New Fungicides to Control Benomyl-Resistant *Penicillium expansum* in Apples

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ABSTRACT

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The in vivo activity of several new fungicides was tested on strains of *Penicillium expansum* resistant and sensitive to benomyl. In simulated commercial storage, CGA-64251, prochloraz, and iprodione showed promise as effective replacements for benomyl and thiabendazole to control benzimidazole-resistant strains of blue mold on apples. When mixed with either diphenylamine or calcium chloride and applied as a postharvest drench, these fungicides did not injure the fruit.

Blue mold, caused by *Penicillium expansum* (Link) Thom, has been the principal postharvest rot of apples, accounting for 80–95% of the apple rots in eastern U.S. markets (1). In 1932, losses to blue mold were at least 4%, which today would cost Michigan apple growers about $2.5 million, based on 1978 yield statistics and an average apple price of 7.9 cents per pound (7).

Good refrigeration and controlled-atmosphere storage methods have tended to reduce blue mold decay. However, the popular practice of dipping or drenching apples with solutions of scald inhibitors before storage has fostered decay, because both practices spread blue mold spores and inoculate the fruit. In the spring of 1972, some Michigan storage facilities reported market losses as high as 50% in apples drenched with scald inhibitors, even though captan fungicide had been added to the mixture.

Fortunately, two benzimidazole fungicides, benomyl and thiabendazole, were introduced commercially in 1972 for postharvest control of blue mold and were added to scald inhibitor solutions. Losses from blue mold decay in 1973 fell significantly. Unfortunately, because of the success of these fungicides in controlling blue mold, the search for new compounds has been minimal.

In 1976, we started recovering benzimidazole-resistant strains of the blue mold fungus from decayed apples drenched with benomyl plus diphenylamine and from the water in hydrohandling lines and dump tanks in packinghouses. In 1978, we recovered the resistant strains in more than 20 packinghouses. In the late spring of 1979 and again in 1980, some packinghouse operators reported losses as high as 25% from blue mold on specific lots of apples coming from controlled-atmosphere storage. In all cases, the fruit had received a postharvest drench containing benomyl.

Recently, benzimidazole-resistant strains of *P. expansum* have been reported on pears in Australia (10) and Oregon (2) and on apples in New York (8). Because no other effective registered fungicide is available, we and other workers initiated fungicide screening trials to find new compounds that would control both resistant and sensitive strains of *P. expansum* (3,6,9). The most promising candidates from our screening trials were evaluated in combination with a scald inhibitor or calcium chloride for effectiveness under simulated commercial storage conditions in 1979. In the past, some combinations had proved phytotoxic; in other cases, fungicides were incompatible with scald inhibitor or calcium chloride (4,5). This report includes the results of our 1979 screening program for decay control and the results of phytotoxicity and decay control tests on apples stored for 6 mo under simulated commercial storage conditions.

MATERIALS AND METHODS

Fungicide screening trials. The following fungicides were tested: benomyl (Benlate 50W), CGA-64251 10W-G [1-[[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl]methyl]-*1H*-1,2,4-triazole], fenapronil (Sisthane 2EC), imazalil (Deccozil EC), iprodione (Rovral 50W), prochloraz (BTS-40 542 40 EC), procymidone (DPX-4424 50 WP), thiabendazole (Mertect 340-F), triadimefon (Bayleton 50W), and vinclozolin (Ronilan 50W). Their effectiveness in controlling blue mold was tested with unblemished Jonathan, Golden Delicious, Red Delicious, and McIntosh apples selected at harvest or from storage and washed in 40-C detergent solution. None of the fruit had received a postharvest fungicide or scald-inhibitor treatment.

The skin of each apple was wounded at 10 points to a depth of 1–2 mm with three pointed finishing nails (1.5-mm diameter) mounted in a cork. The wounded fruit were submerged for 30 sec in a mixed spore suspension prepared from four benzimidazole-resistant and two benzimidazole-sensitive *P. expansum* isolates grown for 7 days on potato-dextrose agar. Spore concentrations were about 50,000 per milliliter. To facilitate spore wetting, a few drops of Triton 1956 surfactant were added to the water, which had been checked for freedom from chlorine. The punctured fruit were dried for 3 hr, then placed in a plastic-mesh bucket and dipped for 5–10 sec in the fungicide suspensions (Table 1), which were made with chlorine-free water.

After draining briefly, the fruit were placed in perforated polyethylene bags and stored at 2 C for 14 days, followed by 7 days at 21 C. They were then examined for decay. We conducted seven tests, each with four replicates of 10 apples, during a 3-mo period after harvest. Treatment means were compared by the Student-Newman-Keuls multiple range test.

Simulated commercial storage tests. The four fungicides that appeared most promising in in vivo screening tests—prochloraz, CGA-64251, procymidone, and iprodione—were tested under commercial storage conditions. Imazalil, an excellent compound for controlling blue mold decay, was not included because it caused severe skin injury on all cultivars in the screening tests.

To test for possible injury and compatibility of the fungicides with diphenylamine or calcium chloride ($CaCl_2$), apples were drenched with the concentration of fungicides that best controlled decay in the screening tests and with four times that amount plus 2,000 μg/ml of diphenylamine (28% liquid concentrate) or 4% $CaCl_2$

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Table 1. Blue mold decay in punctured apples inoculated with a mixture of benomyl-resistant and -sensitive *Penicillium expansum* spores, dipped in fungicide, and stored for 2 wk at 2 C plus 7 days at 21 C, 1979

Postharvest dip treatment	Rate (μg a.i./ml)	Percentage blue mold[z]
Prochloraz	600	0.0 a
	300	0.5 a
	150	2.0 ab
	75	2.0 ab
CGA-64251	180	0.0 a
	120	0.5 a
	60	2.0 ab
Imazalil	600	1.5 ab
	300	3.0 abc
Procymidone	1,200	3.0 abc
	900	8.0 bc
	600	31.0 d
	300	53.0 e
Iprodione	1,800	3.5 abc
	1,200	11.0 c
	600	12.5 c
Vinclozolin	1,800	9.5 bc
	1,200	10.5 c
	600	60.0 e
Fenapronil	1,200	25.5 d
	900	40.0 d
	600	41.0 d
	300	58.5 e
Triadimefon	1,800	66.0 ef
	900	81.0 fg
Benomyl	1,800	66.0 ef
	300	82.0 fg
Thiabendazole	1,800	86.0 g
Control (water only)	...	85.0 g

[z]Means followed by the same letter do not differ significantly ($P=0.05$), according to the Student-Newman-Keuls multiple range test. The data represent the combined results of seven tests replicated four times; each replicate contained 10 apples.

Table 2. Blue mold decay caused by benomyl-sensitive and benomyl-resistant isolates of *Penicillium expansum* in apples following postharvest application of experimental fungicides plus 2,000 μg/ml diphenylamine or 4% calcium chloride during storage for 6 mo at 0 C

Postharvest dip treatment		Cultivar[z]		
Fungicide	Rate (μg a.i./ml)	McIntosh	Golden Delicious	Jonathan
Prochloraz	300	1.3 a	1.5 a	0 a
CGA-64251	120	1.8 a	1.5 a	0 a
Procymidone	900	12.5 b	31.5 b	8.0 b
Iprodione	1,800	1.5 a	3.5 a	1.5 a
Benomyl	300	48.8 c	53.0 c	25.5 c
Control (diphenylamine)	...	64.0 d	53.5 c	...
Control ($CaCl_2$)	...	...	...	38.0 d
Control (water only)	...	47.3 c	41.5 bc	32.5 cd

[z]Figures are mean percentages of blue mold. Means in the same column followed by the same letter do not differ significantly ($P=0.05$), according to the Student-Newman-Keuls multiple range test.

(commercial grade 75–78%). McIntosh and Golden Delicious apples were used for the fungicide-diphenylamine tests because of their susceptibility to scald; Jonathan apples were used for the fungicide-$CaCl_2$ tests because of their susceptibility to internal breakdown. Fruit free of defects were assigned at random to 10 treatments in lots of about 140–150 apples (20 kg). About 30 L of chemical mixture was poured into a perforated pan held above a crate of apples to uniformly drench the fruit. After draining briefly, each crate was enclosed in an unsealed polyethylene bag to reduce fruit moisture loss, then stored at 0 C (Jonathan at 2.2 C) for 6 mo. Each treatment was replicated three times.

Prestorage inoculation tests. The effectiveness of the chemical mixtures in controlling blue mold for an extended storage period was tested with seven lots of apples selected from fruit similar to those obtained for the commercial storage tests. Apples were wounded, inoculated with both the benzimidazole-resistant and benzimidazole-sensitive *P. expansum* spore mixtures as described in the screening tests, then treated with the single-rate fungicide-diphenylamine-$CaCl_2$ mixture (Table 2). Each lot of treated fruit was placed in perforated polyethylene bags, stored at 0 C (Jonathan at 2.2 C) for 6 mo then at 21 C for 6–7 days, and then examined for decay. Each treatment was replicated four times.

Poststorage blue mold inoculation tests. For each treatment lot of apples that had been stored for 6 mo in the simulated commercial storage tests, three replicates of 10 fruit judged to be free of decay and injury were thoroughly wiped with a clean, soft paper towel. Each apple was wounded 10 times, then dipped in a spore suspension of resistant and sensitive strains of *P. expansum* as described previously. The spore concentration used was 50,000 per milliliter for the Jonathan and Golden Delicious apples and 110,000 per milliliter for the McIntosh apples. After inoculation, the apples were placed in a perforated film bag, held at 2 C for 2 wk then at 21 C for 6–7 days, and then examined for decay.

Firmness of the fruit flesh after the 6-mo storage was determined with an Effegi tester (model FT-327, Effegi, 48011 Alfonsine, Italy). Three pared sides of 25 apples from each treatment were measured with a 7.9-mm probe. The pressures averaged 2.97 kg for McIntosh, 3.43 kg for Jonathan, and 3.25 kg for Golden Delicious.

RESULTS

Fungicide screening trials. The screening tests, summarized in Table 1, showed that neither benomyl at six times the recommended rate nor thiabendazole at nearly three times the recommended rate in dip solutions effectively controlled blue mold decay on wounded and inoculated apples. No commercially practical levels of decay control could be expected from increasing fungicide concentration; thus, other fungicides were indicated.

All other fungicides tested except triadimefon effectively reduced blue mold infection caused by the benzimidazole-resistant and -sensitive strains of *P. expansum*. Prochloraz, CGA-64251, and imazalil dips consistently gave excellent control. Increasing the concentration of prochloraz from 75 to 600 μg/ml, of CGA-64251 from 60 to 180 μg/ml, and of imazalil from 300 to 600 μg/ml tended to decrease decay, but not significantly. Procymidone, iprodione, and vinclozolin significantly reduced blue mold, but rather large amounts were required for commercial effectiveness. Although classified as of potential value in earlier trials (3), fenapronil (RH2161-2EC) proved marginal in 1979; the test concentration of 1,200 μg/ml resulted in a decay level of 25%.

The imazalil dip treatments at 300 and 600 μg/ml injured the skin of all three cultivars. Injury was evident on treated fruit after 2 wk of storage at 2 C followed by 7 days at 21 C and ranged from a slightly mottled, brown discoloration to a severe browning that gave the fruit a cooked appearance. No other dip treatment caused apparent fruit injury.

Simulated commercial storage. Suspensions of the fungicides in combination with either diphenylamine or $CaCl_2$ were prepared without difficulty from chemical incompatibility. McIntosh and Golden Delicious apples treated with either single or quadruple concentrations of prochloraz, CGA-64251, procymidone, or iprodione plus diphenylamine and Jonathan apples treated with the same fungicides and concentrations plus $CaCl_2$ showed no appreciable chemical skin injury after 6 mo of storage. None of the fungicide-diphenylamine or fungicide-$CaCl_2$ combinations appeared to affect bitter pit or Jonathan spot. Scald and internal breakdown were absent in all lots.

Prestorage inoculation. Prochloraz, CGA-64251, and iprodione effectively controlled blue mold decay during storage and did not differ significantly in their effects on all three cultivars (Table 2). The procymidone drench treatments were not effective on Golden Delicious but were significantly better than no treatment for McIntosh and Jonathan apples. Benomyl was not effective.

Poststorage inoculation. For most

Table 3. Blue mold decay caused by benomyl-sensitive and benomyl-resistant isolates of *Penicillium expansum* in apples inoculated after being dipped in experimental fungicides plus 2,000 μg/ml diphenylamine or 4% calcium chloride and stored for 6 mo at 0 C

Postharvest dip treatment		Cultivar[z]		
Fungicide	**Rate (μg a.i./ml)**	**McIntosh**	**Golden Delicious**	**Jonathan**
Prochloraz	300	18.0 a	1.7 a	1.0 a
	1,200	14.6 a	2.0 a	0 a
CGA-64251	120	22.3 a	4.7 b	1.7 a
	480	18.0 a	3.7 ab	1.7 a
Procymidone	900	26.0 a	12.0 c	2.3 a
	3,600	27.6 a	10.0 c	1.7 a
Iprodione	1,800	22.6 a	13.6 c	2.7 a
	7,200	16.0 a	3.0 ab	1.0 a
Control (diphenylamine)	...	70.3 b	35.7 de	...
Control ($CaCl_2$)	...	...	...	16.7 b
Control (water only)	...	63.3 b	47.0 e	26.0 b

[z] Figures are mean percentages of blue mold. Means in the same column followed by the same letter do not differ significantly ($P = 0.05$), according to the Student-Newman-Keuls multiple range test.

treatments, the antifungal activity of the chemical residues remaining on or in the fruit was adequate to give significant control of *P. expansum* in inoculated fruit during a 2-wk storage period at 2 C followed by 7 days at 21 C (Table 3). Although Golden Delicious fruit treated with procymidone and iprodione showed significantly more decay than similar fruit treated with prochloraz or CGA-64251, the decay was still much less than for the control treatments.

The higher percentage of decay on treated McIntosh apples than on the other two cultivars may have resulted from the higher spore concentration. Experience in previous tests has shown that tests conducted with spore inoculum concentrations above 100,000 per milliliter rather than about 50,000 per milliliter can reject chemicals that would otherwise be good candidates for blue mold control. Also, poststorage fruit quality was marginal, in that apples of all cultivars were too ripe for commercial acceptance. In general, overmature fruit are more susceptible to *P. expansum* infection than firm-mature fruit. The high spore concentration, advanced fruit maturity, and excessive length of holding, although more severe than normal commercial conditions, did allow us to critically evaluate the chemicals.

DISCUSSION

Until these new materials are cleared for postharvest use, the benzimidazole-resistant strains of blue mold will continue to cause storage losses unless the effectiveness of benomyl and thiabendazole can be prolonged. Their usefulness can be extended by following good packinghouse and storage sanitation practices, cleaning pallet boxes before they are used, and changing fruit drenching solutions often. Frequent changing of drenching solutions is stressed because most fruit destined for storage probably become infected with benzimidazole-resistant blue mold during drenching. The resistant strains may be selected through repeated preharvest benomyl sprays and are part of the soil debris that often accumulates on pallet boxes during the filling process in the orchards (C. L. Burton, *unpublished*). During drenching, this debris is flushed from the boxes into the tank, where it is recycled and increased as the process is repeated. Commercial practice has shown that storage rot problems can be greatly reduced if drenching solutions are changed frequently during fall apple handling (C. L. Burton, *unpublished*).

Other practices that may reduce chances of problems from blue mold resistance include eliminating damaged and rotten fruit daily from the packinghouse premises, changing water in dump tanks frequently during packing operations, and cleaning storage areas and equipment before use. Dropped fruit in orchards with a history of benomyl use should be handled separately and used immediately, and containers should be cleaned soon after disposing of the fruit.

Whether or not restricting the use of benomyl to postharvest application would delay resistance is not known. The uniqueness of the benzimidazole agents, benomyl and thiabendazole, for postharvest disease control would justify special use because no replacement chemicals are available at this time.

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