Occurrence of Thiabendazole-Resistant Strains of *Penicillium italicum* in Egypt

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ABSTRACT

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Blue mold (*Penicillium italicum*) was a more prevalent decay than green mold (*P. digitatum*) on Valencia oranges treated with thiabendazole (TBZ). Some blue mold isolates from decayed fruits were resistant to concentrations of TBZ at least 2,000 times greater than the minimal dose required to inhibit the sensitive isolates. The TBZ-resistant isolates were as virulent as the TBZ-sensitive isolates.

Thiabendazole (TBZ) has been used successfully and extensively for control of postharvest decays in most citrus packinghouses throughout the world, including Egypt. *Penicillium digitatum* Sacc. (green mold) and *P. italicum* Wehmer (blue mold), two fungi responsible worldwide for a major amount of the decay losses of citrus fruits, have consistently developed resistance to thiabendazole and related compounds in different parts of the world (1–14). Strains of *P. digitatum* and *P. italicum* resistant to thiabendazole and benomyl were isolated from citrus fruits that had decayed during shipment from 18 countries, including Egypt, to European markets (9).

During the 1980–1981 season, it was observed in a commercial packinghouse at Tanta, Egypt, that considerable blue mold developed during shipment of oranges treated with 2,500 μg/ml TBZ applied in shipping wax. This suggested that a fungal strain resistant to TBZ had developed so trials were conducted to determine if a resistant strain was present in Egypt.

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MATERIALS AND METHODS

Samples of Valencia oranges that had been processed in the commercial packinghouse at Tanta were evaluated for rot development. Oranges were surface-treated with sodium *O*-phenylphenate (SOPP) as foam wash, covered with shipping solvent wax (Fruitseal-ST-Extra) containing TBZ (2,500 μg/ml), wrapped in tissue paper impregnated with biphenyl, and packed into cartons (about 14 kg each). Fourteen cartons were examined, then held for a simulated transporting period (16 days at 18–22 C) and reevaluated for rot.

Cultures of *Penicillium* spp. were isolated on potato-dextrose agar (PDA) from TBZ-treated and untreated decayed fruits. The untreated decayed fruits were collected from different orchards at Behera, Gharbeih, and Alexandria governorates. The single-spore technique was used for obtaining pure cultures of the isolates.

The *Penicillium* isolates were inocu-

lated into surface-sterilized (5 min in 500 μg/ml sodium hypochlorite and air-dried) Valencia oranges. A 5-mm disk of a 7-day-old PDA culture of the fungus was inserted through a wound (1 cm long and about 1 cm deep) in the tissues of inoculated fruit with a dissecting needle. The inoculated fruits were kept separately in polyethylene bags at 18–22 C for 7 days to raise relative humidity.

TBZ formulated as Tecto (100% a.i. wettable powder, Merck Sharp Dohme) was used for the in vitro tests.

In one test, the disk-diffusion method was used to evaluate the inhibitory effect of TBZ. Filter paper disks (9 mm diam.) were impregnated with a solution of TBZ (5 μg a.i./ml) and placed on PDA plates previously seeded with a spore suspension of the fungus to be tested. The diameter of the zone of inhibited growth surrounding the disk was measured after incubation for 4–5 days at 26 C.

In another test, the effect of TBZ on linear growth of the *Penicillium* isolates was studied. Fresh stock suspensions of TBZ were prepared in sterile distilled water. Aliquots were added aseptically to sterile liquified PDA after cooling to about 48 C to get the desired final concentration of the active ingredient. TBZ was added after the agar was autoclaved and cooled to avoid heat inactivation of the fungicide. Plates of solidified medium were inoculated with 6-mm-diameter mycelial plugs obtained from the edges of 5-day-old cultures and then incubated at 25–28 C for 9 days. Three replicates (plates) were used for each TBZ concentration. Diameters of the resulting colonies were measured daily and growth rates calculated.

RESULTS

Fruits held for a simulated transporting period showed a 1.4% incidence of blue mold and a 0.3% incidence of green mold. About 120 isolates of *Penicillium* spp. were collected from decayed fruits and evaluated for resistance to TBZ by the disk-diffusion method. About 80% of the *P. italicum* isolates from decayed fruits treated with TBZ were resistant to TBZ (5 μg/ml), but all isolates of *P. digitatum* tested were sensitive to TBZ.

The effect of 0.1–1,000 μg/ml a.i. TBZ on linear growth was studied with TBZ-sensitive isolates of *P. italicum* from decayed non-TBZ-treated fruits (two isolates), with TBZ-resistant isolates (six isolates) from TBZ-treated decayed fruits, and with isolates of *P. digitatum* from treated (five isolates) and untreated (six isolates) decayed fruits. TBZ at 0.5 μg/ml completely inhibited the growth of the sensitive green and blue molds. TBZ at 30, 50 and 200 μg/ml slowed the mycelial growth of the resistant isolates of *P. italicum* but most of these isolates were still able to grow at 1,000 μg/ml TBZ.

DISCUSSION

The greater prevalence of blue mold (*P. italicum*) over green mold (*P. digitatum*) on Valencia oranges treated with TBZ can be explained at least in part by the occurrence of TBZ-resistant strains of *P. italicum*. TBZ-resistant blue mold isolates from the decayed fruits were resistant to concentrations of TBZ at least 2,000 times greater than the minimal dose required to inhibit the sensitive isolates.

Isolates tolerant to TBZ, primarily *P. italicum*, were obtained in vitro by serially growing the fungus on media containing increasing concentrations of TBZ (4). Moreover, it was reported (5) that TBZ-resistant strains of *P. italicum* and *P. digitatum* occurred naturally in orchards and packinghouses where the chemical has never been used. Use of TBZ, however, results in an increase in resistant strains due to selectivity for these strains.

Strains of both *P. italicum* and *P. digitatum* retained a constant degree of tolerance to TBZ even after 16 transfers on fungicide-free media (4). This may indicate that the tolerant strains resulted from a genetic change (4). Gutter et al (4) pointed out that these findings stress the potential and prolonged danger inherent in the occurrence of resistant strains.

The TBZ-resistant isolates in our investigation proved to be virulent. Similar results were obtained by Wild and Rippon (14) and Gutter et al (4), who found that mutation and selection that produced benzimidazole resistance has not reduced the pathogenicity of the organism.

It could be suggested that benomyl might be used as an alternate treatment against ordinary TBZ-resistant strains of blue mold on citrus fruits. However, strains have developed resistance to both TBZ and benomyl simultaneously (2,5,12). Thus, once such strains resistant to TBZ and benomyl appear, use of benomyl is of no value.

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