Negatively Correlated Cross-Resistance to Diphenylamine in Benomyl-Resistant *Penicillium expansum*

D. A. Rosenberger and F. W. Meyer

Plant pathologist and research technician, New York State (Geneva) Agricultural Experiment Station, Hudson Valley Laboratory, P.O. Box 727, Highland 12528.

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ABSTRACT

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Combinations of diphenylamine (DPA) at 1,000–2,000 μg/ml and benomyl, thiabendazole, or thiophanate-methyl provided better control of benomyl-resistant *Penicillium expansum* in inoculated apples stored at 2.2–4.4 C than either DPA or the fungicides used alone. The addition of DPA did not significantly affect control achieved with the fungicides when fruit were stored at 16–22 C. Comparisons of mycelial growth rates for three benomyl-resistant and four benomyl-sensitive isolates in the presence of varying concentrations of benomyl, DPA, or benomyl plus DPA showed benomyl-resistant isolates were more sensitive to DPA than benomyl-sensitive isolates. Testing of 20 additional, randomly-selected isolates of *P. expansum* from apple packinghouses showed that DPA concentrations required to reduce mycelial growth by 50% were 24.4, 16.9, and 3.1 μg/ml for the three benomyl-sensitive, eight moderately benomyl-resistant (EC_{50} = 14.2 μg benomyl per milliliter), and six highly benomyl-resistant (EC_{50} >100 μg benomyl per milliliter) isolates, respectively. Three of the 20 isolates were resistant to both benomyl and DPA. Control of benomyl-resistant *P. expansum* by DPA may represent the first commercially significant application of negatively correlated cross-resistance for reducing losses to fungicide-resistant pathogens.

Additional key words: apple storage scald, ethoxyquin, iprodione, postharvest fungicides.

Postharvest dip or drench treatments containing fungicide and a scald inhibitor are commonly used in the United States to prevent damage to apples (*Malus domestica* Borkh.) stored for 3–10 mo in low-oxygen, controlled-atmosphere storages. Storage scald (or superficial scald) is a physiological disorder caused by oxidation of α-farnesene (8) and causes a brown discoloration of the epidermis of affected apple fruit. Storage scald is controlled with postharvest treatments of diphenylamine (DPA) or ethoxyquin (13,18). The scald inhibitor of choice is usually combined with a fungicide to prevent postharvest decays caused by *Penicillium expansum* (Link) Thom and *Botrytis cinerea* Pers.: Fr. The benzimidazole fungicides benomyl, thiabendazole, and thiophanate-methyl are the most effective postharvest fungicides registered for use on apples in the United States, and they are still widely used despite several reports of benzimidazole-resistance in *P. expansum* (1,4,10,14,17).

Although DPA and ethoxyquin have been used for more than 20 yr, the fungicidal properties of these scald inhibitors have never been carefully investigated in postharvest treatments. Our objective in this study was to determine if DPA and ethoxyquin affect the efficacy of registered and experimental fungicides applied to apples in postharvest dip treatments for control of *P. expansum*. Preliminary results of these and other related investigations have been reported (15,16).

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MATERIALS AND METHODS

Apple storage trials. Fungicides, scald inhibitors, and fungicide-scald inhibitor combinations were tested by inoculating wounded apple fruit, immersing fruit in treatment solutions, and evaluating fruit for decay after 66–113 days of storage at cold (2.2–4.4 C) or 13–16 days at ambient (16–22 C) temperature. Fruit were uniformly wounded at three sites on a single face using three 45-mm-long finishing nails mounted in a large cork to produce wounds 2–3 mm deep. Wounded fruit in baskets or field crates were inoculated by dipping for 20 sec in a spore suspension of *P. expansum*. Fruit were allowed to dry approximately 1 hr before they were dipped for 30 sec in well-agitated treatment solutions. Treated fruit were stored wounded-face-upward on spring cushion trays in commercial fiberboard shipping cartons or in custom-made wooden crates designed to allow adequate air circulation during storage. Randomized block designs, either alone or within factorials, were used for all experiments. Treated fruit were divided into replicates based on fruit size or source tree, and replicates were grouped together during storage. The arcsine square root percentage transformation was used for statistical analysis of results.

Isolates of *P. expansum* used for inoculum were originally recovered from decayed fruit or water flotation tanks in commercial storages and were tested for pathogenicity and benomyl-resistance prior to each season's tests. Benomyl-resistance was determined initially by plating isolates on potato-dextrose agar (PDA) amended with 250 μg of benomyl per milliliter. Benomyl-resistant isolates used in treatments were recovered from benomyl-amended plates to assure that isolates were not mixtures of benomyl-resistant and sensitive strains. All but one of the benomyl-resistant isolates used for inoculum had benomyl EC_{50} values greater than 100 μg/ml and all had DPA EC_{50} values ranging from 1.2–12.8 μg/ml as determined by in vitro trials. Isolate P-132, one of the benomyl-resistant isolates used in Trials II and III, was selected because preliminary tests on fungicide-amended agars showed it was more resistant to the benomyl-DPA combination than the isolates used in Trial I. Isolate P-132 had an EC_{50} value of 22.6 μg/ml for benomyl.

Conidia of *P. expansum* used for inoculations were grown on PDA and were collected by flooding 1- to 2-wk-old cultures with distilled water containing 0.2% Tween-20. Concentrations of spore suspensions recovered from plates were determined for each isolate by using a hemacytometer. Separate benomyl-resistant and benomyl-sensitive spore suspensions were prepared by combining approximately equal numbers of conidia from two to four isolates from the same category. Inoculum from resistant and sensitive isolates was then combined to provide the desired proportion of resistant to sensitive conidia in the final inoculum suspension (Table 1). Multi-isolate inoculum suspensions were used to simulate the heterogeneous population of *P. expansum* found in commercial storages.

The fruit of cultivar Empire used in Trials I and II was from experimental orchards and had not been sprayed with fungicides for at least 60 days prior to harvest. Fruit of cultivar Delicious for Trial III was purchased from a commercial orchard that had not been sprayed within 25 days of harvest. Inoculations and treatments were completed within 5 days of harvest in Trials I and II, but fruit for Trial III was held at outdoor temperatures (5–21 C) in a shaded location for 14 days between harvest and inoculation. Prior to each trial, 25 fruit were selected at random and tested for firmness (as a measure of maturity and fruit condition) by using an Effegi pressure tester (2). Details of experimental protocols for Trials I, II, and III are presented in Table 1.

Fungicides used in treatments were benomyl (Benlate 50W; DuPont de Nemours & Co., Wilmington, DE), thiophanate-methyl (Topsin M 70W; Pennwalt Corp., Agchem Division, Fresno, CA), thiabendazole (Mertect 340-F; Merck & Co., Inc., Rahway, NJ), captan (Captan 50W; Stauffer Chemical Co., Agricultural Chemical Division, Westport, CT), iprodione (Rovral 50W; Rhône-Poulenc, Inc., Monmouth Junction, NJ), and etaconazole (Vangard 10W; Ciba-Geigy Corp., Agricultural Division, Greensboro, NC). Scald inhibitors tested were diphenylamine (Shield Liquid DPA-82; Shield-Brite Corp., Kirkland, WA), and ethoxyquin and diphenylamine (Deccoquin 305 Concentrate and No-Scald DPA EC-283, respectively; Pennwalt Corp., Decco Tiltbelt Div., Monrovia, CA). The formulated carrier (composed of solvents and emulsifiers without active ingredient, and hereafter called No-Scald carrier) for No-Scald DPA EC-283 was also supplied by Decco Tiltbelt. No-Scald carrier was used for comparison with No-Scald DPA to determine if observed effects were attributable to DPA or to a wetting-agent effect of the DPA formulation. The No-Scald formulation of DPA was used in all experiments except where otherwise specified.

Treatments were evaluated after the indicated storage period by determining the numbers of fruit with blue-mold decay. Fruit infected with *Botrytis cinerea, Physalospora obtusa* (Schw.) Cooke, or *Alternaria* species were discarded and not included in totals. Fruit infected with extraneous organisms were generally less than 2% of the total fruit in any treatment.

In vitro trials. The effects of various concentrations of benomyl, DPA, and combinations of benomyl and DPA on growth of *P. expansum* in vitro were determined by plating mycelial plugs from nonsporulating cultures on PDA plates amended with the test materials. The commercial formulations of benomyl and No-Scald DPA were used in all tests. Benomyl was incorporated into PDA before autoclaving. DPA was added to the autoclaved PDA after it was cooled to 47 C in a shaking water bath. An initial in vitro test comparing growth of *P. expansum* on PDA amended with the No-Scald carrier showed the formulating materials in No-Scald DPA did not affect the activity of benomyl.

Inoculum plugs of nonsporulating mycelia were obtained by collecting conidia from cultures as described for the apple storage trials, plating 0.1 ml of the conidial suspension on unamended PDA, and removing plugs with a 1-cm-diameter corkborer after 16–30 hr. Plugs were placed inoculated-side-up on test plates, plates were incubated for 7 days, and diameters of colonies were measured across two perpendicular axes. A single plug was placed on each plate in Trial A. Two plugs per plate were used in Trial B. Effects of the amendments on colony growth were determined by subtracting the plug diameter from all measurements and determining percent growth inhibition on amended compared to control plates. The amendment concentrations required to reduce mycelial growth by 50% (EC_{50}) were determined by probit analysis for graded responses (7).

In Trial A, growth of three benomyl-resistant and four benomyl-sensitive isolates was compared by using extensive arrays of amended agars containing benomyl, DPA, and benomyl-DPA combinations at various concentrations. Benomyl-resistant isolates were tested on 24 different amendments and benomyl-sensitive isolates were tested on 29. The experiment was repeated three times with two replicates at each concentration except that two of the sensitive isolates were included in only two of the three repetitions.

Trial B was conducted to determine if isolates with resistance to both benomyl and DPA could be detected in apple storage and packing facilities. Twenty randomly selected and previously untested isolates of *P. expansum* recovered from water flotation tanks in several storages were tested on PDA amended with benomyl at 0.1, 1, 10, and 100 μg/ml and DPA at 1, 5, 10, 50, and 100 μg/ml. Mycelial plugs of each of the 20 isolates were tested in a single experiment on two amended plates at each concentration. Plates were incubated 7 days at 22 C.

RESULTS

Cold storage trials. Results from Trial I were initially analyzed by using a six fungicide × five scald inhibitor factorial design. Because of significant ($P = 0.05$) fungicide-scald inhibitor interactions, Duncan's multiple range test separations were determined for each column and row in the factorial design (Table 2). Neither thiabendazole (TBZ) nor benomyl had any effect on decay development in Trial I because fruit were inoculated with

TABLE 1. Details of experimental design and protocols used for apple storage trials

	Storage trial		
	I	II	III
Apple cultivar	Empire	Empire	Delicious
Mean pressure test (kg)[x]	7.4	8.3	6.5
Date trial initiated	October 1982	October 1983	October 1983
Number of treatment replications	4	4	4
Number of fruit per replicate	25	25	25
Inoculum			
Number of isolates	4 BR:4 BS[y]	2 BR:4 BS	3 BR or 4 BS
BR:BS[y] ratio	20:80	11:89	Not combined
Total concentration (conidia/ml)	50,000	45,000	50,000
Posttreatment storage			
Temperature (C)	2.2	4.4 or 16–22	2.2 or 16–22
Duration (days)	92	66 or 13	113 or 16

[x] Twenty-five fruit were selected at random at the beginning of each trial and were tested for firmness with an Effegi pressure tester.

[y] BR = benomyl-resistant, and BS = benomyl susceptible.

20% benomyl-resistant conidia. All three scald inhibitors and the No-Scald carrier caused a significant increase in decay compared to the control when no fungicides were present. Ethoxyquin reduced the efficacy of all fungicide treatments except iprodione, and No-Scald carrier had no beneficial effect on fungicides except when combined with iprodione. Both formulations of DPA interacted with benomyl and TBZ to effectively control decay. The benomyl plus captan combination was the most effective of the fungicide treatments when used without scald inhibitors but was not significantly improved by adding DPA and was impaired when applied with No-Scald carrier.

P. expansum was reisolated from some of the decayed fruit in Trial I to determine the proportion of decays in which benomyl-resistant isolates were involved. Reisolation from infected fruit and tests for resistance to benomyl at 250 μg/ml were made as described elsewhere (17). DPA inhibited development of resistant isolates when no fungicides were applied and reduced the proportion of decays containing resistant isolates even when benomyl and TBZ were used (Table 3).

In Trial II, the beneficial benzimidazole-DPA interaction was not evident in fruit held at 16–22 C, was affected by DPA concentration, and occurred with thiophanate-methyl as well as with benomyl. Both benomyl and thiophanate-methyl reduced decay compared to the water control in fruit held at room temperature, possibly because of the low proportion (11%) of benomyl-resistant conidia used in the inoculum. Both fungicides were ineffective, however, at 4.4 C (Table 4). Neither concentration of DPA significantly improved fungicide activity and no rate-dependent effects were evident in treatments stored at 16–22 C. In fruit held at 4.4 C, all fungicide-DPA combinations resulted in less decay than comparable treatments with DPA or fungicides alone. Control with fungicide-DPA combinations was better with DPA at 2,000 rather than at 1,000 μg/ml except where DPA was combined with the higher concentration of thiophanate-methyl. The combination of DPA and ethoxyquin has been suggested for some highly scald-susceptible strains of Delicious (13). The addition of ethoxyquin to the fungicide-DPA combinations significantly decreased the efficacy of the DPA plus thiophanate-methyl combination at the lower but not at the higher concentration of thiophanate-methyl. The addition of ethoxyquin did not significantly affect the efficacy of the benomyl-DPA combination.

Results of Trial III confirmed that the benomyl-DPA interaction is not evident in fruit held at room temperature. In fruit held at 2.2 C, DPA alone had no effect on decay in the fruit inoculated with benomyl-sensitive isolates but caused a significant reduction in decay in fruit inoculated with benomyl-resistant isolates (Table 5). The benomyl-DPA combination proved less effective in Trial III than in previous trials in which isolate P-132 was either not used or P-132 conidia comprised only a small part of the total inoculum. In Trial III, fruit inoculated with benomyl-resistant isolates were exposed to 16,650 conidia per milliliter from isolate P-132 whereas in Trial II the inoculum included only 2,500 conidia per milliliter from P-132 (Table 1).

In vitro tests. Growth inhibition tests with the three benomyl-resistant and four sensitive isolates on benomyl- and DPA-amended agars showed benomyl-resistant isolates were more sensitive to DPA than were benomyl-sensitive isolates. Mean percent growth inhibition for the benomyl-resistant and sensitive isolates on the full array of benomyl- and DPA-amended agars is presented in Fig. 1, and the benomyl and DPA EC_{50} values for each isolate are given in Table 6.

The 20 isolates tested in Trial B were divided into four categories based on results of growth inhibition studies. The three benomyl-sensitive isolates were completely inhibited by benomyl at 10 μg/ml and were more than 80% inhibited by benomyl at 1 μg/ml. The 17 remaining isolates in Trial B were all benomyl-resistant but were further classified as highly benomyl-resistant, moderately benomyl-resistant, or dually resistant to both benomyl and DPA. The six highly resistant isolates all showed less than 15% inhibition on plates amended with benomyl at 100 μg/ml. The eight moderately resistant isolates showed less than 25% growth inhibition on benomyl at 1 μg/ml (six of the eight showed no inhibition at 1 μg/ml) but more than 75% inhibition with benomyl at 100 μg/ml. The three dually resistant isolates fit the benomyl-resistance pattern for highly resistant isolates but reacted to DPA like benomyl-sensitive isolates.

Accurate EC_{50} values could not be determined for individual isolates in Trial B because many of the concentrations tested either allowed no growth or produced no inhibition and therefore could not be used in statistical analysis. However, combining the useful data points from all isolates for each category allowed calculation of EC_{50} values for each category (Table 6). Sensitivity to DPA increased from benomyl-sensitive to moderately resistant, and from moderately resistant to highly resistant isolates.

Subinhibitory levels of both DPA and benomyl in agar frequently caused colonies to grow more rapidly than colonies on control plates. Stimulation by subinhibitory concentrations was greatest, however, for dually resistant isolates: DPA at 5 μg/ml resulted in mean colony growth of 107% of control plates and benomyl at 1 μg/ml produced growth equal to 109% of controls.

Actual colony growth rates on unamended PDA were generally lower for benomyl-resistant than for sensitive isolates. Mean colony diameters (including the 10-mm-diameter inoculum plugs) were 57.6, 47.5, 29.4, and 35.9 mm for the benomyl-sensitive, moderately resistant, highly resistant, and dually resistant isolates, respectively, after 10 days at 22 C.

DISCUSSION

Results reported here confirm our earlier reports (15,16) that combinations of benzimidazole fungicides and DPA are more effective for controlling some benomyl-resistant isolates of *P. expansum* than either DPA or the fungicides used alone. In previous work (17), we screened isolates of *P. expansum* for benomyl-resistance on agar containing benomyl at 250 μg/ml. The resistant isolates we used for our initial tests (Trials I and A) were

TABLE 2. Percent of cultivar Empire apples (Trial I) infected with *Penicillium expansum* after storage at 2.2 C for 92 days as affected by postharvest treatment with fungicides and scald inhibitors

Fungicide treatment and concentration	Scald inhibitors added to fungicide treatments				
	None	Ethoxyquin (2,700 μg/ml)	No-Scald carrier[r] (1,000 μl/ml)	No-Scald DPA (1,000 μg/ml)	Shield Liquid DPA (1,000 μg/ml)
Water control	74.2 b X[s]	94.7 c Y	98.5 d Y	91.7 c Y	99.0 e Y
Thiabendazole (529 μg/ml)	69.1 b Y	ND[t]	64.5 bc Y	8.7 a X	12.4 ab X
Benomyl (300 μg/ml)	56.8 b Y	75.2 b Z	71.2 c YZ	1.5 a X	3.5 a X
Benomyl (300μg/ml) and captan (1,200 μg/ml)	21.4 a X	68.1 b Y	51.0 b Y	22.8 b X	17.7 bc X
Captan (1,200 μg/ml)	72.0 b X	89.7 c Y	79.1 c XY	89.7 c Y	88.8 d Y
Iprodione (1,200 μg/ml)	31.1 a Y	47.0 a Y	5.3 a X	5.1 a X	32.4 c Y

[r] The No-Scald DPA formulation without DPA as supplied by the manufacturer.
[s] Means in the same column followed by the same small letter (a, b, ...) or means in the same row followed by the same capital letter (X,Y,Z) are not significantly different as determined by Duncan's multiple range test ($P = 0.05$).
[t] Not tested because product labels indicate incompatibility.

therefore all highly resistant isolates which were highly sensitive to DPA. The subsequent testing of 20 randomly selected isolates in Trial B showed moderately resistant isolates are less affected by DPA than are highly resistant isolates. The differences between moderately and highly resistant isolates in their responses to benomyl and DPA suggest two genetically distinct categories of benomyl-resistant isolates may be present in apple storages.

The increasing sensitivity to DPA associated with increasing levels of benomyl-resistance in benomyl-sensitive, moderately resistant, and highly resistant isolates of *P. expansum* fits the definition of negatively correlated cross-resistance (5). Negatively correlated cross-resistance with benomyl has previously been reported for TBZ (19), several carbamate herbicides (11,12), and methyl *N*-(3,5-dichlorophenyl)-carbamate (9). All of these chemicals are reported to interfere with mitosis and thus have modes of action similar to benomyl. DPA has never been reported to affect mitosis but is known to block the carotene biosynthesis pathway in many organisms including the fungus *Epicoccum nigrum* Link (6). Thus, the benzimidazole-DPA interaction may involve a different mechanism than that responsible for the previously reported cases of negatively correlated cross-resistance.

Negatively correlated cross-resistance has been suggested as a possible means of countering resistance to fungicides (5). The benzimidazole-DPA interaction in apple postharvest treatments may represent the first commercial application (albeit totally accidentally) of negatively correlated cross-resistance. The practical implications of the benzimidazole-DPA interaction cannot be assessed until more is known about the relative incidence, pathogenicity, and ecological fitness of moderately resistant, highly resistant, and dually resistant isolates present in apple storage facilities. A high incidence of dually resistant and/or moderately resistant isolates (with resistance levels and pathogenicity similar to isolate P-132) could reduce the commercial importance of the benzimidazole-DPA interaction. Decay control with the benomyl-DPA combination (with DPA at 1,000 μg/ml) decreased from 98% in Trial I to 81.5% in Trial II. The combination with DPA at 2,000 μg/ml provided 97.5% control in Trial II compared to only 54.4% in Trial III. The decreased efficacy of the benomyl-DPA combination in Trial III may have resulted from an increase in the concentration of P-132 conidia from 5.5% of the inoculum in Trial II to 33% of the benomyl-resistant inoculum in Trial III, an increase in the total concentration of benomyl-resistant spores from 11,000 per milliliter in Trial II to 50,000 per milliliter in Trial III, or a combination of the above two factors.

TABLE 3. Incidence of benzimidazole-resistant (BR) isolates of *Penicillium expansum* in reisolations from fruit of apple cultivar Empire (Trial I) inoculated with 20% BR and 80% benomyl-sensitive conidia and treated with fungicides and/or diphenylamine (DPA)

	Without DPA		With DPA	
Fungicide and concentration	BR isolates/ total no. of reisolations	Percent BR isolates	BR isolates/ total no. of reisolations	Percent BR isolates
Water control	26/50	52	0/50	0
Thiabendazole (529 μl/ml)	25/25	100	6/17	35
Benomyl (300 μg/ml)	23/25	92	5/6	83

TABLE 4. Percent of cultivar Empire apples (Trial II) infected with *Penicillium expansum* following postharvest dip treatment with fungicides and scald inhibitors and storage at room temperature or 4.4 C

	Scald inhibitors added			
		DPA[w]		DPA 2000 μg/ml plus ethoxyquin 2,700 μg/ml
Treatment	None	1,000 μg/ml	2,000 μg/ml	
Room temperature trial[x]				
Water control	40 b[y]	62 b	43 b	48 b
Benomyl 300 μg/ml	17 a	8 a	17 a	14 a
Thiophanate-methyl 420 μg/ml	15 a	14 a	11 a	13 a
Thiophanate-methyl 840 μg/ml	15 a	12 a	6 a	10 a
Cold storage trial[x]				
Water control	81 a	82 b	63 c	61 c
Benomyl 300 μg/ml	67 a Z[y]	15 a Y	2 a X	5 a XY
Thiophanate-methyl 420 μg/ml	61 a Z	28 a Y	5 ab X	23 b Y
Thiophanate-methyl 840 μg/ml	71 a Z	15 a Y	16 b Y	18 ab Y

[w] Diphenylamine.
[x] Treatments stored 13 days at 16–22 C or 66 days in cold storage (4.4 C).
[y] Means for the same temperature and within columns followed by the same small letters (a, b, c) or means within rows followed by the same capital letters (X, Y, Z) are not significantly different (DMRT, $P = 0.05$). Scald inhibitors caused no significant differences between means in the room temperature trials or for the water control in cold storage.

TABLE 5. Percent of cultivar Delicious apples (Trial III) with blue mold decay as affected by postharvest treatments with fungicides or diphenylamine and storage at ambient temperature or 2.2 C

	16 days of storage at ambient temperature[w]		113 days of storage at 2.2 C	
Postharvest treatment	Benomyl-susceptible inoculum[x]	Benomyl-resistant inoculum[x]	Benomyl-susceptible inoculum[x]	Benomyl-resistant inoculum[x]
Water control	21.8 b[y]	38.9 b	41.8 b	62.8 d
Benomyl (300 μg/ml)	1.0 a	43.7 b	0 a	66.5 d
DPA (2,000 μg/ml)	49.0 c	38.9 b	51.0 b	48.0 c
Benomyl (300 μg/ml) & DPA (2,000 μg/ml)	0 a	30.6 b	0 a	28.6 b
Etaconazole (45 μg/ml)	0 a	0 a	0 a	0 a

[w] Storage temperature fluctuated from 16 to 22 C.
[x] Conidial suspensions of both benomyl-susceptible and -resistant isolates of *Penicillium expansum* were adjusted to 50,000 conidial/ml and fruit were dipped into inoculum prior to treatment.
[y] Means within columns followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

TABLE 6. Concentrations of benomyl and diphenylamine (DPA) required to reduce by 50% (EC_{50}) the mycelial growth rates of benomyl-sensitive and several categories of benomyl-resistant isolates of *Penicillium expansum*

	EC_{50} in (μg/ml) for:	
Isolate description	Benomyl	DPA
Trial A		
Benomyl -sensitive		
Isolate P-26	0.13 ± 1.01	37.8 ± 1.4
Isolate P-31	0.07 ± 0.71	22.9 ± 0.8
Isolate P-39	0.08 ± 0.79	78.5 ± 1.7
Isolate P-88	<0.07	39.3 ± 1.6
Benomyl-resistant		
Isolate P-8	>100	12.8 ± 1.9
Isolate P-23	>100	<2.0
Isolate P-24	>100	4.4 ± 0.9
Trial B[x]		
Benomyl-sensitive	0.14 ± <0.001	24.4 ± 0.6
Moderately-resistant	14.2 ± 0.3	16.9 ± 0.4
Highly-resistant	>100	3.1 ± 0.9
Dually-resistant	>100	27.2 ± 0.8

[x] EC_{50} values were calculated from probits for percent growth inhibition for three benomyl-sensitive, eight moderately-resistant, six highly-resistant, and three dually-resistant isolates.

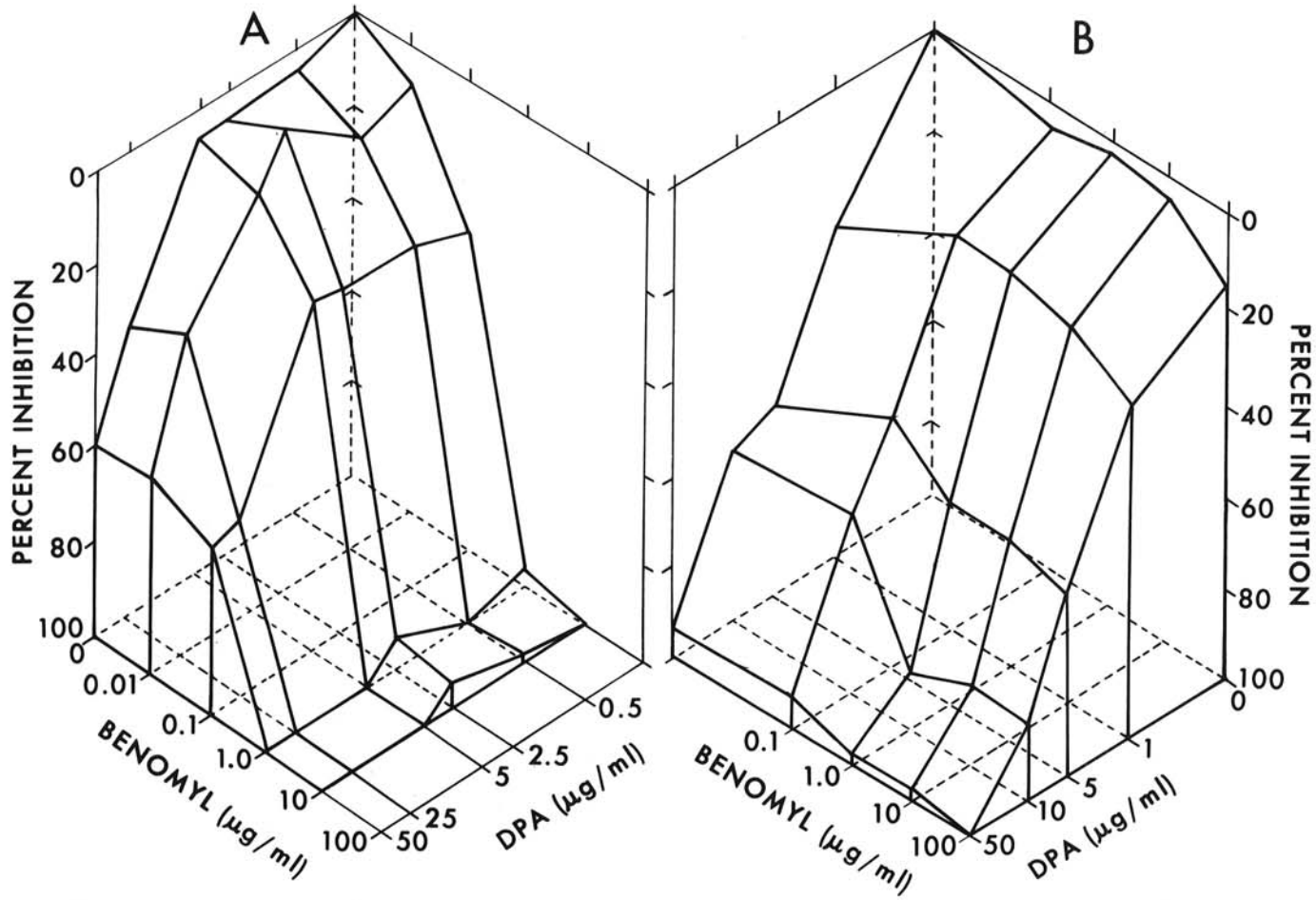

Fig. 1. Effects of various concentrations of benomyl, diphenylamine (DPA) and benomyl-DPA combinations incorporated into potato-dextrose agar on mean growth of **A,** four benomyl-sensitive isolates and **B,** three benomyl-resistant isolates of *Penicillium expansum*.

Additional storage trials comparing disease control in fruit inoculated separately with moderately resistant, highly resistant, and dually resistant isolates are needed to more clearly establish the disease-causing potential of isolates in each category.

The use of No-Scald carrier to investigate the effects of DPA wetting agent on fungicides clearly showed that the interaction with benzimidazoles is a function of the DPA itself and is not a wetting-agent effect. However, the interaction of both the carrier and No-Scald DPA but not Shield Liquid DPA, with iprodione indicates the efficacy of iprodione could be increased by use of appropriate wetting agents.

The commercial scald inhibitors we tested are formulated with good wetting agents to allow uniform coverage of the waxy cuticle on apples. The wetting agents may also be involved in some of the treatment effects we observed. The increased decay in treatments involving ethoxyquin, DPA, and No-Scald carrier in the absence of fungicides (Trial I, Table 2) probably resulted from increased penetration of the nail-hole wounds by water and spores. In some cases, combining two or three formulated compounds, each with its own wetting agents, may have resulted in excessive wetting agents and undesirably low surface tension in the final treatment solution. The reduced surface tension may have increased solution runoff and resulted in decreased fungicidal residues on the fruit. This phenomenon could explain the following observations: ethoxyquin generally caused a decrease in fungicide effectiveness compared to the fungicides used alone (Table 2), the benomyl-DPA combination was less effective when captan or ethoxyquin was added (Tables 2 and 5), and a DPA rate response occurred when DPA was combined with thiophanate-methyl at 420 μg/ml but not with thiophanate-methyl at 840 μg/ml (Table 5). Both the beneficial and the negative interactions between compounds combined in postharvest treatments illustrate the importance of testing fungicides in combination with other products commonly combined with the test materials in commercial practices.

The effect of temperature on the activity of postharvest fungicides has been noted before (3,17,20), but the failure of the DPA-benzimidazole combinations at warm temperatures provides evidence for what may be a commercially significant temperature-fungicide interaction. Further work is needed to clarify the mechanism involved in temperature-dependent responses of benomyl-resistant isolates of *P. expansum* to benomyl and DPA.

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