29 were obtained from two imazalil-free packinghouses in central Florida at Winter Haven and Haines City. Plates of potato-dextrose agar (PDA) were exposed for 1 min at the dump area in the first packinghouse, and individual colonies that developed were used to obtain isolates 1–19. Isolates 20–29 were obtained from individual Dancy tangerines infected by *P. digitatum* that were collected from the dump site at the second packinghouse. Isolates 30–47 were obtained from individually infected Hamlin oranges or Marsh grapefruit taken from two groves near Arcadia, in southwestern Florida. After isolation, each culture was single-spored and stored on silica gel at 4 C (8).

Imazalil dissolved in water was incorporated into warm sterilized PDA, and 15 ml was poured into a 100 (diameter) × 15 mm culture plate. Each isolate was tested at five concentrations (ng/ml) that caused slight to extensive but never complete inhibition, and growth was compared with that in control plates. Disks (4 mm in diameter) of inoculum of *P. digitatum* were obtained from actively growing cultures after the spores were removed with sterile water containing 0.04% Triton X-100. This was done to prevent the inadvertent dropping of spores onto the test media during transfer. The inoculum was placed on the control and imazalil media

at four equidistant locations at the edge of the culture plate. The plates were incubated at 25 C for 5–7 days, and radial growth of each colony was measured within the area from the edge of the disk toward the center of the culture plate. The concentration of imazalil that reduced growth by 50% (ED_{50}) was determined by the linear relationship between the probit of the percentage of growth reduction and the logarithm of the fungicide concentration. Best fit of the data was determined by linear regression (1).

RESULTS AND DISCUSSION

Mean ED_{50} values of isolates 1–19, 20–29, and 30–47 were 65, 51, and 48 ng/ml, respectively. ED_{50} values of the 47 isolates of *P. digitatum* ranged from 27 to 146, with a mean of 55 and a standard deviation of 18. The ED_{50} distribution frequency of the 47 isolates was 4, 33, 8, 1, 0, 0, and 1 at sensitivity ranges of 20–40, 41–60, 61–80, 81–100, 101–120, 121–140, and 141–160 ng/ml, respectively. These ED_{50} values are comparable to those of wild-type isolates recovered elsewhere with ED_{50} values of less than 100 ng/ml imazalil (7). Biotypes of *P. digitatum* with reduced imazalil sensitivity found in California had ED_{50} values of approximately 1,000 ng/ml imazalil, and decay or sporulation caused by those isolates was not effectively controlled with standard imazalil in wax treatments of 2 g/L (4).

Because of the directional selection (6) of imazalil resistance, isolates of *P. digitatum* with ED_{50} values between 100 and 1,000 should become more prevalent in a population where resistance is developing. If such biotypes occurred, it would signal the need to evaluate the pathogenic fitness of these isolates (6) and, if they were competitive, to initiate strategies to combat resistance (2). Such a sampling policy could be established in a routine, quality-control program provided by companies that service the Florida fresh citrus industry or by the quality-control personnel of individual packinghouses.

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