Effects of Sequential Calcium Chloride, Ziram, and Yeast Orchard Sprays on Postharvest Decay of Pear

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ABSTRACT

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The most effective nutritional, fungicidal, and biological control treatments previously evaluated for control of postharvest decay in pear were evaluated for 3 years as factorial treatments to determine the best combinations for an integrated program. Calcium chloride sprays during the growing season reduced incidence of side rot in each year and of blue mold in 1 year, while ziram was effective against side rot in 1 year and blue mold in 2 years. Ziram, but not calcium chloride, provided control of gray mold and bull's-eye rot. Application of the yeast *Cryptococcus infirmo-minutus* to pear fruit 1 week before harvest at a concentration of 1.0 to 1.5×10^8 CFU/ml resulted in establishment of large populations of yeast on fruit surfaces, but did not reduce postharvest fungal decay incidence in 3 years of testing. In 1 year, ziram sprays applied 2 weeks before harvest significantly reduced yeast populations on fruit subsequently treated with *C. infirmo-minutus*. Sequential treatments with calcium chloride and ziram are indicated in an integrated program to take advantage of their differential effectiveness to broaden the range of control of pear postharvest decay pathogens.

Additional keywords: *Alternaria*, *Botrytis cinerea*, *Neofabraea*, *Penicillium expansum*

Stored pears are susceptible to decay caused by several fungi, and postharvest decay is a source of significant economic losses in the pear industry of the United States (9). Although the traditional focus of pear decay control has been fungicide application in the packinghouse (9,13), improvements in decay control have been achieved through orchard applications of calcium chloride (15,16), the fungicide ziram (2,8,12), and application of biocontrol agents (1). These diverse control methods can each contribute to decay reduction, but the levels of control that have been observed experimentally are relatively small. To date, they represent the most effective orchard-applied nutritional, fungicidal, and biological control treatments, respectively, evaluated for control of pear decay. The objective of this study was to evaluate the effectiveness of an integrated program consisting of sequential orchard treatments with calcium chloride, ziram, and the yeast biocontrol agent *Cryptococcus infirmo-minutus* in controlling postharvest pear decay. In addition, the effects of calcium and ziram treatments on

the populations of applied yeast on fruit surfaces were studied.

MATERIALS AND METHODS

Plot design. In a block of pear trees (cv. Golden Russet Bosc on rootstock OHxF 333) planted in 1986 at a 2×5 m spacing, treatment combinations were assigned to two-tree replicate units, with five replicate units per treatment combination in a completely randomized design. In each of 3 years of study (1998 to 2000), all possible combinations of two levels of calcium (calcium chloride or none), fungicide (ziram or none), and yeast biocontrol agent (*C. infirmo-minutus* or none) were arrayed through the plot. Treatment combinations were re-randomized each year. At least one nontreated tree was left between replicate units to reduce drift or contamination among treatments.

Calcium treatments. Trees (fruit) in the calcium treatments were sprayed to runoff with CaCl_2 solutions at 17.5 g per liter of water, equivalent to 6 g of actual Ca per liter. Calcium applications began in early July each year and were re-applied every 2 weeks for a total of three sprays annually.

Fungicide treatments. Ziram 76 DF (Micro Flo Co., Lakeland, FL; common name: ziram, zinc methylthiocarbamate) fungicide was applied 2 weeks before harvest each year at a rate of 4.8 g per liter of water. Treatments were applied by handgun sprayer to runoff. Ziram applications took place approximately 2 weeks after the

third (last) calcium chloride application each year and 1 week before application of the yeast biological control agent.

Biological control agent. *C. infirmo-minutus* strain YY6 (CIM) was originally isolated by Chand-Goyal and Spotts from the surface of pear fruit from Yakima, WA (6). In 1998, the yeast was maintained at -20°C on yeast malt dextrose broth (YMDB: 3 g of malt extract, 3 g of yeast extract, 5 g of peptone, and 10 g of dextrose per liter of medium) until use. The yeast was activated by dispensing 1 ml of thawed suspension into 75 ml of sterile YMDB in a 250-ml flask. After 2 days of incubation at 20°C on a shaker, 0.1-ml aliquots of yeast suspension were placed on petri dishes containing yeast malt dextrose agar (YMDA: YMDB plus 18 g per liter of agar). After 2 days of incubation at 20°C , yeast colonies were removed by scraping the dishes with a sterile rubber spatula, and the yeasts were suspended in sterile distilled water. CIM suspensions were adjusted to 2% transmittance at 550 nm using a Spectronic 20 spectrophotometer to give a final concentration of 1.0 to 1.5×10^8 CFU/ml. Spectrophotometric determinations of yeast concentrations were confirmed by dilution plating of the treatment suspensions. For treatments in 1999 and 2000, a dry formulation of CIM provided by Ecogen, Inc. (Langhorne, PA) was used. The concentration of CIM in the formulation was determined by dilution plating to be 4.7×10^{10} CFU g^{-1} in 1999 and 3.0×10^{10} CFU g^{-1} in 2000. The concentrated formulation was mixed with water to give a final treatment concentration of 1.0×10^8 CFU ml^{-1} . Trees were thoroughly wetted with yeast suspensions applied by handgun sprayer 1 week prior to harvest in each year.

Population sampling. CIM populations on the fruit surfaces were determined at harvest from each replicate receiving calcium chloride + yeast, ziram + yeast, and yeast only, as well as from each replicate receiving no treatment. Three uniform-sized fruit were collected from each replicate and promptly transported to the laboratory. The three fruit were combined in beakers containing 900 ml of 0.05 M sterile phosphate buffer (SPB) with 0.006% (vol/vol) Tween 20. The fruit and buffer were stirred for 5 min using a magnetic stirrer, then placed in a sonicating bath for 5 min (5). A 1-ml sample was removed from each beaker immediately after sonica-

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tion, and 10-fold dilutions were made to 10^{-5} CFU ml⁻¹ in SPB. Samples of each dilution were then placed on petri dishes containing a selective, dilute YMDA medium (1.5 g of malt extract, 1.5 g of yeast extract, 2.5 g of peptone, 5 g of dextrose, and 18 g of agar per liter of medium, with 100 ppm chloramphenicol, 50 ppm ampicillin, and 2 ppm dichloran). Chloramphenicol and ampicillin were included to inhibit bacterial growth; dichloran was used to inhibit radial growth of filamentous fungi. The plates were incubated for 2 to 3 days at room temperature. After incubation, population levels were determined using the plate-dilution frequency technique of Harris and Sommers (7). The yeast used in this study exhibits distinct colony color and morphological characteristics, which were used to visually distinguish the yeast colonies from other indigenous yeasts (1,5). Only CIM colonies were counted in the population samples.

Postharvest wounding and storage. At commercial maturity, 20 fruit were harvested from each replicate unit and cooled for 1 week at 0°C. Each fruit was then wounded with a sterile nail (3.5 mm diameter by approximately 5 mm depth) to simulate stem punctures, which are common entry points for decay pathogens (11).

Each fruit was wounded in five locations. The fruit were not washed or surface-sterilized, so that naturally occurring pathogens and field-applied treatments on the fruit surface were not intentionally removed or inhibited. Three to four months after each harvest, the fruit were removed from storage and visually evaluated for the effects of the treatment programs on post-harvest decay. Incidence of decay was recorded as the percentage of wounds infected by each decay type: blue mold (*Penicillium expansum*), side rot (*Phialophora malorum*, *Cladosporium herbarum*, or *Alternaria* spp. [14]), gray mold (*Botrytis cinerea*), and bull's-eye rot (*Neofabraea* spp.).

Statistical analysis. All decay data were treated as factorial and subjected to analysis of variance using the General Linear Model of Minitab statistical software (State College, PA). Percentage values were transformed to arcsine (square root [x]) prior to analysis. Yeast population counts were transformed to log₁₀, and means were compared by Fisher's protected LSD following ANOVA, using Minitab software.

RESULTS

CIM sprays resulted in the establishment of substantial populations on the surfaces

of pear fruit (Table 1). At harvest, 1 week after yeast applications, populations of CIM ranged from 2.6 to 11.2×10^6 CFU per fruit in 1998, 0.7 to 2.1×10^6 in 1999, and 0.6 to 20.8×10^6 in 2000. In all 3 years, calcium chloride treatments did not significantly ($P > 0.05$) affect CIM populations on the fruit surfaces. Ziram sprays reduced CIM populations in 1998 but not in 1999 or 2000 (Table 1). CIM colonies were not recovered from fruit sampled from replicates not treated with CIM, with the exception of a few colonies recovered on petri dishes in 2000 (D. Sugar, unpublished data).

Where fruit were wounded in the presence of natural inoculum, calcium chloride sprays were associated with reduced incidence of side rot in 1998 and 2000 and of blue mold and side rot in 1999 (Table 2). No appreciable blue mold incidence was observed in any treatment in 1998. Ziram sprays reduced incidence of gray mold in 1998, blue mold, side rot, gray mold, and bull's-eye rot in 1999, and blue mold, gray mold, and bull's-eye rot in 2000. Total decay incidence was reduced by ziram in 1998 and by both calcium chloride and ziram in 1999 and 2000. Yeast treatments did not affect incidence of any type of decay, and there were no significant interactions ($P > 0.05$) among treatment variables (Table 2).

DISCUSSION

In a previous study (1), we found that single applications of CIM and *Rhodotorula glutinis* provided significant postharvest decay control in pear fruit treated in the orchard 3 weeks before harvest. CIM also controlled decay in fruit treated 1 day before harvest. Although similar populations of CIM were established on pear fruit following orchard sprays in the present study, significant decay control was not

Table 1. Populations of *Cryptococcus infirmo-miniatus* recovered in washes of Bosc pears at harvest

	log ₁₀ CFU/fruit ²		
	1998	1999	2000
No calcium or ziram	6.90 a	6.00	6.55
Calcium	6.93 a	6.16	6.68
Ziram	6.37 b	5.78	6.42
P value	0.020	0.270	0.396

² A water suspension of *C. infirmo-miniatus* containing 1×10^8 CFU/ml was sprayed on fruit 1 week before harvest. The fruit had previously been sprayed with ziram (1 month before harvest), calcium chloride (mid-growing season), or neither. Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test ($P \leq 0.05$).

Table 2. Effect of treatments with (+) or without (–) field applications of calcium chloride (Ca), ziram (Z), and the yeast *Cryptococcus infirmo-miniatus* (Y) on postharvest decay of Bosc pears

Treatment sequence	Percentage of wounds infected ²													
	1998				1999				2000					
	Side rot	Gray mold	Bull's-eye rot	Total decay	Blue mold	Side rot	Gray mold	Bull's-eye rot	Total decay	Blue mold	Side rot	Gray mold	Bull's-eye rot	Total decay
+ Ca + Z + Y	1.2	0.8	1.6	3.6	2.8	0.8	0.2	0.8	4.6	1.2	5.0	0.2	0.8	7.6
+ Ca + Z – Y	1.6	1.6	4.6	7.8	1.6	0.2	0.4	0.2	2.4	0.6	1.6	0.4	0.0	3.0
+ Ca – Z + Y	1.3	7.4	9.8	19.6	4.0	3.2	2.4	5.0	15.2	0.2	3.0	0.6	9.8	14.8
+ Ca – Z – Y	1.6	7.8	9.4	18.8	3.0	4.8	1.4	4.4	13.8	0.4	3.6	0.8	6.6	12.8
– Ca + Z + Y	7.2	1.8	2.0	11.0	3.0	3.2	0.0	0.8	7.0	1.6	12.0	0.6	0.2	15.4
– Ca + Z – Y	4.2	1.8	3.2	9.2	4.4	4.4	0.2	0.2	9.6	0.4	12.2	0.0	2.8	17.0
– Ca – Z + Y	5.0	7.8	3.0	15.8	6.6	8.0	2.8	4.4	22.2	0.2	15.6	1.0	6.0	24.2
– Ca – Z – Y	2.0	4.6	4.6	11.2	9.8	10.4	2.0	0.8	23.2	0.2	25.4	1.4	5.6	35.6
<i>P</i> values for main effects and interactions														
Ca	0.003	0.935	0.197	0.946	0.019	<0.001	0.652	0.345	0.014	0.744	<0.001	0.169	0.991	<0.001
Z	0.464	<0.001	0.074	<0.001	0.028	<0.001	<0.001	0.016	<0.001	0.007	0.091	<0.001	<0.001	0.002
Y	0.773	0.980	0.452	0.795	0.636	0.334	0.369	0.236	0.984	0.133	0.964	0.922	0.715	0.979
Ca × Z	0.692	0.309	0.331	0.053	0.212	0.433	0.499	0.345	0.513	0.577	0.247	0.169	0.248	0.776
Ca × Y	0.155	0.782	0.693	0.255	0.186	0.489	0.821	0.484	0.488	0.430	0.295	0.118	0.253	0.234
Z × Y	0.818	0.562	0.501	0.291	0.693	0.548	0.263	0.484	0.921	0.085	0.184	0.169	0.791	0.229
Ca × Z × Y	0.592	0.823	0.505	0.954	0.752	0.963	0.986	0.484	0.796	0.577	0.964	0.169	0.365	0.975

² After harvest, all fruit were artificially wounded with sterile nails, allowing natural inoculum on the fruit surface to initiate decay at wounds.

detected. It is possible that the unintended drift of ziram between treatments affected the efficacy of CIM in controlling postharvest decay, but samples at harvest indicated the presence of substantial populations, capable of forming colonies on the sampling medium. It is also possible that the pathogen inoculum levels on the fruit surfaces, which were not sampled, were higher in the present experiment than in the previous study, resulting in a differential response to yeast treatments. CIM appears to be most effective when used in a postharvest application (17). Orchard applications of *Candida sake* reduced incidence and severity of blue mold decay of apple (18), and orchard-applied mixtures of *Aureobasidium pullulans*, *R. glutinis*, and *Bacillus subtilis* (10) reduced blue mold, gray mold, and bull's-eye rot in apple.

Total decay incidence appeared to increase over the 3 years of this study, primarily as a consequence of an increase in side rot (Table 2). One factor influencing this trend may have been rainfall. No rainfall occurred between the ziram or yeast sprays and harvest in 1998 and 1999, but in 2000 rainfall of 5.1 mm was recorded over 3 days in the final week before harvest.

Multiple spray applications of calcium chloride in the orchard have been reported to reduce postharvest *Alternaria* infection as well as postharvest development of bitter rot following orchard infection by *Colletotrichum* spp. in apple (3,4). In pear, calcium chloride orchard sprays have been shown previously to enhance fruit resistance to blue mold decay and side rot, but not gray mold or bull's-eye rot (15). Ziram has been applied as a preharvest spray to pears intended for long-term storage, with the primary targets of bull's-eye rot and side rot (2,8).

In the present study, calcium chloride treatment was effective against blue mold and side rot in 1 and 3 years, respectively, while ziram was effective in reducing incidence of gray mold in all 3 years. Only ziram provided control of gray mold and bull's-eye rot. The total decay incidence associated with treatment programs that included both calcium chloride and ziram over the 3 years ranged from 2.4 to 7.8%, with an average of 4.8% (Table 2). Total decay resulting from programs that included neither calcium chloride nor ziram ranged from 11.2 to 35.6%, with an average of 22.0%. Although there were no significant interactions between calcium and ziram treatments, and thus no evidence of synergy between the treatments, the differential efficacy of these treatments among postharvest pathogens indicates that a sequential treatment program can provide additive benefit by broadening the range of postharvest decay control.

LITERATURE CITED

1. Benbow, J. M., and Sugar, D. 1999. Fruit surface colonization and biological control of postharvest diseases of pear by preharvest yeast applications. *Plant Dis.* 83:839-844.
2. Bertrand, P., and MacSwan, I. 1977. Control of postharvest decay in Oregon tree fruit crops. *Proc. Oreg. Hortic. Soc.* 68:71-73.
3. Biggs, A. R. 1999. Effects of calcium salts on apple bitter rot caused by two *Colletotrichum* spp. *Plant Dis.* 83:1001-1005.
4. Biggs, A. R., Ingle, M., and Solihati, W. D. 1993. Control of *Alternaria* infection of fruit of apple cultivar Nittany with calcium chloride and fungicides. *Plant Dis.* 77:976-980.
5. Chand-Goyal, T., and Spotts, R. A. 1996. Enumeration of bacterial and yeast colonists on apple fruits and identification of epiphytic yeasts on pear fruit in the Pacific Northwest United States. *Microbiol. Res.* 151:427-432.
6. Chand-Goyal, T., and Spotts, R. A. 1996.

Control of postharvest pear diseases using natural saprophytic yeast colonists and their combination with a low dosage of thiabendazole. *Postharvest Biol. Technol.* 7:51-64.

7. Harris, R. F., and Sommers, L. E. 1968. Plate-dilution frequency technique for assay of microbial ecology. *Appl. Microbiol.* 16:330-334.
8. Kienholz, J. R. 1956. Control of bull's-eye rot on apple and pear fruits. *Plant Dis. Rep.* 40:872-877.
9. Kupferman, E. A. 1998. Postharvest applied chemicals to pears: A survey of pear packers in Washington, Oregon, and California. *Tree Fruit Postharvest J.* 9:3-24.
10. Leibinger, W., Breuker, B., Hahn, M., and Mendgen, K. 1997. Control of postharvest pathogens and colonization of the apple surface by antagonistic microorganisms in the field. *Phytopathology* 87:1103-1110.
11. Spotts, R. A., Sanderson, P. G., Lennox, C. L., Sugar, D., and Cervantes, L. A. 1998. Wound healing and staining of mature d'Anjou pear fruit. *Postharvest Biol. Technol.* 13:27-36.
12. Sugar, D. 1999. Control of postharvest diseases of pears with orchard sprays. *Proc. Wash. Tree Fruit Postharvest Conf.* 15:76.
13. Sugar, D. 2002. Management of postharvest diseases. Chapter 9 in: *Fruit Quality and its Biological Basis*. M. Knee, ed. Sheffield Academic Press, Sheffield, UK.
14. Sugar, D., and Powers, K. 1986. Interactions among fungi causing postharvest decay of pear. *Plant Dis.* 70:1132-1134.
15. Sugar, D., Powers, K. A., and Hilton, R. J. 1991. Enhanced resistance to side rot in pears treated with calcium chloride during the growing season. *Plant Dis.* 75:212-214.
16. Sugar, D., Roberts, R. G., Hilton, R. J., Righetti, T. L., and Sanchez, E. E. 1994. Integration of cultural methods with yeast treatment for control of postharvest fruit decay in pear. *Plant Dis.* 78:791-795.
17. Sugar, D., and Spotts, R. A. 1999. Control of blue mold decay in pear by four laboratory-grown yeasts and two registered biocontrol products. *Plant Dis.* 83:155-158.
18. Teixidó, N., Viñas, I., Usall, J., and Magan, N. 1998. Control of blue mold of apples by preharvest application of *Candida sake* grown in media with different water activity. *Phytopathology* 88:960-964.