Postharvest Chemical Control of Penicillium Blue Mold of Apple

B. L. TEPPER, Graduate Research Assistant, and K. S. YODER, Assistant Professor, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg 24061, and VPI & SU Fruit Research Laboratory, Winchester 22601

ABSTRACT

Tepper, B. L., and Yoder, K. S. 1982. Postharvest chemical control of Penicillium blue mold of apple. Plant Disease 66:829-831.

Apples of six cultivars were selected for uniform size and ripeness, punctured, and inoculated with conidia of benzimidazole-sensitive *Penicillium expansum*, dipped in fungicide preparations either 4 or 24 hr after inoculation, and stored for 1 wk at 19 C or 11 wk at 0.5 C before decay incidence was rated. Treatment with CGA 64251 was 80% effective at 3.8 μg/ml and 98% effective at 7.5 μg/ml. Benomyl at 300 μg/ml and captan + benomyl at 600 and 300 μg/ml, respectively, were as effective as CGA 64251 at 7.5 μg/ml. Only 38% control was obtained with bitertanol at 900 μg/ml. Fungicide treatments 4 hr after inoculation gave better rot control than treatments applied 24 hr after inoculation. CGA 64251, benomyl, and captan + benomyl controlled rot effectively for the 11-wk storage period. Median lethal doses (μg/ml) for germination of *P. expansum* conidia on glass slides were: captan + benomyl, 0.07 + 0.07; benomyl, 0.10; CGA 64251, 0.25; captan, 1.04; and bitertanol, 3.98.

Penicillium blue mold of apple caused by *Penicillium expansum* Lk. ex Thom is reported to be the most prevalent postharvest disease in the major apple-growing regions of the world (8,9,12). Roy (12) estimated that from 25 to 50% of postharvest disease losses were from blue mold.

P. expansum penetrates the fruit surface through insect or harvest injuries, scab lesions, or open lenticels. Bulk unloading of apples into water dip tanks containing scald inhibitors increased decay incidence by exposing fruit to high levels of inoculum. Spore concentrations in dip tanks increased over time because of the washing of spores from the surface of infected fruit (1).

Attempts to reduce the increasing decay problem focused on the use of postharvest chemical dip treatments along with the scald inhibitor. Thiabendazole and benomyl were effective in controlling blue mold, whereas captan and several experimental materials were ineffective (2,3,7). Combinations of radiation and heated benomyl dips (50 C) provided complete control, but treated fruit was injured and lost flavor and texture (12). Heated (45 C) suspensions of benomyl or thiabendazole controlled blue mold without fruit injury (13). Heated tap water (3 min at 45 C) dips alone did not adequately control decay. In recent postharvest dip studies, CGA 64251 (7.5 μg/ml) and prochloraz (875 μg/ml) effectively controlled blue mold (4,11,14).

Recent development of benzimidazole-tolerant strains of *P. expansum* in several apple-growing regions increases the need for fungicides with other modes of action to control these strains (8–10,15).

Reported here are tests of CGA 64251, bitertanol, benomyl, and captan as inhibitors of spore germination and as postinoculation dips for control of *P. expansum* in long-term storage of apple cultivars.

Accepted for publication 9 December 1981.

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.

0191-2917/82/09082903/$03.00/0

MATERIALS AND METHODS

Spore germination tests. The fungicides benomyl (Benlate 50W), bitertanol (Baycor 50W), captan (50W), and CGA 64251 (Vangard 10W; 1-[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-ylmethyl] -1*H*,1,2,4-triazole; Ciba-Geigy Corp., Greensboro, NC 27409) were suspended in absolute ethanol and diluted with sterile distilled water to give a final ethanol concentration of 0.1% for all fungicide concentrations used. *P. expansum* was grown in petri dishes on Difco potato-dextrose agar (PDA) incubated at 20–21 C for 11 days. Cultures were then flushed with 10 ml of sterile distilled water and filtered through

sterile cheesecloth. Spore density was adjusted to 1.5×10^5 spores per milliliter, 2.5 ml of the suspension was pipetted into a sterile 20-ml test tube containing 2.5 ml of Difco nutrient yeast extract (0.3%, w/v), and 5 ml of the selected fungicide stock suspension was added to give final fungicide concentrations of 10, 1, 0.1, or 0.01. μg/ml.

The mixture of spore suspension, yeast extract broth, and fungicide was agitated to obtain a uniform mixture, and 0.2 ml was pipetted into a cell of a serological ring slide. There were three replicates per treatment. The controls upon which LD_{50} values were based contained 2.5 ml of spore suspension, 2.5 ml of yeast extract, 5 ml of sterile distilled water, and 0.1% ethanol.

Spores were incubated for 24 hr at 21 C. The numbers of germinated and ungerminated spores in each treatment were then counted. Three fields of focus containing at least 100 spores per field in each of the three cells per treatment were counted. The test was repeated on four replicate days per treatment. The data for LD_{50} values were analyzed by the nonparametric Spearman-Karber procedure (6).

Fruit dip tests. Fruit treated with commercial fungicides throughout the growing season was selected at harvest for uniform size and ripeness. Golden Delicious, Delicious, and Stayman apples were each randomized into three 20-fruit replicates. Rome Beauty, York Imperial, and Winesap were treated as one test with a single, 20-fruit replicate for each cultivar. Apples were punctured and inoculated to a depth of 3 mm with a 3-mm-diameter dowel rod dipped into a suspension at 1.3×10^5 conidia per milliliter of benomyl-sensitive *P. expansum*. Cultures grown in petri dishes on PDA and incubated at 20–21 C for 11 days were flushed with 10 ml of sterile distilled water and filtered through sterile cheesecloth.

The fungicides benomyl, bitertanol, captan, and CGA 64251 were suspended in tap water. At intervals of 4 or 24 hr after inoculation, fruit was dipped for 30 sec in the treatment and allowed to air-dry for 1 hr, then placed onto packing trays inside apple cartons with polyethylene liners. Treated fruit was then placed into cold storage (0.5 C) for 11 wk or held at ambient temperature (16–22 C) for 1 wk. Decay incidence was evaluated upon removal from storage.

Four control treatments were included in the test: punctured and inoculated, punctured and uninoculated, unpunctured and uninoculated (clean), and unpunctured but dipped into an inoculum suspension. All controls were tested in both storage conditions.

The statistical design for the fruit dip test was a randomized complete block of three replicates per cultivar for Golden Delicious, Delicious, and Stayman, and one combined test with one replicate each of Rome Beauty, York Imperial, and Winesap. Data were analyzed by means of the Waller-Duncan K-ratio *t*-test (6).

RESULTS

Spore germination tests. The sensitivity of *P. expansum* conidia to the fungicides was determined by the Spearman-Karber analysis for LD_{50} values (Table 1). The lowest LD_{50} was obtained with captan + benomyl at 0.07 + 0.07 μg/ml, followed by benomyl at 0.10, CGA 64251 at 0.25, captan at 1.04, and bitertanol at 3.98 μg/ml. The low LD_{50} for captan + benomyl was closely associated with that of benomyl; however, the fiducial interval suggests that the addition of captan contributed to a reduction of the LD_{50}.

Qualitative effects of the compounds on the germination processes were observed (Table 2).

Fruit dip tests. CGA 64251 at 45 μg/ml, benomyl at 300 μg/ml, and captan + benomyl at 600 + 300 μg/ml gave good control of blue mold on both Delicious and Golden Delicious (Table 3). The CGA 64251 and benomyl treatments gave comparable control during the short-term warmer storage period, but control by captan + benomyl was significantly poorer. Captan + benomyl equalled benomyl in control during the long-term cold storage. CGA 64251 on Golden Delicious fruit controlled decay best when applied 4 hr after inoculation, with the fruit stored for 11 wk at 0.5 C. No significant differences were noted between the CGA 64251 (30 μg/ml), benomyl, and benomyl + captan treatments when applied to Delicious 4 hr after inoculation and when the fruits were stored 11 wk.

Dip treatments of Golden Delicious and Delicious fruit in bitertanol (225 μg/ml) or captan (600 μg/ml) were ineffective in controlling decay for all test periods and postinoculation intervals.

There was a general increase in percentage of decay when the postinoculation treatment interval was increased from 4 to 24 hr with all the test fungicides. The effect was more apparent on Delicious than on Golden Delicious.

Lesion diameter on infected fruit was greater after long-term cold storage than after short-term warm storage with all treatments. Lesions on Stayman fruit treated with bitertanol (900 μg/ml) 4 hr after inoculation and stored 7 days were smaller than lesions on fruit treated with bitertanol at 450 μg/ml and than on punctured, inoculated control fruit.

Bitertanol and captan treatments of Stayman fruit were relatively ineffective under all test conditions (Table 4). CGA 64251 at 3.8 μg/ml gave poorer control than at 7.5 μg/ml; captan + benomyl (600 + 300 μg/ml) and CGA 64251 (7.5 μg/ml) gave good control under all test conditions on Stayman fruit (Table 4). As was the case with Golden Delicious and Delicious, rot control was poorer when treatments were applied to Stayman fruit 24 hr rather than 4 hr after inoculation. The punctured and inoculated controls developed 98–100% decay.

Table 1. Inhibition of germination of *Penicillium expansum* conidia by selected fungicides

Treatment	Inhibition (%) (μg/ml) 10.0	1.0	0.1	0.01	LD_{50} (μg/ml)[y]	Point of fiducial interval Lower (2.5%)	Upper (97.5%)
Bitertanol	85	1	0	0	3.98	3.81	4.21
CGA 64251	100	100	13	0	0.25	0.24	0.25
Captan	100	65	0	0	1.04	0.98	1.10
Benomyl	100	97	59	0	0.10	0.09	0.11
Captan + benomyl[z]	100	99	57	15	0.07 + 0.07	0.07	0.08

[y] Based on 84% germination of control. Spearman-Karber analysis. Means of four replicates.
[z] Tested as equal concentrations of both compounds.

Table 2. Effects of selected fungicides on *Penicillium expansum* germination processes

Treatment	Fungicide concentration (μg/ml) 0.01	0.1	1	10
Benomyl	No effect	Reduced germ tube growth	Swelling of conidia; stunting and curving of germ tubes	
CGA 64251	Slight stimulation of germ tube growth	13% inhibition of germination; reduced germ tube growth	Complete inhibition of germination	
Captan	Stimulation of germ tube growth		65% inhibition of germination	Complete inhibition of germination
Captan + benomyl	15% inhibition of germination; no growth reduction in unaffected germ tubes	Stunting, curving of germ tubes; 57% inhibition of germination	Complete inhibition of germination	

Table 3. Effect of chemical dip treatments on the incidence of Penicillium blue mold on apples at different inoculation-treatment intervals

Treatment	Rate (μg/ml)	Fruit with infection[y]							
		Golden Delicious				Delicious			
		1-wk storage, 22 ± 4 C		11-wk storage, 0.5 C		1-wk storage, 22 ± 4 C		11-wk storage, 0.5 C	
		4 hr[z]	24 hr	4 hr	24 hr	4 hr	24 hr	4 hr	24 hr
Bitertanol 50W	150	98 c	100 d	100 c	97 d	82 d	98 e	100 d	100 e
Bitertanol 50W	225	93 c	98 cd	98 c	100 d	63 c	98 e	88 c	100 e
CGA 64251 10W	15	5 a	8 ab	2 a	42 b	3 b	30 b	22 b	47 c
CGA 64251 10W	30	0 a	3 a	7 a	20 a	2 a	5 a	8 a	22 a
CGA 64251 10W	45	0 a	5 a	2 a	12 a	0 a	12 ab	3 a	28 ab
Captan 50W	600	93 c	92 c	95 c	88 c	48 c	77 d	77 c	83 d
Benomyl 50W	300	5 a	8 ab	23 b	18 a	0 a	33 c	3 a	37 abc
Captan 50W + benomyl 50W	600 + 300	15 b	20 b	20 b	22 a	8 b	35 c	7 a	38 bc
Control (punctured and inoculated)	...	100 c	100 d	100 c	98 d	100 e	100 e	100 d	100 e

[y] Randomized complete block design with three replicates, 20 fruits per replicate. Column mean separation by Waller-Duncan K-ratio *t*-test ($P = 0.05$).
[z] Inoculation-treatment interval.

Table 4. Effect of chemical dip treatments on the incidence of Penicillium blue mold on Stayman apples at different inoculation-treatment intervals

Treatment	Rate (μg/ml)	Fruit with infection (%)[y]			
		1-wk storage, 22 ± 4 C		11-wk storage, 0.5 C	
		4 hr[z]	24 hr	4 hr	24 hr
Bitertanol 50W	450	78 e	100 c	85 c	95 e
Bitertanol 50W	900	62 d	100 c	78 c	93 e
CGA 64251 10W	3.8	20 b	52 b	45 b	53 d
CGA 64251 10W	7.5	2 a	17 a	2 a	20 ab
CGA 64251 10W	15	3 a	10 a	3 a	25 bc
Captan 50W	600	43 c	60 b	32 b	40 cd
Captan 50W	1,200	35 bc	52 b	32 b	42 cd
Benomyl 50W	300	2 a	10 a	5 a	8 a
Captan 50W + benomyl 50W	600 + 300	3 a	10 a	3 a	12 ab
Control	...	100 f	100 c	98 c	100 f

[y] Randomized complete block design with three replicates, 20 fruits per replicate. Column mean separation by Waller-Duncan K-ratio *t*-test ($P = 0.05$).
[z] Inoculation-treatment interval.

A test of higher rates of bitertanol and lower rates of CGA 64251 was conducted on single replicates of York Imperial, Rome Beauty, and Winesap apples treated 4 hr after inoculation and stored for 1 wk at 22 ± 4 C. In this test, as in the previous ones, CGA 64251 at 7.5 μg/ml, benomyl at 300 μg/ml, and captan + benomyl at 600 + 300 μg/ml effectively controlled the decay; however, bitertanol at 900 μg/ml and captan at 600 μg/ml were not effective.

DISCUSSION

Postharvest fungicide dips of CGA 64251, benomyl, and captan + benomyl effectively controlled Penicillium blue mold. CGA 64251 showed much greater potential than bitertanol, and it is equal to or better than benomyl because of the low concentrations needed for good control in dip tests.

In the spore germination studies, benomyl had more in vitro activity in 0.1 μg/ml than CGA 64251. However, because the sterol-inhibiting mode of action of CGA 64251 is different from that of benomyl, CGA 64251 is a good alternative fungicide to counteract the development of benzimidazole-resistant strains of *Penicillium* (5).

The lack of control by bitertanol in the fruit dip tests and the lack of inhibition at 1 μg/ml in the germination studies indicates that this fungicide would not be commercially acceptable for control of Penicillium blue mold.

Although captan was ineffective as a postinoculation dip treatment, its value in present commercial postharvest dip programs is demonstrated by its inhibition of spore germination at concentrations as low as 10 μg/ml, which is 1.6% of the commercial use rate (600 μg/ml). This inhibition of spore germination effectively reduces the buildup of inoculum, including benzimidazole-resistant inoculum, in tanks where antiscald treatments are applied. Thus, captan is more effective as a protectant than as a postinoculation treatment (eradicant). The use of captan also improves control of other diseases, particularly those caused by *Alternaria* and *Rhizopus* sp., upon which the benzimidazole fungicides have little effect.

The time of application of a fungicide as a postharvest dip is critical because a delay from 4 to 24 hr after inoculation results in a large increase in percentage of decay. Commercial dip or drench programs using both CGA 64251 and benomyl would effectively control Penicillium blue mold and reduce the possibility of strains developing tolerance to either chemical.

ACKNOWLEDGMENTS

We wish to thank G. E. Mattus and R. J. Stipes for facilities and supplies and M. K. Roane for helpful advice.

LITERATURE CITED

1. Blanpied, G. D., and Purnasiri, A. 1968. *Penicillium* and *Botrytis* rot of McIntosh apples handled in water. Plant Dis. Rep. 52:865-867.
2. Blanpied, G. D., and Purnasiri, A. 1970. Thiabendazole control of postharvest apple decay. HortScience 5:476-478.
3. Cargo, C. A., and Dewey, D. H. 1970. Thiabendazole and benomyl for the control of postharvest decay of applies. HortScience 5:259-260.
4. Conway, W. S. 1981. Blue mold. Fungic. Nematic. Tests 36:1.
5. Delp, C. J. 1980. Coping with resistance to plant disease control agents. Plant Dis. 64:652-657.
6. Finney, D. J. 1971. Statistical Method in Biological Assay. 2nd ed. Charles Griffin and Co., London. 668 pp.
7. Hardenburg, R. E., and Spalding, D. H. 1972. Postharvest benomyl and thiabendazole treatments alone and with scald inhibitors to control blue and gray mold in wounded apples. J. Am. Soc. Hortic. Sci. 97:154-158.
8. Koffman, W., Penrose, L. J., Menzies, A. R., Davis, K. C., and Kaldor, J. 1978. Control of benzimidazole-tolerant *Penicillium expansum* in some fruit. Sci. Hortic. 9:31-39.
9. Morris, S. C. 1980. Dip pasteurization: A solution for resistance to fungicides. HortScience 15:757-758.
10. Rosenberger, D. A. 1980. Control strategies for benomyl-resistant postharvest decays of apples. Proc. N.Y. State Hortic. Soc. 125:98-102.
11. Rosenberger, D. A., and Meyer, F. W. 1981. Blue mold postharvest decay. Fungic. Nematic. Tests 36:14.
12. Roy, M. K. 1975. Radiation, heat and chemical combines in the extension of shelf life of apples infected with blue mold rot (*Penicillium expansum*). Plant Dis. Rep. 59:61-64.
13. Spalding, D. H., Vaught, H. C., Day, R. H., and Brown, G. A. 1969. Control of blue mold rot development in apples treated with heated and unheated fungicides. Plant Dis. Rep. 53:738-742.
14. Tepper, B. L., and Yoder, K. S. 1981. Blue mold: *Penicillium expansum*. Fungic. Nematic. Tests 36:19-20.
15. Yoder, K. S. 1979. Detection of benomyl-resistant *Venturia inaequalis* and *Penicillium* sp. on apples in Virginia. Va. J. Sci. 30:59.