Resistance to Benzimidazole Fungicides of *Penicillium italicum* and *P. digitatum* Isolated from Packinghouses and Orchards in Egypt

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ABSTRACT

El-Goorani, M. A., El-Kasheir, H. M., Kabeel, M. T., and Shoeib, A. A. 1984. Resistance to benzimidazole fungicides of *Penicillium italicum* and *P. digitatum* isolated from packinghouses and orchards in Egypt. Plant Disease 68: 100-102.

A survey of the incidence and occurrence of strains of *Penicillium italicum* and *P. digitatum* resistant to thiabendazole in Egyptian packinghouses and orchards was carried out. About 89% of the *P. italicum* isolates from decayed fruits treated with thiabendazole were moderately resistant or resistant to the fungicide. In general, *P. italicum* isolates were more resistant to thiabendazole than *P. digitatum* isolates. About 19 and 2.7% of the *P. digitatum* isolates from decayed fruits treated with thiabendazole were moderately resistant and resistant, respectively, to the fungicide. Most *P. italicum* isolates resistant to thiabendazole showed resistance to benomyl. The *P. italicum* isolates, with a few exceptions, were sensitive to imazalil or prochloraz. Two isolates of *P. italicum* were moderately resistant to imazalil, with a minimal inhibitory concentration of about 10 μg a.i./ml. Two additional isolates of *P. italicum* were moderately resistant to prochloraz, with a minimal inhibitory concentration of about 5 μg a.i./ml. All of these isolates proved virulent.

Benzimidazole fungicides have been used extensively during the past 10 yr to control postharvest decays of citrus fruits (3). However, *P. digitatum* Sacc. (green mold) and *P. italicum* Wehmer (blue mold) have consistently developed resistance to benzimidazole fungicides in the United States (1,10,18), Israel (6,7), Australia (17,21,22), Japan (12), and Taiwan (23). Strains of *P. digitatum* and *P. italicum* resistant to benzimidazole fungicides were also isolated from citrus fruits that had decayed during shipment from 18 countries to European markets (16).

During the 1980–1981 season, strains of *P. italicum* resistant to thiabendazole were found in Egypt on Valencia oranges that had been processed in a packinghouse at Tanta and covered with shipping wax containing 2,500 μg thiabendazole per milliliter. These strains were resistant to thiabendazole concentrations of 2,000 times greater than those required to inhibit sensitive types (5).

Because the occurrence of *Penicillium* spp. with resistance to thiabendazole is a potentially serious situation, the purposes of this investigation were to survey occurrence of thiabendazole-resistant strains of *Penicillium* spp. developed on rotted navel oranges at different packinghouses and orchards in Egypt and to determine the differential resistance of these molds to various levels of benzimidazole and other fungicides.

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MATERIALS AND METHODS

Measurements of decay in navel orange samples at different packinghouses and orchards were performed. Samples were collected from the following locations:

Tanta and Damanhour packinghouses. Samples of navel oranges that had been processed in commercial packinghouses at Tanta and Damanhour were evaluated for rot development. Thirty cartons of about 14 kg each were examined in each packinghouse. Cartons were examined initially, then held for a simulated transporting period (15 days at 18–22 C) and reevaluated for rot.

The standard practice for chemical treatment at the packinghouses involved the following steps: At the Tanta packinghouse, oranges were surface-treated with sodium *O*-phenylphenate (SOPP) (2%) as a foam wash, covered with shipping solvent wax (Fruitseal-ST-Extra) containing thiabendazole (2,500 μg/ml), wrapped in tissue paper impregnated with biphenyl, and packed into cartons. At the Damanhour packinghouse, oranges were surface-treated with SOPP (0.5% at 38 C for 3 min) as a tank wash, covered with shipping wax (emulsion type, Citra shine SP) containing thiabendazole (4,000 μg/ml) + SOPP (1.5%), wrapped in tissue paper impregnated with biphenyl, and packed into cartons.

Alexandria Port. Samples of rotted fruits were collected from cartons that had been processed in different packinghouses and stored for different periods in outside sheds. Most of the cartons examined were processed in the Belbis packinghouse. The standard practice for chemical treatment at the Belbis packinghouse was similar to those followed at the Tanta packinghouse.

Orchards. Rotted samples were collected from different orchards at Alexandria, Behera, and Gharbeih governorates, where benomyl or thiabendazole had not been used as a tree spray.

Cultures of *Penicillium* spp. were isolated on potato-dextrose agar (PDA) medium from decayed fruits. The single-spore technique was used for obtaining pure cultures of the isolates.

Surface-sterilized (5 min in 500 μg/ml sodium hypochlorite and air-dried) navel oranges were inoculated with the *Penicillium* isolates. 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.

Fungicides used for in vitro tests were thiabendazole (Tecto 100WP), benomyl (Benlate 50WP), imazalil 20EC, and prochloraz 40EC. The effect of these fungicides on linear growth of the *Penicillium* isolates was determined. Fresh stock suspensions of each fungicide were prepared in sterile distilled water. Aliquots were added aseptically to sterile liquified PDA after cooling to about 48 C to provide the desired final concentration of the active ingredient. Plates of solidified medium were inoculated with 6-mm-diameter mycelial plugs obtained from the edges of 5-day-old cultures, then incubated at 25–28 C for 9 days. Three replicates (plates) were used for each fungicide concentration. Diameters of resulting colonies were measured daily, growth rates calculated, and minimal inhibitory concentrations (MIC) determined.

Isolates were classified into sensitive (S), moderately resistant (MR), and resistant (R) groups as follows: for thiabendazole, S = MIC less than 1 μg/ml, MR = MIC ranging from 2 to less than 100 μg/ml, and R = MIC greater than 100 μg/ml; for the other fungicides, S = MIC less than 1 μg/ml, MR = MIC ranging from 2 to 10 μg/ml, and R = MIC

Table 1. Number of isolates of *Penicillium italicum* and *P. digitatum* sensitive (S), moderately resistant (MR), and resistant (R) to different fungicides

Source of isolates[a]	No. of isolates	Thiabendazole S	Thiabendazole MR	Thiabendazole R	Benomyl S	Benomyl MR	Benomyl R	Imazalil S	Imazalil MR	Imazalil R	Prochloraz S	Prochloraz MR	Prochloraz R
Penicillium italicum													
Tanta packinghouse (thiabendazole-treated decayed fruit)	33	4	8	21	2	23	8	32	1	0	32	0	0
Alexandria Port (thiabendazole-treated decayed fruit)	43	4	23	16	7	26	10	42	1	0	43	0	0
Orchards (untreated decayed fruit)	39	24	15	0	39	0	0	39	0	0	37	2	0
P. digitatum													
Tanta packinghouse (thiabendazole-treated decayed fruit)	21	17	3	1	21	0	0	21	0	0	21	0	0
Alexandria Port (thiabendazole-treated decayed fruit)	16	12	4	0	16	0	0	16	0	0	16	0	0
Orchards (untreated decayed fruit)	40	38	2	0	40	0	0	40	0	0	40	0	0

[a] Isolates were obtained from navel oranges that had decayed in orchards and during prolonged storage at different packinghouses.

Table 2. Zones of inhibition obtained with disks of navel orange rind using *Penicillium digitatum* sensitive to thiabendazole as a bioassay for thiabendazole residue

Source of samples	Zone of inhibition (mm diameter) Average[a]	Range	Standard deviation
Damanhour packinghouse[b]	52.5	45–60	6.38
Tanta packinghouse[c]	20.0	0–35	14.68
Control	0.0	0.0	0.00

[a] Average of 36 disks from 36 fruits.
[b] Fruit covered with shipping wax (emulsion type) containing thiabendazole (4,000 μg/ml) + sodium *O*-phenylphenate (SOPP).
[c] Fruit covered with shipping solvent wax containing thiabendazole (2,500 μg/ml).

ranging from greater than 10 to 100 μg/ml.

Rind tissues were tested for residual fungicide(s) by bioassay. Samples of navel oranges that had been processed in the Tanta and Damanhour packinghouses were used in this experiment. Assays were made from fruits from each packinghouse 2 days after processing. Disks (14 mm in diameter and about 3 mm thick) were removed from the outer part of the fruit rind and placed directly on PDA plates seeded with an isolate (no. 19) of *P. digitatum* sensitive to thiabendazole. Disks were placed so that the exterior rind surface was in contact with the medium surface. Disks removed from similarly treated fruits without adding fungicide(s) to the wax served as the control. Zones of inhibition were read after 5 days of incubation at 24–27 C.

RESULTS

Navel oranges (processed at the Tanta and Damanhour packinghouses) held in storage at 18–22 C but otherwise treated as if for export were examined for decay. Samples from the Damanhour packinghouse were free of rot. However, fruits from the Tanta packinghouse showed 2.8% incidence of blue mold, 1.3% incidence of green mold, and about 1% incidence of other rots of relatively minor importance, namely brown rot (*Phytophthora citrophthora* (R. E. Sm. et E. H. Sm.) Leonian, Alternaria rot (*Alternaria* sp.), sour rot (*Geotrichum candidum* Lk. ex Pers.), and Botryodiplodia rot (*Botryodiplodia theobromae* Pat.).

About 89% of the *P. italicum* isolates from decayed fruits treated with thiabendazole collected from different packinghouses were moderately resistant or resistant to thiabendazole or benomyl (Table 1). About 38% of the *P. italicum* isolates from decayed fruits collected from the orchards were moderately resistant to thiabendazole. However, all isolates of *P. italicum* from decayed fruits collected from orchards were sensitive to benomyl (Table 1).

Isolates of *P. italicum*, with a few exceptions, were sensitive to imazalil or prochloraz (Table 1). Two isolates of *P. italicum* were moderately resistant to imazalil, with a minimal inhibitory concentration of about 10 μg a.i./ml. Two other isolates of *P. italicum* were moderately resistant to prochloraz, with a minimal inhibitory concentration of about 5 μg a.i./ml. All these isolates proved to be virulent.

In general, *P. italicum* isolates were more resistant to thiabendazole and benomyl than *P. digitatum* isolates (Table 1). About 19 and 2.7% of the *P. digitatum* isolates from decayed fruits treated with thiabendazole were moderately resistant and resistant respectively to the fungicide. Five percent of the *P. digitatum* isolates from decayed fruits collected from the orchards were moderately resistant to thiabendazole (Table 1). All isolates of *P. digitatum* tested were sensitive to benomyl, imazalil, and prochloraz.

Testing thiabendazole-resistant strains for cross-resistance to benomyl. Results show that 81 of 111 isolates of *P. italicum* were moderately resistant or resistant to thiabendazole and were in turn moderately resistant or resistant to benomyl. Twenty-four isolates were moderately resistant to thiabendazole but were sensitive to benomyl. Five isolates were sensitive to thiabendazole and moderately resistant to benomyl. One isolate was sensitive to thiabendazole and resistant to benomyl. However, all nine isolates of *P. digitatum*, which were moderately resistant to thiabendazole, were sensitive to benomyl.

Rind tissue bioassays. All disks tested from oranges collected from the Damanhour packinghouse produced high fungicidal activity (Table 2). This indicates that the combination of SOPP and thiabendazole produced high levels of fungicide residues on the exterior surface of the rind. However, low fungicidal activity was detected on the disks from oranges collected from the Tanta packinghouse. Considerable variation was also found in residues among fruits sampled from this packinghouse. This indicates that the method of applying thiabendazole to fruit must have had some defects, resulting in nonuniform application of the fungicide (Table 2) (Fig. 1).

DISCUSSION

In general, *P. italicum* isolates found on decayed fruits from Egyptian

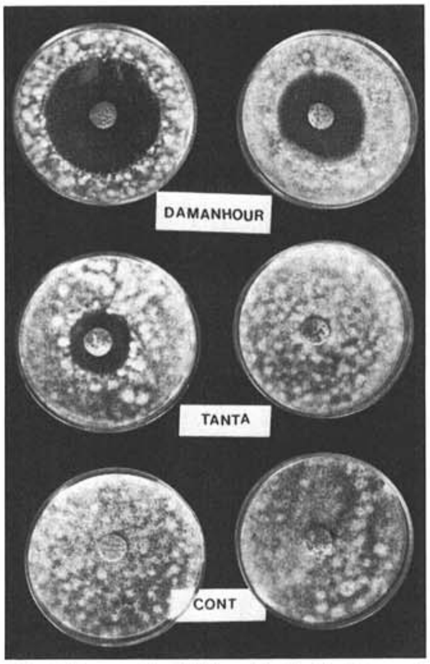

Fig. 1. Zones of inhibition obtained with navel orange rind disks using *Penicillium digitatum* sensitive to thiabendazole as a bioassay for thiabendazole.

packinghouses and orchards were more resistant to thiabendazole than *P. digitatum* isolates. Occurrence of both blue and green molds with resistance to thiabendazole is a potentially serious situation.

Most *P. italicum* isolates resistant to thiabendazole showed resistance to benomyl, however, a few isolates of *P. italicum* and *P. digitatum* that were resistant or moderately resistant to thiabendazole did not show cross-resistance to benomyl. The phenomenon of cross-resistance found among isolates of benzimidazole-resistant strains of blue and green molds has been reported by several investigators (6,7,10,12,16,18,22). This phenomenon emphasizes the need to study the effect of nonbenzimidazole fungicides on growth of isolates of green and blue molds resistant and sensitive to benzimidazole. We found that the isolates of *P. italicum* and *P. digitatum*, with the exception of a few *P. italicum* isolates, were sensitive to imazalil and prochloraz.

The ability of imazalil to control strains of blue and green molds resistant to benzimidazoles as well as to suppress sporulation of these fungi on diseased fruits makes it a desirable postharvest fungicide (1,3,8,11,14,15). Prochloraz appears to be highly effective against the same diseases as imazalil (20).

Certain investigators pointed out that imazalil-resistant strains of *P. italicum* and *P. digitatum* have never appeared (2,13). Genetic and biochemical investigations on imazalil provide hope that development of resistance of *Penicillium* spp. will be slow and involve mutants with diminished virulence (4). However, in this study, we found two isolates of *P. italicum* that were moderately resistant to imazalil (MIC about 10 μg a.i./ml).

Considerable variation was found in residues from fruit to fruit in samples from the Tanta packinghouse, indicating that the method of applying thiabendazole to fruit resulted in nonuniform application of the fungicide. In addition, it is clear that chemical treatments at the Damanhour packinghouse gave better control of postharvest diseases of oranges than the chemical treatments at the Tanta packinghouse. The foam treatment is known to be less effective in decay control than the bath treatment (3). In addition, use of SOPP dip alone at a concentration of 1 or 2% gave satisfactory results in controlling benzimidazole-resistant strains of *P. italicum* and *P. digitatum* (8). In combination, SOPP and thiabendazole might be expected to have an effect greater than that of thiabendazole alone. Other workers have found that a combination of both fungicides gave consistently better control than either fungicide alone (9,19).

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