Control of Brown Rot and Blue Mold of Sweet Cherry with Preharvest Iprodione, Postharvest *Cryptococcus infirmo-minutus*, and Modified Atmosphere Packaging

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ABSTRACT

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The effectiveness of preharvest iprodione and postharvest *Cryptococcus infirmo-minutus* treatments alone and in combination for control of decay of sweet cherry fruit was studied. Also, the effect of a modified atmosphere on brown rot control was evaluated as a part of the iprodione-*C. infirmo-minutus* combinations. A single preharvest application of iprodione at 1.13 kg a.i./ha reduced brown rot in stored sweet cherry fruit in both years of this study. Significantly better control of brown rot was obtained when cherry fruit that received a preharvest iprodione application also were treated with a postharvest dip in a suspension of *C. infirmo-minutus* containing 0.5 to 1.5×10^8 CFU/ml. Brown rot was reduced by modified atmosphere packaging (MAP) alone and further reduced as a result of a *C. infirmo-minutus*-MAP synergism. Incidence of brown rot was reduced from 41.5% in the control to 0.4% by combining preharvest iprodione and postharvest *C. infirmo-minutus* treatments with MAP.

Additional keywords: biocontrol, *Monilinia fructicola*, *Penicillium expansum*, *Prunus*, yeast

Approximately half of the market losses in Western sweet cherry fruit have resulted from disease (1). Several postharvest diseases, including blue mold (*Penicillium expansum* Link), brown rot (*Monilinia fructicola* G. Wint. Honey), *Alternaria* rot (*Alternaria alternata* (Fr.:Fr.) Keissl.), Rhizopus rot (*Rhizopus stolonifer* (Erhenb.:Fr.) Vuill.), and gray mold (*Botrytis cinerea* Pers.:Fr.), cause most of the decay (1). In a more recent survey, blue mold was the most damaging disease of sweet cherry at the New York terminal market and occurred in 35% of all shipments (2). Extended storage increases disease severity (17).

Postharvest disease control consists of an integrated combination of pre- and postharvest fungicide applications, sanitation, cold storage, and modified storage atmosphere (28,30). While several fungicides can be used preharvest on sweet cherry, few postharvest fungicides are registered (23). Elevated atmospheric CO₂ levels have been shown to reduce fruit decay of cherry during long-term storage (10,12,19,20).

Several recent studies have focused on biological control of postharvest diseases of stone fruits. Brown rot and Rhizopus rot of peach have been controlled with *Bacillus subtilis* (24) and *Enterobacter cloacae* (31), respectively. *Bacillus subtilis* also has controlled postharvest brown rot and *Alternaria* rot of sweet cherry (29). Control of brown rot of peach and nectarine has been achieved with *Pseudomonas corrugata* and *P. cepacia* when applied up to 12 h after inoculation with the pathogen (27). Application of *Aureobasidium pullulans* and *Epicoccum purpurascens* to sweet cherry blossoms reduced the number of latent infections of brown rot in green fruit (32). *Candida oleophila* reduced the level of infection caused by *P. expansum* in harvested nectarines (15). *C. oleophila* was effective in air and controlled atmosphere storage and when combined with the fungicide dichloran (15).

Recently, we found that three naturally occurring, saprophytic yeasts isolated from the surface of pear fruit (5) were effective for control of five postharvest diseases of pear (3,6). In addition, these yeasts controlled blue mold of apple and brown rot of sweet cherry fruit (4,6). Control of brown rot of sweet cherry was achieved when a combination of *Cryptococcus laurentii* or *C. infirmo-minutus* with a low dose of iprodione was applied postharvest (4). Iprodione was used extensively as a postharvest treatment of cherry until March 1996, when the product label was voluntarily changed by the manufacturer to cancel postharvest use on sweet cherry and to

restrict applications to no fewer than 7 days before harvest.

The primary objective of this study was to determine the effectiveness of preharvest iprodione and postharvest *C. infirmo-minutus* (*Cim*) treatments alone and in combination for control of decay of sweet cherry fruit. In addition, the effect of a postharvest modified atmosphere storage on brown rot control was evaluated as a part of the iprodione-*Cim* combinations.

MATERIALS AND METHODS

Iprodione (Rovral 50WP, Rhone-Poulenc Ag Co., Research Triangle Park, NC) was applied to five sweet cherry trees cv. Lambert on 21 or 28 June 1996 and 20 June 1997. Iprodione was applied at the rate of 1.13 kg a.i./ha in 757 liters of water (7.57 liters per tree) using a handgun sprayer operating at a pressure of 21 kg/cm². Five nonsprayed trees were left as controls. In 1996, all trees were sprayed with chlorothalonil at 2.36 kg a.i./ha on 8 and 15 April and 28 May. In 1997, all trees received bloom applications of chlorothalonil at 1.70 and 1.18 kg a.i./ha on 15 and 24 April, respectively.

Healthy appearing sweet cherry fruits were harvested on 3 July 1996 and 27 June 1997 at commercial maturity and treated the day of harvest. Cherry fruits were neither wounded nor surface-sterilized.

C. infirmo-minutus strain YY6, isolated from the surface of a pear fruit from Yakima, Washington, in 1993 (5), was stored at -70°C using the procedure of Gibson and Khoury (13). This yeast was activated by dispensing 70 µl of thawed suspension into 70 ml of yeast malt dextrose broth (YMDB: 3.0 g of Bacto yeast extract, 3.0 g of Difco malt extract, 5.0 g of Bacto peptone, and 10.0 g of Bacto dextrose per liter of medium) in a 200-ml flask. The *Cim* suspension was incubated on a rotary shaker at 125 rpm for 48 h at 23 ± 1°C, then spread (0.1 ml per petri dish) on yeast malt dextrose agar (YMDA) medium (YMDB plus 18.0 g of Bacto agar per liter). After 48 h of incubation at 23 ± 1°C, *Cim* cells were removed with a sterile rubber spatula, suspended in sterile distilled water (SDW), and vortexed for 1 to 3 min. The *Cim* cells were centrifuged at 1.0×10^4 rpm ($G = 1.06 \times 10^4$) for 15 min, and the supernatant was discarded. The cells were resuspended in SDW, and the concentration was adjusted to 1.45×10^8 CFU/ml in 1996 and 0.47×10^8 CFU/ml in

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1997 (2% transmittance at 550 nm using model Spectronic 20 spectrophotometer, Bausch and Lomb Optical Co., Rochester, NY). Yeast concentration was verified by dilution plating on YMDA.

In 1996, conidia of *M. fructicola* were harvested into SDW from a 12-day-old culture grown on acidified PDA. In 1997, conidia were removed from infected cherry fruit in the orchard by vigorously scraping fruit in SDW with a rubber spatula. Germination of conidia was 94%. In both years, spore concentrations were determined with a hemacytometer and adjusted to 1.0×10^4 conidia per ml of treatment suspension; suspensions were used within 2 to 3 h.

The postharvest treatments applied to sweet cherry fruits were: *M. fructicola* alone (control) and *Cim* combined with *M. fructicola*. These treatments were applied to fruit from each of the three preharvest treatment regimes (no spray and iprodione at 12 or 5 days preharvest) in 1996 and two regimes (no spray and iprodione at 7 days preharvest) in 1997. There were five replicates of 50 cherry fruits in each preharvest-postharvest treatment combination. Fruits were placed in containers with metal screens, immersed for 30 s in treatment suspensions, then drained for 1 min.

Each replicate of 50 fruits was stored in air at 2.8°C in a 3.78-liter polyethylene bag that had 18 6-mm-diameter holes along three sides. Twenty of these bags were placed into a larger folded but unsealed polyethylene bag inside a cardboard cherry box. In 1997, five replicate bags per treatment also were stored in a modified atmosphere package (MAP) sold under the trademark View Fresh (Orchard View Farms, The Dalles, OR), a polyethylene bag whose exact characteristics are proprietary. Prior to storage at 0.5°C, the bag was evacuated, injected with a 50-50 gas mix of CO₂ and N₂, and sealed using a CVP Systems gas injection system (Model A-300, Dowensgrove, IL). The atmosphere of the bag was determined by gas chromatography just prior to opening for fruit evaluation after 42 days of storage, and the percentages of oxygen and carbon dioxide were 5.1 and 11.4, respectively. Disease incidence was evaluated after 20 and 42 days storage in air and MAP, respectively.

In 1996, iprodione residues that washed off the fruit into the postharvest treatment suspension were determined by enzyme-linked immunosorbent assay (ELISA) using a Carbaryl RAPID Assay kit (Ohmicron, Newtown, PA) modified for iprodione. Iprodione residue in the suspensions in which the fruit were dipped was 2.2 ± 0.6 µg/ml.

In 1997, iprodione residues on fruit and in treatment suspensions were determined by gas liquid chromatography by Horizon Laboratories, Columbia, Missouri. The mean and standard error of the iprodione residue on the surface of fruit from trees sprayed with iprodione was 2.2 ± 0.3 µg/g.

Iprodione residues on fruit from unsprayed trees and in water after dipping all fruit from unsprayed trees was less than 0.05 µg/g, the minimum detectable amount. Iprodione residue in water after dipping all fruit from both unsprayed and iprodione-treated trees was 2.6 µg/ml.

RESULTS AND DISCUSSION

In 1996, rain occurred on 18, 19, 23, 24, and 25 June as cherry fruit ripened and caused splitting in about 95% of the fruit. Although the tissue at the split areas appeared to dry, considerable blue mold from natural inoculum and brown rot from controlled inoculation developed in this tissue. All treatments reduced blue mold, and all except *Cim* alone reduced brown rot (Table 1). *C. infirmo-minutus* combined with iprodione applied 5 days before harvest (PHI) was significantly better than the 12-day PHI iprodione alone (blue mold and brown rot) and the 5-day PHI iprodione alone (brown rot). *C. infirmo-minutus* combined with the 12-day PHI iprodione was not different from the 5-day iprodione alone or the *Cim* combined with the 5-day PHI iprodione for both blue mold and brown rot.

For air-stored fruit in 1997, iprodione and the combination of preharvest iprodione with postharvest *Cim* reduced brown

rot incidence, but *Cim* alone had no effect when compared with the control (Table 2). Untreated fruit stored for 42 days under MAP conditions had 40% less brown rot than those stored for 20 days in refrigerated air (Table 2). For MAP-stored fruit, both the iprodione and the *Cim* treatments alone significantly reduced brown rot incidence (Table 2). The combination of the iprodione and *Cim* was more effective than iprodione alone.

Preharvest applications of iprodione are effective for control of postharvest brown rot fruit decay and have been recommended for several years in the Pacific Northwest (23). A single preharvest application of iprodione reduced brown rot in stored sweet cherry fruit in both years of this study. Dicarbimides such as iprodione inhibit the germination of conidia as well as mycelial growth (26). Sensitive strains of *M. fructicola* are affected by less than 1 µg of iprodione per ml (8). Iprodione residues in water or yeast suspensions in which cherry fruit were dipped ranged from 2.2 to 2.6 µg/ml in 1996 and 1997, respectively. Although the resistance status of strains of *M. fructicola* used in this study was not determined, it appears that most were sensitive to iprodione, since the fungicide gave significant ($P = 0.05$) decay control.

Table 1. Control of decay of sweet cherry fruit with combinations of preharvest iprodione and postharvest *Cryptococcus infirmo-minutus* applications in 1996

Treatment ^x	Percent fruits decayed ^w	
	Blue mold ^y	Brown rot ^z
<i>C. infirmo-minutus</i>	4.0 b	16.0 de
<i>C. infirmo-minutus</i> + iprodione 5 day PHI	0.0 a	2.4 a
<i>C. infirmo-minutus</i> + iprodione 12 day PHI	0.0 a	3.2 ab
Iprodione 5 day PHI	2.4 ab	6.8 bc
Iprodione 12 day PHI	4.4 b	8.4 cd
Nontreated	19.6 c	20.8 e

^w Mean disease incidences for five replicate, 50-fruit samples per treatment. Means followed by the same letter within columns are not significantly different at $P = 0.05$ according to least significant difference (LSD) test. Data were transformed to arcsine $\sqrt{\text{percent}}$ before analysis.

^x Aqueous suspensions of *C. infirmo-minutus* contained 1.45×10^8 CFU/ml. Iprodione was applied 5 and 12 days before harvest (PHI) at 1.13 kg a.i./ha.

^y Blue mold developed from natural inoculum.

^z Postharvest treatment suspensions (*C. infirmo-minutus* and sterile distilled water) contained 1×10^4 conidia of *Monilinia fructicola* per ml.

Table 2. Effect of preharvest iprodione, postharvest *Cryptococcus infirmo-minutus*, and modified atmosphere packaging on control of brown rot of sweet cherry fruit

Treatment ^{y,z}	Percent decay ^{w,x}	
	Air	Modified atmosphere
<i>C. infirmo-minutus</i>	41.3 e	2.0 abc
<i>C. infirmo-minutus</i> + iprodione	1.2 ab	0.4 a
Iprodione	2.0 abc	4.8 c
Nontreated	41.5 e	24.7 d

^w Decay evaluated after 20 and 42 days storage in air at 2.8°C or modified atmosphere (5.1% oxygen, 11.4% carbon dioxide) at 0.5°C, respectively.

^x Mean disease incidences of five replicate, 50-fruit samples per treatment. Means followed by the same letter are not significantly different according to least significant difference (LSD) test at $P = 0.05$. Data were transformed to arcsine $\sqrt{\text{percent}}$ before analysis.

^y Aqueous suspensions of *C. infirmo-minutus* contained 0.47×10^8 CFU/ml. Iprodione was applied 7 days before harvest at 1.13 kg/ha.

^z Postharvest treatment suspensions (*C. infirmo-minutus* and sterile distilled water) contained 1×10^4 conidia of *Monilinia fructicola* per ml.

Significantly better control of brown rot usually was obtained when cherry fruit that received a preharvest iprodione application also were treated postharvest with *Cim*. Whereas residues of iprodione in this study were adequate to affect *M. fructicola*, *Cim* is resistant to 50 µg iprodione/ml in YMDA (T. Chand-Goyal, *unpublished*). In previous studies with pome fruits, three yeasts, including *Cim*, combined with 15 µg of thiabendazole per ml gave significantly better disease control than either thiabendazole or the yeasts alone (3,4).

Although *C. infirmo-miniatus* alone did not control brown rot in air-stored cherry fruit, the yeast alone did control blue mold caused by *P. expansum*. While conidia of *M. fructicola* germinate in less than 30 h at 0°C, *P. expansum* conidia do not germinate in 96 h at this temperature (11). The slower germination rate of *P. expansum* may give *Cim* time to grow and colonize wound sites. In previous studies with biocontrol of blue mold of apple and pear, *Cim* rapidly colonized wound sites and appeared to control decay by competition for nutrients (3,4).

Brown rot also was reduced by MAP storage. The CO₂ level in the MAP was 11.5% after 42 days of storage. In previous studies, decay of sweet cherry has been reduced by CO₂ atmospheres of 7.5 to 25% (10,12,19,20). Carbon dioxide is considered fungistatic to *M. fructicola*, since decay develops when fruit are returned to air (7,9). Many yeasts require CO₂ as a carbon source for growth (25), and about 10% CO₂ enhances sporulation in yeast fungi (22). In addition, when CO₂ dissolves in water, carbonic acid is produced and pH is lowered. Lower pH has been reported to favor yeast fungi (21), and *Cim* will grow in wounds of freshly harvested Golden Delicious apple at pH 3.1 (T. Chand-Goyal, *unpublished*). Thus, the *Cim*-MAP synergism for control of brown rot may be related to a combination of suppression of *M. fructicola* and stimulation of *Cim* by the high CO₂. In a recent study with d'Anjou pear fruit, the combination of *Cim* and MAP (2 to 6% O₂, 0.5 to 1% CO₂) reduced blue mold better than MAP without yeast or treatment with *Cim* followed by storage in air (16).

In recent years, an integrated strategy to control decay in pome and stone fruits has been advanced (28,30). In this study, the combination of preharvest iprodione with postharvest *Cim* reduced decay by 88.5 to 97.1%, and fruit treated with this combination and stored in modified atmosphere packaging had a reduction in brown rot of 98.2%. The cherry industry is interested in reduction of decay by nonchemical means and maintenance of fruit quality during long-term storage and distant transport. In many instances, slower surface transportation is used rather than faster but more expensive air transport. Implementation of

the preharvest iprodione–postharvest yeast-MAP system would increase decay control during long-term storage and transport of sweet cherry fruit.

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