Development, Persistence, Survival, and Strategies for Control of Thiabendazole-Resistant Strains of *Penicillium expansum* on Pome Fruits

D. Prusky, Malka Bazak, and Ruth Ben-Arie

Department of Fruit and Vegetable Storage, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.

Contribution from the ARO, 888-E, 1983 series.
Accepted for publication 28 January 1985.

ABSTRACT

Prusky, D., Bazak, M., and Ben-Arie, R. 1985. Development, persistence, survival, and strategies for control of thiabendazole-resistant strains of *Penicillium* and *expansum* on pome fruits. Phytopathology 75: 877-882.

Both the proportion of isolates of *Penicillium expansum* resistant to thiabendazole (TBZ) at 40 μg/ml and the persistence of this characteristic after a single transfer on apples increased during 3 consecutive years of postharvest treatment with TBZ. Resistance to TBZ was maintained both when the isolates were grown for 20 transfers on a fungicide-free medium or when they were inoculated into and recovered from untreated apple fruits for five transfers. Fruit decay and initiation of sporulation always developed more slowly after inoculation with resistant strains than with sensitive ones. In the absence of fungicides, fruit decay caused by mixtures of sensitive and resistant strains developed at a rate similar to that caused by the sensitive strain alone. The proportion of resistant spores collected from decayed fruit inoculated with a mixture of strains, with no TBZ selection pressure, showed a rapid decline. Three fungus control strategies were compared for the control of a highly resistant population of *P. expansum*: uniform treatment with a mixture of fungicides, heterogeneous treatment in which two fungicides were applied separately to different lots of fruit placed in the same storeroom, and a combination of heterogeneous and mixed treatments in which a mixture of fungicides and a single fungicide were applied to different lots of fruits placed in one storeroom. All three strategies significantly reduced decay incidence in relation to the control; however, the last of these three strategies caused the most rapid decline in the proportion of resistant strains.

Additional key words: control strategies in storage, resistance to fungicides.

Pome fruits, harvested during a relatively short season, are usually dipped in a fungicide suspension immediately after harvest, and are stored at 0 C for periods of up to 1 yr. The standard antifungal postharvest treatment in Israel in the 1960s was a 20-sec dip in captan. During the last decade the benzimidazole fungicides were introduced and thiabendazole (TBZ) was widely used because of its effectiveness in controlling blue mold, which is caused by *Penicillium expansum* Link (Thom.) (2). The benzimidazoles were shown to be effective in preventing infection of fruit injuries incurred during harvest and handling before and after the treatment (7). Their eradicative properties can be attributed to penetration through the waxy cuticle of the host and arrival at the infection site (1). This characteristic was responsible for the persistence of the fungicide over long storage periods, but at the same time it became the cause for its continuous selection pressure on populations of *Penicillium* spp. Years of continuous use of TBZ and homogeneous selection pressure have resulted in decreased fungicide effectiveness and increased crop losses (14). New control strategies are directed at reducing the population of thiabendazole-resistant strains and providing better control of Penicillium rot. Benzimidazole-resistant strains of *P. expansum* have been reported on harvested apples and pears in Oregon (4), New York (17), Australia (11), and Israel (14,15).

This paper describes an investigation of the parasitic fitness of a resistant population of *P. expansum* in storerooms in Israel and the use of this characteristic for the development of fungus control strategies, which both enhance a decline in the resistant population and provide disease control.

MATERIALS AND METHODS

Source of fruit and inoculum. Apple fruits (*Malus domestica* 'Calville de San Sauveur' and 'Jonathan') and pear fruits (*Pyrus communis* 'Spadona') were used in the experiments. Fruits were stored at 0 C for 4–6 mo followed by 5 days at room temperature. Isolates of *P. expansum* were obtained from naturally rotted stored fruits. Spores of the fungus originating from a fruit were transferred to potato-dextrose agar (PDA) and to PDA amended with thiabendazole (TBZ) (2-[4-thiazolyl]-benzimidazole) formulated as Tecto 60 WP; Merck & Co., Inc., Rahway, NJ). Conidia were harvested by adding a small amount of water to each culture plate and gently rubbing the sporulating mycelial mat with a bent glass rod. The number of spores was determined by using a hemocytometer and inoculum concentrations were adjusted to 10^6 spores per milliliter. Pathogenicity of the resistant isolates was tested by wound-inoculating apples with the newly produced conidia. Surface disinfested (90% ethanol) apples were inoculated either by pricking the fruit to a depth of 2 mm with a sterile three-needle probe and subsequently covering the spot with 15 μl of conidial suspension, or by touching the culture of *P. expansum* with the three-needle probe and then pricking the fruit surface. Following inoculation, the fruits were held at 25 C in polyethylene bags to maintain high humidity. The single-spore technique was used to obtain initial cultures of the resistant pathogenic strains. Cultures were grown on PDA at 25 C. Stock cultures were held at 0 C and tested for pathogenicity every 3 mo.

Assay for resistance. TBZ at 40 μg a.i. per milliliter was incorporated into the PDA media prior to sterilization. That concentration was chosen because it is twice the concentration required to control all sensitive members of the fungal population, and does not affect the development of resistant members. Control media did not contain the fungicide. A suspension containing 50–100 conidia was spread on each plate and colony development at 25 C was observed after 5 days. Only colonies that had reached 5 mm in diameter were counted. The occurrence of resistant isolates in rotted apples was determined by growing the fungus isolated from the rotted tissue on TBZ-amended medium and observing the culture for the effects of TBZ on inhibition of hyphal elongation and growth of the mycelium (9).

Sampling of natural spore populations. Four methods for sampling spore populations in the orchard, packinghouse, and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

storage room were tested: isolation of fungi from rotten fruits; exposure of plates for a given length of time; washing fruits brought to the laboratory with 20 ml of sterile water, from which an aliquot was then plated; and exposure of pricked apples that had been dipped in TBZ at 600 μg/ml a.i. Control apples were pricked but not dipped. For each assay, the proportion of resistant isolates present was estimated by comparing the number of colonies developing on PDA plates or fruits with and without fungicide. Five plates and/or four punctured fruits were used for each sampling.

Control strategies on a laboratory scale. Fruits were stored in plastic trays at 10 C in 50 × 70 × 80-cm polyvinyl chloride tents through which air was flushed at ~10 L/min. The air stream entered the tents through an inlet at the bottom, passed through a wet sponge below the fruit and exited through an outlet above the fruit. Each tent contained 10 plastic trays (replicates) each containing 10 fruits. Spadona pears were prick-inoculated with 15 μl of a 1:1 mixture of sensitive and resistant conidial suspensions (10^6/ml) and incubated at high humidity and room temperature for 1 day. The original spore suspensions from which the mixture was prepared consisted of conidia from seven different isolates resistant to TBZ at 40 μg/ml, and sensitive conidia from two isolates. Resistance of the isolates was stable at 40 μg/ml. Two weeks after inoculation the proportion of resistant strains was determined by testing the spores produced from decayed fruits on PDA and TBZ-amended PDA. These decayed fruits were then returned to the tents and a new batch of healthy, pricked fruit was placed in the trays together with the decayed fruit for natural touch-inoculation and the development of a second generation of the fungus. Spore distribution within the tent was enhanced by operating a 10-cm-diameter fan for 24 hr. When disease symptoms were evident on the newly introduced fruits (second generation), the old decayed fruit were removed. This procedure was repeated several times depending on the number of generations being checked. During 4–5 mo under commercial storage conditions at 0 C, *P. expansum* usually passed through one, or occasionally two, full generations. The time from infection to full sporulation in the laboratory-scale experiment at 10 C (1–2 wk) was considered to be one generation and equivalent to one commercial storage season. Different control strategies were tested by enclosing differently treated fruit within the same tent. The three strategies which were compared to untreated controls were: i) a mixture of two fungicides, ii) a heterogeneous fungicide treatment in which five fruits per tray were treated with one fungicide and the remaining five were treated with another fungicide, iii) a combination of heterogeneous and mixed fungicide treatment in which five fruits per tray were treated with one fungicide and the remaining five were treated with a mixture of two fungicides.

Each fungicide was used only for two generations of *P. expansum*, to simulate 2 yr of commercial use. In the heterogeneous treatment (strategy ii), the fungicide introduced in each generation was alternated with the fungicide previously used and in the following generation with another new fungicide to which the fungal population had never been exposed. In the heterogeneous mixed-fungicide treatment (strategy iii) each fungicide was used as a single treament during the first generation only and in the second generation was applied in a mixture with captan. Fungicide dips were applied for 30 sec at the beginning of the experiment 24 hr after artificial inoculation. For the second and third generation, the fungicides were applied to freshly pricked healthy fruits before they were transferred to the tent for natural infection. The fungicides and rates of active ingredient tested were TBZ at 600 μg/ml a.i.; 1,2,3,6-tetrahydro-*N*-(trichloromethylthio) phthalimide (captan, 50 WP, Makhteshim, Israel) at 2,500 μg/ml a.i.; 3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carbozamide (iprodione, 50 WP, Rhône-Poulenc, France) at 500 μg/ml a.i.; 1-(β-allyloxy-2,4 dichlorophenethyl) imidazole (imazalil sulfate, 75 WP; Janssen Pharmaceutica, Belgium) at 800 μg/ml a.i.; and 1-[(2-2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl) methyl]-1H-1,2,4-triazole (etaconazole, 10 WP; Ciba-Geigy, Switzerland) at 500 μg/ml. This experiment was repeated twice.

Control strategies in semicommercial trials. Different fungus control strategies were applied to freshly harvested, naturally inoculated fruits by dipping boxes containing 100 fruits in the fungicide suspension for 30 sec. Each treatment consisted of ten boxes. Fruits were stored under commercial conditions in controlled atmosphere storerooms at 0 C. Boxes of fruit that had received the three different treatments and the control were separated in the same storeroom by covering the boxes with large plastic bags to prevent spore movement from one treatment group to another. The two fungicides used in these experiments were iprodione and TBZ, applied singly or as a mixture. Each treatment was applied to five boxes of fruit in strategies ii and iii, whereas for the untreated control and strategy i, all 10 boxes in the plastic bag received the same treatment.

RESULTS

Development and persistence of resistance to TBZ. The first indication of resistance of *P. expansum* to benzimidazole compounds was observed in Israel in 1977. Until then, the population of this fungus had been uniformly sensitive to TBZ with an ED_{50} of 5.5 μg/ml. Following a season of unusually high rot incidence in a pear storeroom, resistant strains showing an ED_{50} of 2,000 μg/ml were isolated from the air spora of that room. Isolates from decayed fruit showed an increase from 30% of resistant strains in the first season to 58% in the second storage season and to 78% in the third season. The newly produced conidia were inserted into wounds in pears to determine their pathogenicity and reisolated on TBZ-amended PDA; 25% of those isolated in the first year and 77 and 100% of those isolated in the following 2 yr were resistant to TBZ at 40 μg/ml.

A survey to determine the incidence of resistant strains in apple orchards, packinghouses, and storerooms showed no resistant strains of *Penicillium* spp. in the orchard, on the surface of the fruits, in the atmosphere of the packinghouse, or on initially decayed fruits arriving from the orchard. However, exposure in the storeroom of petri plates containing PDA amended and unamended with TBZ at 40 μg/ml or pricked fruits dipped or not dipped in TBZ, yielded an average of 50 and 45% resistant strains, respectively, throughout the season. At the end of the season, after the storerooms had been evacuated, exposure of plates in five commercial storerooms indicated an average of 75% resistant strains.

Parasitic properties of the resistant strains. Resistance to TBZ at 40 μg/ml persisted in 10 isolates that were transferred for 20 generations on unamended TBZ-PDA and for five subsequent generations through untreated apples. Seven isolates remained pathogenic but three became nonpathogenic after the fifth generation. A difference in the virulence of the resistant and sensitive strains was evident from the rate of rot development and the time from inoculation to commencement of sporulation. The growth rate of the sensitive isolates was faster than that of the resistant isolates (Fig. 1). Sporulation in the sensitive isolates started 3 days before sporulation of the resistant isolate. Although TBZ treatments (600 μg/ml) 24 hr after inoculation did not totally control the growth of the sensitive isolates (probably because of the high inoculum load and the time lapse between inoculation and treatment), both decay development and sporulation were delayed by 2 days, whereas the rate of decay by the resistant isolates was not affected (Fig. 1). In the absence of treatment with the fungicide, the rate of decay development on apples inoculated with mixtures of sensitive and resistant strains of *Penicillium* in various ratios (1:0, 1:1, and 3:7) (Fig. 2) did not differ significantly from the rate incited by the sensitive strain alone, even when the percent of resistant strains comprised the majority of the initial inoculum (Fig. 2). However, TBZ treatment 1 day after mixed inoculation (ratios 1:1 and 3:7 for sensitive:resistant isolates) selectively affected the development of the isolates in the mixture and resulted in a decay rate similar to that of the resistant strain alone.

The growth rates of resistant and sensitive strains seeded on agar disks on PDA were similar and were not affected by each other. Competition between resistant and sensitive strains, following inoculation in a 1:1 ratio, was bioassayed by testing the susceptibility of the newly produced spores. Conidia collected 1 day after sporulation had commenced showed that resistant isolates did not compete with susceptible isolates in the absence of TBZ (Table 1). To test the possibility of slow development and later appearance of the conidia of the resistant strains, the spore composition was examined 6 days after sporulation had begun by sampling different spots of the sporulating mat. Again, no resistant conidia were detected. Similar results were obtained when the initial ratio (3:7) was greatly in favor of the resistant strains. Treatment with TBZ selectively affected the competition between isolates and 100% resistant spores were detected on sporulating rotted fruit (Table 1). Similar competition experiments under simulated storage conditions at 20 C, in which 10 strains were involved (five resistant and five sensitive) and in which reinfection of the fruit took place by natural exposure of healthy, wounded fruits to the decayed ones, showed a decrease in the percent of the resistant population from the initial 50% to about 10% after eight generations (Fig. 3). However, the proportion of resistant isolates rebounded quickly following a single application of TBZ.

Control programs for *P. expansum* on a laboratory scale. The alternative chemicals that were used differed in effectiveness against TBZ-susceptible and TBZ-resistant isolates in vitro. The ED_{50} for iprodione on susceptible and resistant isolates was 0.13 and 0.84 μg/ml, for imazalil was 0.01 and 0.03 μg/ml, and for etaconazol was 0.01 and 0.17 μg/ml, respectively. The possibility of a decline in the resistant population of *Penicillium* during three successive generations was tested for the different fungus control strategies. In these tests, the fungicides were compared relative to decline in resistant populations on untreated control fruits. The decrease in the resistant population was significant in the untreated fruits, from the initial frequency of 50 to 4% after the third generation (Fig. 4). The fungicides used in testing the strategies during the first generation were: TBZ, iprodione, and captan. The TBZ and iprodione mixture (strategy i) increased the percentage of resistant strains in decaying fruits from the initial 50–65%. In the heterogeneous treatment of fruits (strategy ii) with TBZ and iprodione, the initial level of resistant strains was maintained, while heterogenous-mixed treatment of fruits with either the mixture of TBZ + captan or iprodione (strategy iii) decreased the percent of resistant isolates to 35%. The second generation of the fungus, developed under the nonselective effects of iprodione and imazalil,

TABLE 1. Percent of thiabendazole-resistant (40 μg/ml) spores of *Penicillium expansum* collected at days 1 and 7 after initiation of sporulation from decayed apple fruits that had been inoculated with a mixed population of TBZ-susceptible (S) and TBZ-resistant (R) isolates

Ratio (S:R)	TBZ (600 μg/ml)	TBZ-resistant strains (%) Day 1	TBZ-resistant strains (%) Day 7
1:0	−	0	0
1:1	−	0	0
3:7	−	0	0
0:1	−	100	100
1:1	+	100	100
3:7	+	100	100

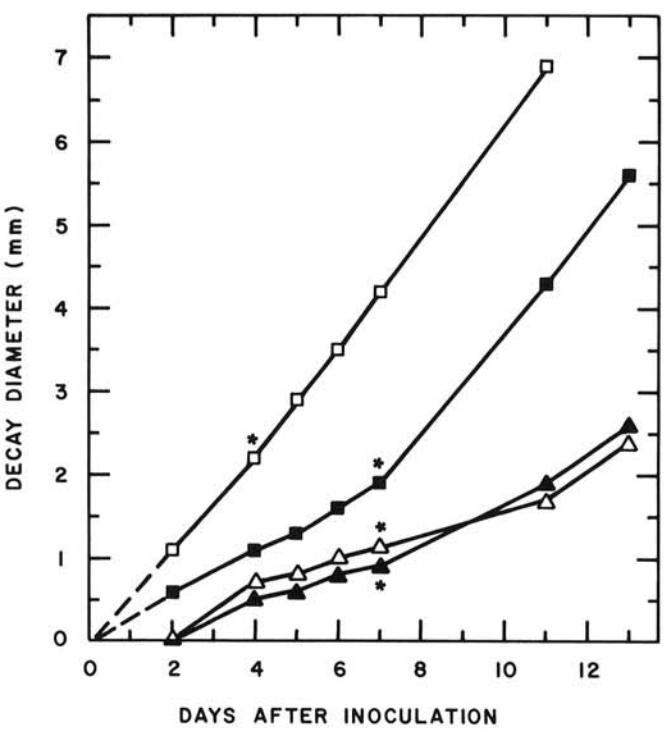

Fig. 1. Decay development and initiation of sporulation of thiabendazole (TBZ)-susceptible and TBZ-resistant strains of *Penicillium expansum* on untreated or treated fruits of apple cultivar Calville de San Sauveur. Symbols: □ = susceptible strain; △ = resistant strain; ■ = susceptible + TBZ 600 μg/ml 24 hr after inoculation; ▲ = resistant + TBZ 600 μg/ml 24 hr after inoculation; and * = start of sporulation.

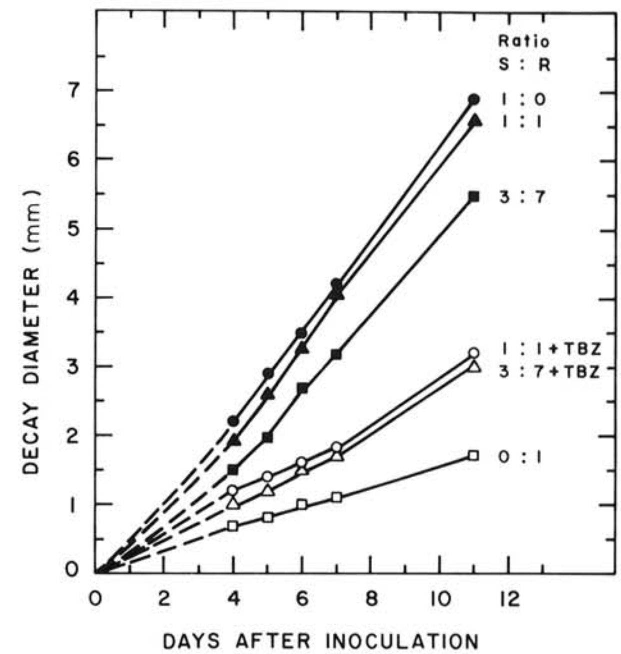

Fig. 2. Effect of inoculation with mixed populations of thiabendazole (TBZ)-susceptible and TBZ-resistant isolates of *Penicillium expansum* on the rate of development of decay in fruits of apple cultivar Calville de San Sauveur, untreated or treated with TBZ at 600 μg/ml 24 hr after inoculation. The suspension of sensitive conidia was composed of equal amounts of conidia from two different strains, and the suspension of the resistant conidia was composed of spores from three isolates resistant to TBZ at 40 μg/ml.

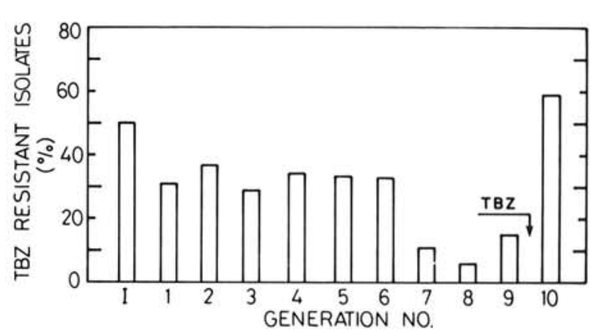

Fig. 3. Proportion of thiabendazole (TBZ)-resistant spores of *Penicillium expansum* recovered after 1–10 generations of natural airborne transfer, starting with an initial inoculation (I) of apple fruits with a 1:1 mixture of spores of resistant and sensitive strains.

resulted in a rapid decrease in the percent of resistant isolates, especially in the mixture treatment (strategy i) which showed a 44% reduction in resistant isolates. During the third generation, the fungicides used were imazalil and etaconazol and the tendency to declining resistance of the fungus to TBZ generally continued. In the presence of nonselective fungicides the resistant population on the whole declined at a rate similar to its decline in the untreated control. Inclusion of TBZ-treated fruits in the tent containing the nontreated fruits at the end of the first, second, and third generation, resulted in a significant selection effect on the population (Fig. 5) causing a rise in resistant isolates from relatively low values of 4–25% to 75–80% in the subsequent generation.

Control strategies in semicommercial trials. To determine the effect of different strategies on the reduction of the resistant population, orchards were chosen in which fruit harvested the previous season had suffered considerable losses from storage decay, of which 90% was found to be caused by TBZ-resistant isolates of *P. expansum*.

After one commercial season, the average percentage of resistant isolates was reduced to the same level as in the absence of any fungicide treatment by treatment of fruit with a mixture of TBZ and iprodione followed by a single application of iprodione (Table 2). The heterogeneous treatment, with TBZ and iprodione applied separately, was less effective. The application of TBZ and iprodione as a mixture had no beneficial effect in reducing the

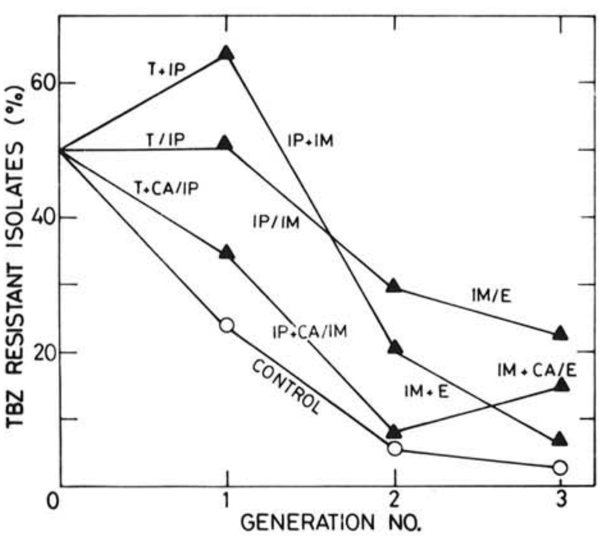

Fig. 4. Effect of different control strategies against *Penicillium expansum* on the percent of thiabendazole (TBZ)-resistant strains isolated from decayed cultivar Spadona pear fruits during successive generations of natural tansfer. Initial inoculation of the pears was with a 1:1 ratio of resistant and susceptible strains. Fungicide treatments were applied as described in Materials and Methods. Abbreviations: T = TBZ, IP = iprodione, IM = imazalil, E = etaconazol, and CA = captan.

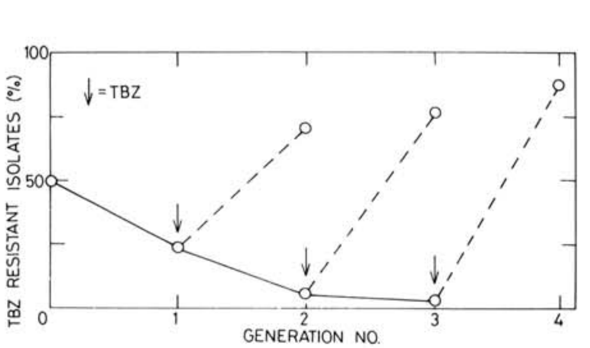

Fig. 5. Percent of resistant strains of *Penicillium expansum* isolated from decayed cultivar Spadona pear fruits during four successive generations of natural transfer with no fungicide treatment (——) or following TBZ treatment (- - -).

resistant population. In spite of the differential behavior of the resistant population, all the control strategies significantly reduced decay severity compared to the untreated fruits (Fig. 6). TBZ treatment of the fruits resulted in 3–9% rot and 100% of resistant isolates.

DISCUSSION

The appearance of resistant strains of *P. expansum* in Israel was first recorded on an isolated farm following 1 yr of inadequate decay control (3). The relatively low incidence of resistant strains and their initial instability indicated that resistance to TBZ had not been fully established in the fungal population when it was first discovered. One year later, resistance and reduced efficiency of disease control were observed throughout Israel. The failure to detect resistance in previous years may have been due to low levels of resistance or the deficiency of country-wide monitoring. The continued use of TBZ enhanced the selection of resistant strains and their proportion increased to 77 and 100% of the isolates from decayed fruits in two subsequent years. Persistence of resistance after a single transfer on apples suggested a step-by-step selection of pathogenic strains (6). The resistance of the isolates observed after 20 transfers on fungicide-free PDA indicated that these resistant strains resulted from a genetic change and conforms with Dekker's definition of resistance (6). Similar persistence of resistance for different numbers of transfers has been described for other pathogens (5,9).

P. expansum was resistant to a maximal concentration of TBZ at 2,000 $\mu g/ml$. However, resistant isolates were considered those that developed in 40 $\mu g/ml^{-1}$. This concentration was considered for our studies, since the actual residual concentration after a 600 $\mu g/ml^{-1}$ commercial treatment is about 5–10 $\mu g/ml^{-1}$ (1), indicating that isolates developing at that concentration are resistant under commercial conditions.

Our results indicate (Fig. 1) that resistant strains are slow growers at 20 C compared to susceptible strains. However, under commercial conditions of apple storage at 0 C for 3–8 mo, a high percentage of fruits decayed by resistant strains was detected, indicating that reduced virulence is not a factor limiting expression of its pathogenicity under these conditions. Eckert and Wild (8) did not find any decrease in virulence of benzimidazole-resistant strains compared to susceptible strains of *P. digitatum*; however,

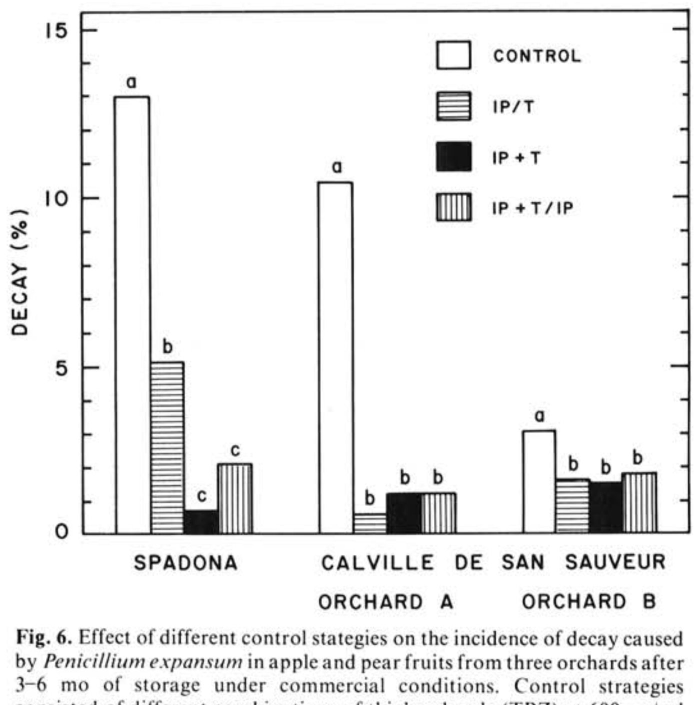

Fig. 6. Effect of different control stategies on the incidence of decay caused by *Penicillium expansum* in apple and pear fruits from three orchards after 3–6 mo of storage under commercial conditions. Control strategies consisted of different combinations of thiabendazole (TBZ) at 600 $\mu g/ml$ and iprodione at 500 $\mu g/ml$. Small letters indicate statistical separations of the means according to Duncan's multiple range test, $P = 0.05$.

they observed a weak competitive behavior of the resistant strains in a mixture. In our studies, reduced virulence (Figs. 1 and 2) was accompanied by weak competitiveness of the resistant strains of *P. expansum*.

Resistance to benzimidazole fungicides has been linked to reduced pathogenicity in some strains of *Cladosporium* (6). However, strains of *Venturia inaequalis* resistant to benomyl (19) are capable of surviving in a mixed population in the absence of benzimidazoles, suggesting a similar fitness of both strains. In our study, resistant isolates of *P. expansum* were less fit, implying that the population may tend to return to its original state of balanced adaptation if the selection pressure by the fungicide were to be relaxed (Figs. 3 and 5). However, the continuous selection pressure in the storage environment and a subsequent reassortment of genes could lead to the evolution of resistant strains in parasitically more fit strains. Various findings suggest that resistant strains might evolve towards increased virulence and ecological fitness: the existence of different levels of virulence in the population of *Penicillium* sp. (10,12,17,20) and changes in virulence of the population of *Penicillium* under selection pressure (4). This kind of evolution could be delayed according to Eckert and Wild (8) by a pleiotropic interaction between the gene(s) for fungicide resistance and the genes for an essential physiological function. In our study the problem was addressed by the cessation of the use of TBZ (9) and the introduction of nonselective control treatments. However, levels of resistance much lower than 4% are essential before the fungicide can be reused for more than one season, since the resistant population may become predominant after one TBZ application (Fig. 5). A significant persistence of the resistant isolates was observed in competition experiments conducted at 20 C in comparison to those done at 10 C (Figs. 3 and 4); however, our data are insufficient to demonstrate a commercially significant temperature effect on the parasitic fitness of resistant isolates.

Even though benzimidazoles are used in the field to control orchard diseases, the presence of resistant isolates in the orchard or in the packinghouse was negligible. Low percentage of resistant isolates were present in empty storerooms prior to filling. The high incidence of resistant strains found during storage and their increase at the end of the season was probably the result of selection pressure on the low resistant population introduced or present in the storeroom during the prolonged exposure of the pathogen to the fungicide. Proper sanitation can reduce the size of the pathogen population but it might not have much effect in cases where a rapid buildup of a resistant population may be achieved from a very low initial population.

Development of resistance to new fungicides with new biochemical targets has resulted in the application of mixtures of selective and nonselective fungicides (11,18). Postharvest treatments of pome fruits involve the dip treatment of hundreds of bins stored in one single storeroom, and affords the possibility of a sequence of different fungicide dip treatments to fruit located in the same storeroom. Our results indicated that among the fungus control strategies tested, decay of *P. expansum* was controlled equally well by the mixture and heterogeneous fungicide treatments on fruits stored in the same storeroom (Fig. 6). The differences between the strategies are based mainly on the selective pressure of the treatment on the resistant population. Mixtures of a nonselective fungicide with a fungicide such as TBZ resulted in a totally selective treatment for benzimidazole-resistant isolates (Fig. 6). To prevent this kind of homogeneous selection pressure for resistant strains, strategies with heterogeneously treated fruits in a closed system were compared. The strategy with two fungicides applied separately to fruits stored in one storeroom (strategy ii) did not enhance the decline of the resistant proportion of the population when the inoculation was with a 1:1 ratio of resistant:sensitive strains. However, it decreased the proportion of resistant strains when the initial percent was much higher (Table 2). Strategy iii, which employed a mixture of two fungicides (TBZ + captan, iprodione + captan, and imazalil + captan) and a newly introduced fungicide (iprodione, imazalil, and etaconazole, respectively), resulted in a more rapid decrease in the percentage of resistant strains. The selective effect of this treatment appeared to

TABLE 2. Effect of different control strategies on the percent of resistant strains of *Penicillium expansum* isolated from naturally decayed apple and pear fruits from three orchards after one storage season under commercial controlled-atmosphere conditions

			Fungicidal treatment			
Fruit	Cultivar	Orchard	None	TBZ + iprodine[a]	TBZ/ iprodione[b]	TBZ + iprodione/ iprodione[c]
Apple	Calville de San Sauveur	1	46.9	100.0	50.0	9.1
		2	50.8	75.0	80.0	42.8
Pear	Spadona	3	27.8	100.0	75.0	75.0
Average			42.0	92.0	62.0	42.0

[a] Fruits were dipped in a mixture of thiabendazole and iprodione.
[b] The fungicides were applied separately and fruits were stored together.
[c] A mixture and a single fungicide were applied separately and treated fruits were stored together.

be minimal since the reduction of the resistant population was similar to the natural decrease of the resistant population in mixtures with the sensitive wild type without any control measures. The variety of fungicides with different modes of action is, in all probability, more likely to prevent the buildup of a specific type of resistance. The use of any one fungicide for only two generations also further decreased the possibility of new resistance developing. Although these strategies might appear to be commercially impractical, several factors support their use: the high rate of decay (up to 10%) developing in stored apples in Israel, the development of resistance in *P. expansum* to TBZ in the storerooms and to iprodione in the laboratory (21), and decay produced by *P. crustosum* in stored apples treated with imazalil (16). These factors require a series of nonselective fungicide treatments. Fungus control strategies might be worthless where the rate of decay is low; in these cases a decrease in the resistant population might be obtained by a protectant, nonselective fungicide such as captan (17).

Although the possibility of resistance to captan was not tested, the use of this fungicide has declined in Israel because of its limited efficacy in the past (2). Recent findings concerning the resistance of *Botrytis* to captan (13) indicate that the single use of one fungicide in the dipping tank, even if it be a nonselective protectant, is risky. The best strategy for pome fruit treatments under the described conditions seems to be the one that enhances the decline of a resistant population, prevents exposure to one fungicide for an extended period of time, and achieves good control of the pathogen. In this study, we have shown that the most effective strategy to reduce the size of the resistant population is based on alternate dips of all the fruit stored in a single room, in two or more fungicides with differential modes of action.

LITERATURE CITED

1. Ben-Arie, R. 1975. Benzimidazole penetration, distribution, and persistence in postharvest-treated pears. Phytopathology 65:1187-1189.
2. Ben-Arie, R., and Guelfat-Reich, S. 1973. Preharvest and postharvest applications of benzimidazoles for control of storage decay of pears. HortScience 8:181-183.
3. Ben-Arie, R., and Prusky, D. 1979. Control of storage rots of Spadona pears. Alon HaNotea 9:631-633 (in Hebrew, with English summary).
4. Bertrand, P. F., and Saulie-Carter, J. L. 1978. The occurrence of benomyl-tolerant strains of *Penicillium expansum* and *Botrytis cinerea* in the mid-Columbia region of Oregon and Washington. Plant Dis. Rep. 62:302-305.
5. Bollen, G. J. 1979. Side effects of pesticides on microbial interactions. Pages 451-481 in: Soilborne Plant Pathogens. S. B. Schippers and W. Gams, eds. Academic Press, New York.
6. Dekker, J. 1976. Acquired resistance to fungicides. Annu. Rev. Phytopathol. 14:405-428.
7. Eckert, J. W. 1978. Control of postharvest diseases. Pages 269-352 in: Antifungal Compounds. Vol. 1. M. R. Siegel and H. D. Sisler, eds. Marcel Dekker Inc., New York.
8. Eckert, J. W., and Wild, B. L. 1983. Problems of fungicide resistance in

Penicillium rot of citrus fruits. Pages 525-555 in: Pest Resistance to Pesticides. G. P. Georghiou and T. Saito, eds. Plenum Publishing Corp., New York.
9. Gutter, Y., Shachnai, A., Schiffmann-Nadel, M., and Dinoor, A. 1981. Biological aspects of citrus molds tolerant to benzimidazole fungicides. Phytopathology 71:482-487.
10. Houck, L. G. 1977. Problems of resistance to citrus fungicides. Proc. Int. Soc. Citriculture 1:263-269.
11. Koffman, W., Penrose, L. J., Menzies, A. R., Davies, K. C., and Kalder, J. 1978. Control of benzimidazole-tolerant *Penicillium expansum* in pome fruit. Sci. Hortic. 9:31-39.
12. Kuramoto, T. 1976. Resistance to benomyl and thiophanate-methyl in strains of *Penicillium digitatum* and *P. italicum* in Japan. Plant Dis. Rep. 60:168-171.
13. Pepin, H. S., and MacPherson, E. A. 1982. Strains of *Botrytis cinerea* resistant to benomyl and captan in the field. Plant Dis. 66:404-405.
14. Prusky, D., Bazak, M., and Ben-Arie, R. 1980. Tolerance of *Penicillium expansum* in Israel to postharvest fungicide treatments. Proc. 5th Congr. Union Phytopathol. Mediterr. 1:158-159.
15. Prusky, D., and Ben-Arie, R. 1983. Tolerance of *Penicillium expansum* to postharvest benzimidazole treatments. (Abstr.) Page 427 in: 4th Proc. Int. Congr. of Plant Pathology, August 1983, Melbourne, Australia.
16. Prusky, D., and Ben-Arie, R. 1985. Effect of imazalil on the pathogenicity of *Penicillium crustosum* causing storage rots of pome fruits. Plant Dis. 69:416-418.
17. Rosenberger, D. A., and Meyer, F. W. 1979. Benomyl-tolerant *Penicillium expansum* in apple packinghouses in eastern New York. Plant Dis. Rep. 63:37-40.
18. Rosenberger, D. A., Meyer, F. W., and Cecilia, C. V. 1979. Fungicide strategies for control of benomyl-tolerant *Penicillium expansum* in apple storages. Plant Dis. Rep. 63:1033-1037.
19. Shabi, E., and Gilpatrick, J. D. 1981. Competition between benomyl-resistant and benomyl-sensitive strains of *Venturia inaequalis* on apple seedlings treated with benomyl and captan. Neth. J. Plant Pathol. 87:250-257.
20. Wild, B. L. 1980. Resistance of citrus green mold, *Penicillium digitatum* Sacc., to benzimidazole fungicides. Ph.D. dissertation. University of California, Riverside. 89 pp.
21. Ziffer, J. 1981. The effect of captan and rovral on thiabendazole-resistant strains of *Penicillium* rots of apples. Alon HaNotea 11:753-755 (in Hebrew, with English summary).