Biological Aspects of Citrus Molds Tolerant to Benzimidazole Fungicides

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ABSTRACT

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A survey of the incidence and occurrence in Israel of strains of the green and blue molds of citrus (*Penicillium digitatum* and *P. italicum*, respectively) tolerant to benzimidazoles revealed that their incidence was low in the orchards, medium in the packinghouses, and high in the storage rooms. Strains were found that are tolerant to concentrations of benzimidazoles 500–1,000 times greater than those required to inhibit the sensitive "wild types." The antifungal properties of benomyl were, in all cases, greater than those of thiabendazole at equal concentrations. All tolerant isolates under study were also tolerant to other compounds of the benzimidazole group—carbendazim, cypendazole, fuberidazole, and thiophanate-ethyl. A relatively constant degree of tolerance was maintained even after 16 weekly transfers to a fungicide-free medium or after inoculation to and recovery from untreated citrus fruits. Our results suggest that tolerant strains were capable of surviving extended periods along with the susceptible strains even in the absence of selection pressure.

Additional key words: fungal resistance, citrus fruit decay.

Standard antifungal treatments practiced for many years in citrus packinghouses in Israel generally consisted of a 3-min dip in a solution of sodium *o*-phenylphenate (SOPP) followed by waxing the fruit to prevent postharvest decay. During the last decade, the thiabendazole (TBZ) treatment was introduced, mainly due to its great effectiveness in controlling both green and blue molds caused by *Penicillium digitatum* Sacc. and *P. italicum* Wehmer, respectively, as well as stem-end rots (induced by *Diplodia natalensis* P. Evans).

In 1970, soon after the introduction of TBZ, tolerant strains, primarily of the blue mold, were obtained in vitro in this laboratory by serially growing the fungus on media containing increasing concentrations of TBZ (*unpublished*). Quite independently, the occurrence of strains of molds tolerant to benzimidazoles was observed later when fruits that had been processed in the packinghouse and covered with wax containing 4,000 μg per ml TBZ, occasionally decayed in spite of the antifungal treatment (10). The problem was not a local one, since tolerant strains of the green and blue molds were also isolated recently from citrus fruits that had decayed during shipment from 16 different countries to the European markets (17).

Since the occurrence of tolerant strains was becoming more frequent, it was important to survey their occurrence at various sites where fruit was being handled and to estimate the potential danger of the occurrence of the tolerant strains in Israel. Also, we studied their properties in comparison with those of the "wild type."

The phenomena of cross-tolerance to different benzimidazoles was observed in the citrus molds and described both in Israel by Gutter (12) and elsewhere by Harding (14) and Kuramoto (16). Similar phenomena were also described among other fungal pathogens (2,5,15,22). Therefore, it was essential to study the extent of cross-tolerance and its practical implications regarding postharvest treatment of citrus fruits.

The tolerance of certain fungal pathogens to fungicides may perhaps decrease under field conditions after the withdrawal of the compounds from use for some time, or in the laboratory after repeated subculturing on a fungicide-free medium, as described by Bollen and Scholten (3) for *Botrytis cinerea,* by Bollen (2) for *Penicillium* spp., and by Richardson (18) for *Fusarium solani.* Information on the persistence of fungal tolerance to benzimidazoles is of great importance in planning efficient measures for the postharvest control of green and blue molds of citrus fruit.

Another aspect that has practical implications is the competition between susceptible and tolerant strains in the absence of a "selection pressure." This problem was studied by Bollen and van Zaayen (4) and Wuest et al (26) in species of *Verticillium,* and by Ben-Yephet et al (1) in *Ustilago hordei.* It was not studied in species of *Penicillium* pathogenic to citrus fruits.

Information gathered along these lines should be helpful in assessing the danger of mold strains tolerant to benzimidazoles and in planning potential strategies for control of postharvest decay of citrus fruits.

MATERIALS AND METHODS

Sampling. Different methods of sampling airborne spores in the orchard, packinghouse, or storage room were tested. These included the use of a sampler of airborne bacteria (Casella London Ltd., Regent House, London N1 7ND, U.K.), an impinger (that pulls fixed volumes of air through a liquid trap), and modifications of these methods. Finally, however, the method of exposing petri plates containing media for given lengths of time was adopted because of its simplicity and the possibility of widening the range of the survey.

At each of the test sites, the proportion of tolerant isolates was estimated by comparing the number of colonies on PDA plates with and without the fungicide. Three plates of each medium were exposed for 4 min in the packinghouses and storage rooms and for 10–20 min in the orchard. These exposure periods ensured a large, but still countable, number of colonies per plate.

Media. To reduce bacterial contamination, which may strongly inhibit *P. digitatum* (13), the pH of the potato-dextrose agar (PDA) medium was adjusted to 3.5 ± 0.2 by adding hydrochloric acid.

TBZ at concentrations of 5, 50, and 500 μg active ingredients per milliliter, and benomyl at 2, 20, and 200 μg per milliliter, were incorporated into the medium.

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Cultures. Isolates under study, which included both the wild types sensitive to benzimidazoles and tolerant strains, were inoculated into citrus fruits and subsequently reisolated to ensure the purity and pathogenicity of the fungus. Single-spore cultures were prepared and used throughout this study.

The isolates used in this work are listed in Table 1.

Fungicides. The various compounds used were: TBZ (2-[4′-thiazolyl] benzimidazole) formulated as Tecto 60 WP, Merck Chemical Division, Rahway, NJ 07065; benomyl (methyl 1-[butylcarbamoyl]-2-benzimidazolecarbamate) Benlate 50 WP, E.I. du Pont de Nemours & Co., Wilmington, DE 19898; carbendazim (2-[methoxycarbonylamino]-benzimidazole) Bavistin 50 WP, BASF, Ludwigshafen 6700, West Germany; cypendazole (1-[5-cyanopentylcarbamoyl-2-methoxycarbonylamino]-benzimidazole) Folcidine 50 WP, Bayer Pflanzenschutz Leverkusen, West Germany; fuberidazole (2-[2′-furyl] benzimidazole) 40 WP, Bayer Pflanzenschutz Leverkusen, West Germany; and thiophanate-ethyl (1,2-*bis*-[3′-ethoxycarbonyl-2′-thioureido]-benzene) Topsin NF 35, Cercobin, 50 WP, Nippon Soda Co., Ohtemachi, Chiyoda-Ku, Tokyo, Japan.

All concentrations were expressed as micrograms of the active ingredient per milliliter of PDA medium.

Assay of tolerance. TBZ arrests the elongation and growth of the mycelium more effectively than it inhibits spore germination (9). Thus, a "colony-count method" was used. Spore dilutions were prepared. These were placed on PDA containing various concentrations of the fungicides. The number of colonies developing was compared with those in the fungicide-free controls. This method measured the effects of the fungicides on both spore germination and mycelial growth.

The inhibition of fungal growth ("vegetative growth method") also was assayed. Agar disks (4 mm in diameter) containing mycelium of the test fungi were placed on PDA containing various concentrations of the fungicide and incubated at 25 C. The diameter of the colony was measured daily and fungal growth was expressed as area occupied by the mycelium. A linear regression of the total area of the developing colony (after subtracting the area of the agar disk) over time was calculated. Three replications were employed in all tests.

The results obtained by the two methods described above were compared.

Fruit inoculation. A 0.5 × 0.5-cm section of the peel was excised to the depth of the albedo. The pathogen (usually as a spore suspension) was introduced and the section of peel was replaced and sealed with paraffin or adhesive tape. This method is hereafter referred to as "window inoculation."

RESULTS

The incidence of tolerant strains. The survey was made at three types of locations: citrus orchards, packinghouses, and storage rooms. In spite of the long (10–20 min) exposure of the PDA plates in the orchards, the numbers of colonies that developed during subsequent incubation of the exposed plates were small compared with those that developed on similar plates exposed for only 4 min in packinghouses or storage rooms.

The percentage of tolerant colonies of *P. digitatum* and *P. italicum* on PDA containing 50 μg of TBZ per milliliter compared with the total fungal population found on unamended PDA, was 1–9% in citrus orchards, 5–35% in packinghouses, and 85–100% in storage rooms.

Decay in storage rooms averaged less than 7% of the fruit after prolonged refrigerated storage, but the majority of isolates collected were tolerant. This high incidence of tolerant strains in the air spora of the storage rooms was also found when isolates were prepared from randomly sampled fruits that had decayed after prolonged storage (Table 2). Seventy to 100% of the isolates were tolerant to the higher concentration (500 μg per ml) of TBZ.

A more detailed survey of the proportion of fungicide- tolerant isolates obtained from a packinghouse was done during the citrus packing season. Plates with media containing three different concentrations of TBZ or benomyl were exposed three times a day (at about 0800 hours, 1200 hours, and 1500 hours) not far from the place where the fruit was dumped. The percentage of colonies growing on these media out of the total number recovered on unamended medium is summarized in Fig. 1.

Variation was observed among the various exposures, suggesting that the operation of temporary local air currents or variations in

TABLE 1. Characteristics of the *Penicillium* species isolates used in this study of fungicide tolerance

Species	Isolate designation	Character	Origin	Maximal concentration allowing growth (μg a.i. per ml)	
				TBZ[a]	benomyl
P. digitatum	A	Susceptible	Untreated, decayed fruit	1.0	0.5
	M	Tolerant	Plate[b]—50 μg TBZ per ml	5,000	300
	N	Tolerant	Plate—1,000 μg TBZ per ml	5,000	300
P. italicum	B	Susceptible	Untreated, decayed fruit	0.5	0.3
	P	Tolerant	Plate—100 μg TBZ per ml	5,000	500
	R	Tolerant	Plate—50 μg TBZ per ml	5,000	400
	C[c]	Tolerant	Plate—500 μg TBZ per ml	5,000	500

[a] TBZ = thiabendazole.
[b] Potato-dextrose agar plate culture containing the indicated amount of thiabendazole (TBZ).
[c] Isolate C is a new mutant of *P. italicum*, beige in color, highly tolerant to benzimidazoles, identified and described as *P. italicum* var. *avellaneum* Samson and Gutter by Samson et al (19).

TABLE 2. Number of isolates of *Penicillium digitatum* and *P. italicum* obtained from citrus fruits that had decayed during prolonged storage in three refrigerated rooms[a]

Medium[b] with:	Room A		Room B		Room C	
	P. digitatum	*P. italicum*	*P. digitatum*	*P. italicum*	*P. digitatum*	*P. italicum*
No TBZ	38	17	36	40	7	63
50 μg TBZ per ml	38 (100)[c]	15 (88)	35 (97)	40 (100)	7 (100)	63 (100)
500 μg TBZ per ml	38 (100)	13 (76)	30 (83)	28 (70)	7 (100)	55 (87)

[a] Isolates from randomly sampled rotten fruits were prepared on PDA containing TBZ and on unamended medium to estimate the incidence and degree of tolerance.
[b] Potato-dextrose agar containing the indicated concentration of thiabendazole (TBZ).
[c] The percentage of the isolates tolerant to the given concentrations of fungicides is given in parentheses.

the cleanliness of the incoming fruit influenced the composition of the air spora. Nevertheless, the proportion of the tolerant spores of the green and blue molds out of the total number of isolates recovered from the packinghouse ranged between 5 and 35%, with an incidence of 15% in most of the exposures. No significant differences in either the size of the total number of isolates observed or in the frequency of the tolerant strains were found between the green and blue molds. Only the last exposure (22 April) suggested that a decrease in the occurrence of the green mold was correlated with an increase in the blue mold.

Effect of thiabendazole and benomyl on the sensitive and tolerant strains of the green and blue molds. The effect of various concentrations of TBZ and benomyl, ranging from 0.01 to 10,000 μg/ml, was studied using the colony-count and vegetative growth methods. The results obtained by the colony-count method are summarized in Fig. 2, and show that concentrations of 5 μg of TBZ per milliliter or 1 μg of benomyl per milliliter inhibited completely the formation of colonies of the sensitive green and blue molds. However, concentrations of 300–500 μg of benomyl per milliliter were required to arrest formation of colonies of the tolerant strains while they were not inhibited by concentrations of TBZ as high as 5,000 μg per milliliter, which proves again that benomyl is more active than TBZ against both sensitive and tolerant strains of citrus molds. Tolerant colonies growing at high concentrations of both compounds (over 500 μg of TBZ per milliliter and 250 μg of benomyl per milliliter) were stunted and did not produce conidia, but gave normal colonies upon transfer to a fungicide-free medium.

The vegetative growth method showed inhibition of both sensitive and tolerant strains at lower fungicide concentrations than did the colony-count method. The results obtained with the vegetative growth and colony-count methods with a tolerant strain of *P. italicum* are compared in Fig. 3. At certain concentrations of both TBZ and benomyl, the vegetative growth was reduced to 10–20%, but there was little or no influence on colony count. Also, sometimes, the rates of growth of the tolerant strains, particularly at very low concentrations of both compounds (eg, less than 5 μg per milliliter), were higher than on the fungicide-free medium (Fig. 3).

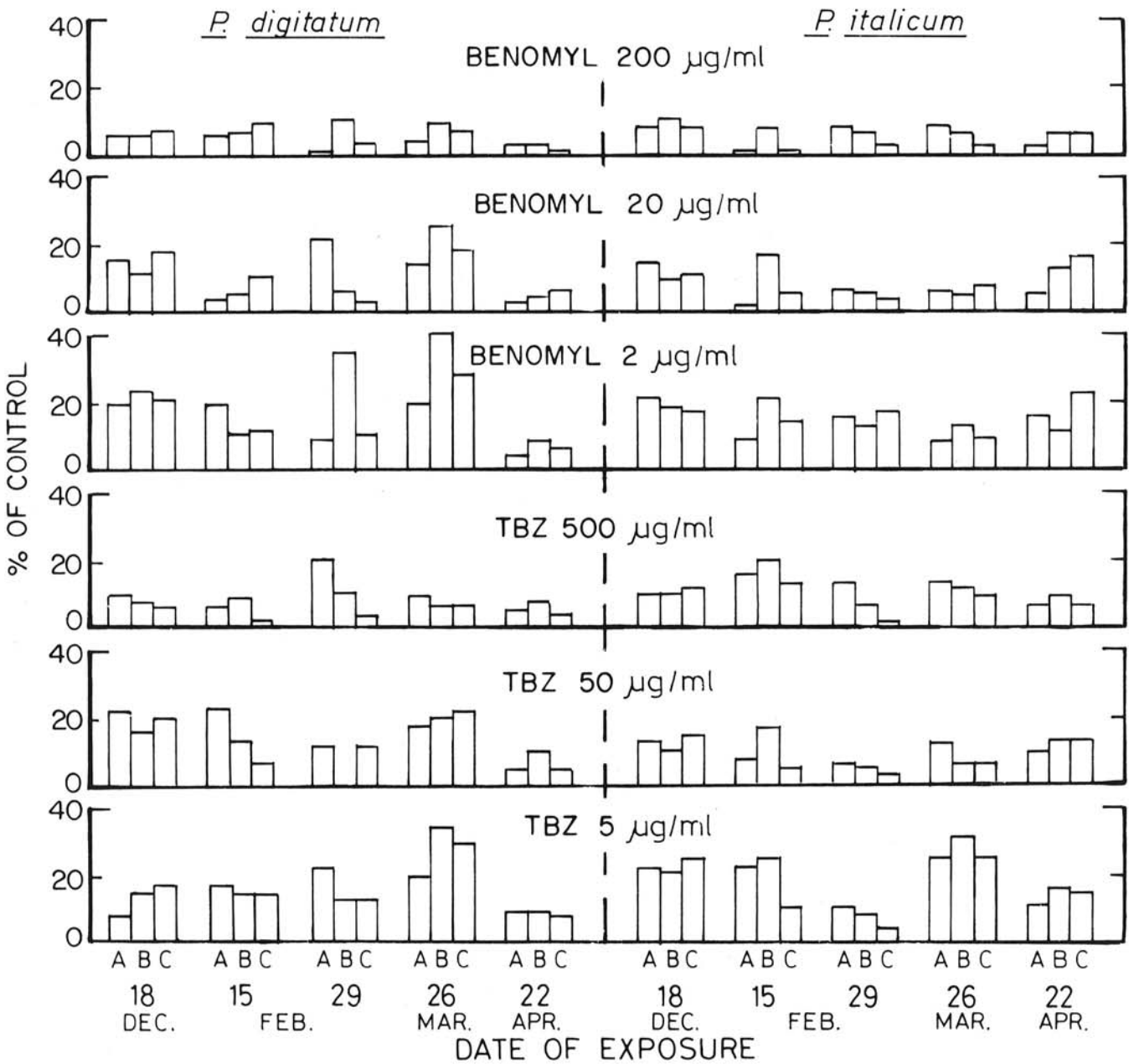

Fig. 1. Incidence of tolerance in the population of *Penicillium digitatum* and *P. italicum* found in the citrus packinghouse during the season. Height of columns indicates percentage of established colonies on potato-dextrose agar (PDA) medium containing different concentrations of benomyl and of thiabendazole (TBZ) (micrograms a.i. per milliliter) in comparison with controls on fungicide-free medium. The survey was carried out by exposing triplicates of PDA plates for 4 min three times a day at **A,** 0800 hours; **B,** 1200 hours; and **C,** 1500 hours.

Cross-tolerance. The response of the TBZ- and benomyl-sensitive and tolerant strains of both molds to four other benzimidazoles (carbendazim, cypendazole, fuberidazole, and thiophanate-ethyl) was compared with their response to TBZ and benomyl. The concentrations tested ranged 0.05–5,000 μg/ml. Strains tolerant to TBZ and benomyl were tolerant to the other four benzimidazoles (Table 3); however, it should be added that the compounds tested differed in effectiveness.

Persistence of tolerance. To determine whether strains maintained fungicide tolerance, single-spore cultures of four tolerant strains (two of *P. digitatum* and two of *P. italicum*) were transferred to PDA and subcultured weekly on fresh unamended PDA medium. In parallel, the same four strains were window-inoculated on untreated citrus fruits, spores were collected and used to inoculate fruits again each week. These subculturings were done over a period of 16 wk. Every 4 wk, the cultures were also grown on media containing 0, 50, and 500 μg of TBZ per milliliter and their degree of tolerance was assayed by the colony-count method.

The results of passing the isolates through citrus fruits and assaying their tolerance are summarized in Fig. 4. After 16 transfers to a fungicide-free medium, the isolates retained fungicide tolerance and there was no loss in pathogenicity compared with that of the original wild type.

Similar results were obtained when the isolates were subcultured at weekly intervals on a PDA medium for 16 wk.

Competition between tolerant strains and the wild type. A study was made of the survival of tolerant strains in a mixed population with isolates of the wild type (susceptible to the benzimidazoles) in the absence of selection pressure, under conditions in which the tolerant strains would not be favored by having benzimidazoles incorporated into the medium.

A suspension of a mixture of spores of five isolates of *P. italicum* tolerant to benzimidazoles (similar in tolerance to isolates P and R [see Table 1 for a description]) was mixed at a proportion of 1:1 with a suspension of spores of the susceptible wild type and window-inoculated into untreated Shamouti oranges. After

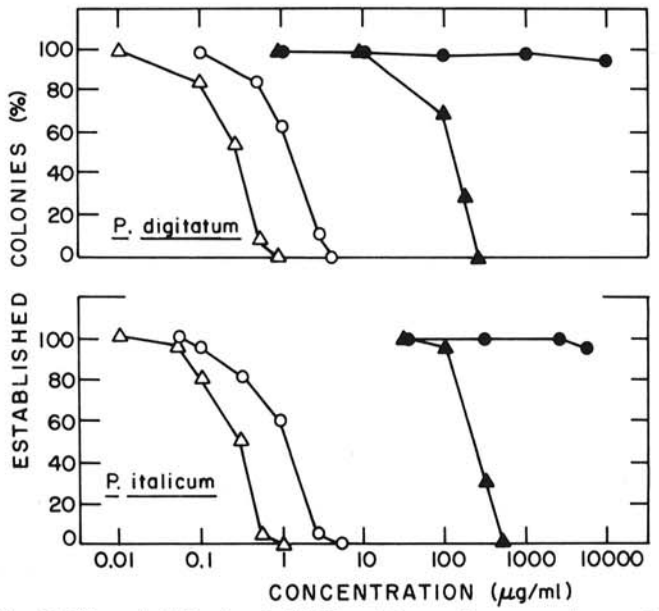

Fig. 2. Effect of thiabendazole (TBZ) and benomyl concentration on the tolerant and sensitive strains of *Penicillium digitatum* and *P. italicum*. Spore suspensions of the different isolates were incubated on potato-dextrose agar plates containing various concentrations of TBZ and benomyl, ranging from 0.01 to 10,000 μg per milliliter. The numbers of established colonies (in percent of the controls on fungicide-free medium) are presented in relation to the fungicide concentration (log scale). The various strains were selected from the general survey. ● = TBZ-tolerant; o = TBZ-sensitive; ▲ = benomyl-tolerant; Δ = benomyl-sensitive strains.

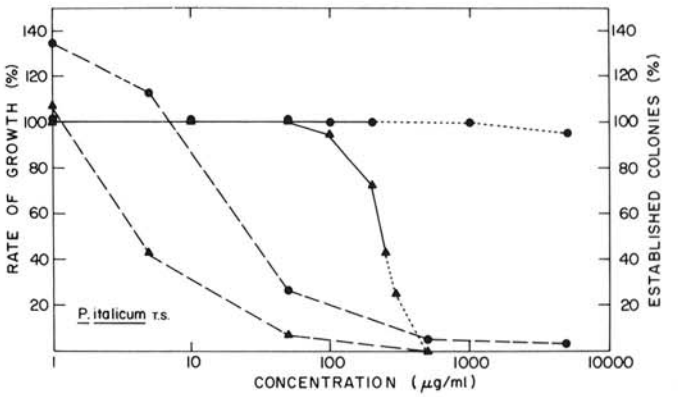

Fig. 3. Comparison of the effect of thiabendazole (TBZ) and benomyl on the rate of growth and number of established colonies of a tolerant strain of *Penicillium italicum*. Potato-dextrose agar (PDA) containing various concentrations of TBZ or benomyl, ranging from 1 to 10,000 μg/ml, was used. PDA disks (4 mm in diameter) with mycelium of the test isolate were placed on the above media, incubated at 25 C, diameter of the colony was measured daily and the fungal growth was expressed as area occupied by the mycelium ("vegetative growth method"). Simultaneously, spore suspensions of the same isolate were prepared, spread on the above media and the number of developing colonies compared with those in the fungicide-free medium ("colony-count method"). The two methods were compared and the results expressed in percent on PDA medium containing different concentrations (log scale) of TBZ or benomyl in comparison with the controls on fungicide-free medium. ● = thiabendazole; ▲ = benomyl; — = colony-count method; --- = vegetative growth method; ···· = mycelium without sporulation.

TABLE 3. Effectiveness of various benzimidazole fungicides on sensitive (S) and tolerant (T) strains of *Penicillium digitatum* and *P. italicum*[a]

		Percentage of colonies (vs. unamended controls) that grew on PDA amended with (μg a.i. per ml)											
		0.05		0.5		5		50		500		5,000	
Fungicide	Fungus	S	T	S	T	S	T	S	T	S	T	S	T
Thiabendazole	*P. digitatum*	100	100	85	100	0	100	0	100	0	100[b]	0	100
	P. italicum	100	100	80	100	0	100	0	100	0	100[b]	0	100[b]
Benomyl	*P. digitatum*	100	100	9	100	0	100	0	80	0	0	0	0
	P. italicum	100	100	5	100	0	100	0	100	0	0	0	0
Carbendazim	*P. digitatum*	100	100	89	100	75	90	15	100	0	100	0	69
	P. italicum	100	100	89	100	77	97	18	90	0	99	0	95
Cypendazole	*P. digitatum*	41	100	23	100	0	100	0	96	0	0	0	0
	P. italicum	59	100	20	94	0	92	0	63	0	41	0	42[b]
Fuberidazole	*P. digitatum*	100	100	92	100	86	100	21	100	0	100	0	100
	P. italicum	100	100	96	100	84	100	20	91	0	91	0	83
Thiophanate-ethyl	*P. digitatum*	100	100	90	100	88	91	50	100	0	98	0	10
	P. italicum	100	100	89	100	85	100	44	100	0	80	0	75

[a] Isolates were grown on potato-dextrose agar medium containing different concentrations of the various benzimidazoles and the results are given in percentages of the number of colonies developing on these media as compared with controls on fungicide-free media.
[b] Sporulation was inhibited.

incubation for approximately 1 wk, the spores were collected from the decayed fruits and inoculated into fresh fruits. Simultaneously other conidia were plated on fungicide-free PDA as well as on PDA plates containing 50 μg of TBZ per milliliter or 20 μg of benomyl per milliliter. The proportion of the tolerant spores in the total population was determined by using the colony-count method. This procedure was repeated four times over a period of 4 wk; the results are summarized in Table 4.

There was no substantial change in the original proportion of susceptible and tolerant isolates over the 4-wk period.

DISCUSSION

Mold strains were tolerant to concentrations of benomyl 500 times and of TBZ 1,000 times greater than the minimal dose required to inhibit the sensitive wild types. The magnitude of tolerance is roughly of the same order as that noted by Bollen and Scholten (3), Geeson (8), and Tate and Samuels (21) for pathogens isolated from hosts other than citrus.

A survey of isolates, carried out at different sites, revealed no significant differences among different populations in the proportions of the green and blue molds and in the percentage of tolerant strains among them. This was quite unexpected in view of the much greater incidence of the decay caused by *P. digitatum* compared with that induced by *P. italicum* throughout the season. This can be explained, at least in part, by the following observations: *P. italicum* is more prone to form resistant strains (12); the effectiveness of most fungicides on *P. digitatum* is somewhat greater than on *P. italicum* (*unpublished*); spores of the blue mold are smaller in size and greater in number per area of fruit (7, pages 391–392), thus counterbalancing their smaller rate of growth as compared with the green mold (7, page 394); and often

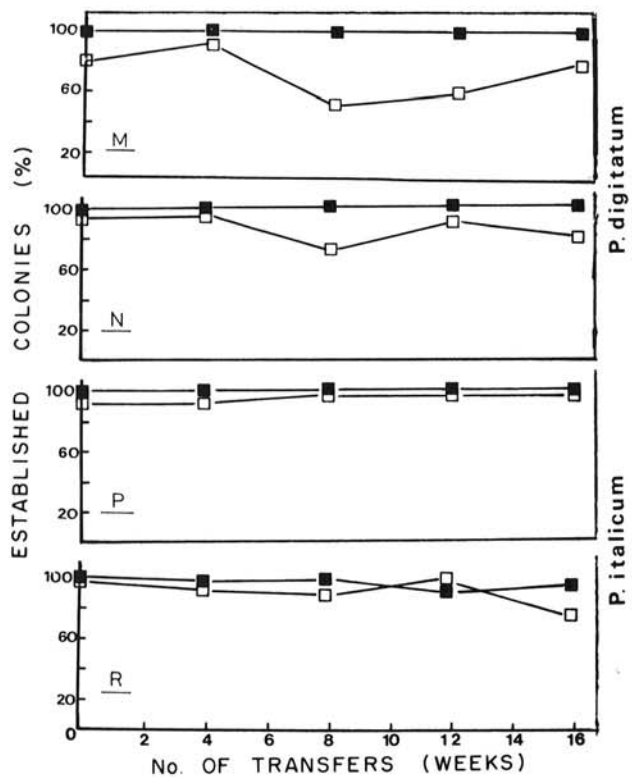

Fig. 4. The persistence of tolerance to thiabendazole (TBZ) in strains of *Penicillium digitatum* (isolates M and N) and *P. italicum* (isolates P and R) after numerous inoculations on untreated fruit. The four isolates were window-inoculated weekly on citrus fruits and spores were collected and used to inoculate fresh fruits again. This was done over a period of 16 weeks. Every 4th wk, the spores were transferred to PDA containing 50 (■) and 500 (□) μg of TBZ a.i. per milliliter to assay the degree of tolerance by counting the number of colonies developing at these concentrations. The results are expressed in percentage of established colonies on TBZ medium in comparison with the controls on fungicide-free medium.

good control of the green mold in citrus fruit was followed by a marked increase in the blue mold (11).

Sensitive as well as tolerant molds were isolated in low frequency in the orchard. Since benzimidazole sprays are not used in citrus orchards in Israel, the source of the tolerant strains may be traced back to bulk bins containing rotten fruits returned from packinghouses to the orchard. Alternatively, spores may be disseminated by winds from packinghouses to adjacent orchards.

The number and types of isolates collected when culture media were exposed in the packinghouse (Fig. 1) varied greatly, indicating that many factors influencing the population were involved, such as sanitation, air currents, temperature, precipitations, and layout of the packinghouse.

An increase in the concentration of both TBZ and benomyl was often correlated with a lower percentage of tolerant strains of both molds (Fig. 1, Table 2). Not all strains isolated initially on plates with the lower concentration of the compound survived the higher concentration. This indicates some gradation in tolerance, as mentioned also by Kuramoto (16) and Geeson (8). Since the antifungal properties of benomyl were greater than those of TBZ, this gradation was conspicuous when the fungi were exposed to benomyl (Fig. 1). In most cases, once a strain was tolerant, it grew on plates when challenged with the highest concentration of the fungicide used in these experiments. Under such extreme conditions, the colonies were stunted and sporeless, but still grew normally on PDA; hence, the compounds could be considered to have fungistatic activity or at least to inhibit sporulation of tolerant isolates.

The high incidence of tolerant strains found in the storage rooms can be explained by the prolonged exposure of the pathogen to the fungicide. These conditions probably induced a selection pressure (25). The length of such exposure should be minimized if the formation of tolerant strains is to be prevented.

Another way to reduce the chances of formation of tolerant strains is to improve sanitation, including careful handling, avoiding wounding of the fruit, cleanliness in packinghouses, and not returning moldy fungicide-treated fruit in bins to the orchards.

Fungitoxicity was more evident when evaluated by using the vegetative growth method than the colony-count method. Therefore, the colony-count method is of more value in evaluating the effectiveness of a compound.

Strains tolerant to TBZ or benomyl were tolerant to other compounds of this group (as reported by others [16,20,24]), in spite of differences in the specific antifungal activity of each compound. Thus, once such strains appear, the use of other benzimidazoles is of no value and fungicides with different molecular structures or modes of action will have to be introduced.

Strains of both green and blue molds retained a relatively constant degree of tolerance to benzimidazoles even after 16

TABLE 4. The proportion of susceptible and tolerant isolates recovered from a mixed population of blue mold (*Penicillium italicum*) spores collected from decayed fruits following four successive inoculations into fresh Shamouti orange fruits[a]

Transfer no.	Number of colonies in controls[b]	Percent of tolerant colonies on: 50 μg per ml TBZ[c]	20 μg per ml benomyl
0	147	46.5	48.2
1	192	51.8	58.3
2	91	50.8	56.0
3	107	54.3	45.7
4	209	53.5	52.1

[a] Shamouti oranges were window-inoculated with a suspension of spores of susceptible and tolerant strains of *P. italicum* mixed at a proportion of 1:1. After 1 wk of incubation, spores from the decayed fruit were collected and plated on PDA medium containing 50 μg TBZ per milliliter or 20 μg benomyl per milliliter to assay the proportion of susceptible and tolerant strains. The collected spores also were inoculated into fresh fruits. This procedure was repeated four times.

[b] Average of six replicates.

[c] TBZ = thiabendazole.

transfers on a fungicide-free substrate (Fig. 4). This may indicate that the tolerant strains resulted from a genetic change and conform to Dekker's definition of resistance (5). Similar persistence of tolerance, for different lengths of time, were described (eg, 2,3,23) for other pathogens. These findings stress the potential and prolonged danger inherent in the occurrence of tolerant strains. Also, the tolerance to benzimidazoles was not linked with any decrease in pathogenicity. This is in contrast to the situation with pimaricin, in which increased tolerance of *Cladosporium* was associated with reduced pathogenicity (6).

The observation that the tolerance of the green and blue molds (in the absence of the fungicides) is persistent and apparently a result of a genetic change, does not necessarily imply that such strains will survive when no benzimidazoles are used. Our results showed that tolerant spores were capable of surviving in a heterogenic population (a mixture with spores of the wild type) under conditions of no selective advantage due to the presence of benzimidazoles (Table 4). This suggests that no immediate improvement may be anticipated with the cessation of the use of benzimidazoles.

The practical implications of some of the above findings are: that the tolerant strains retain pathogenicity after numerous transfers on nutrient medium not containing fungicides, that they are capable of surviving in the absence of benzimidazoles, and that the use of other compounds of the benzimidazole group would not increase control when strains with tolerance to any one of them are involved. Under these conditions, strict sanitation and the use of fungicides other than benzimidazoles should be beneficial practices in control.

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