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Timing and sequence of postharvest fungicide and biocontrol agent applications for control of pear decay

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Abstract

Postharvest decay of pear fruit often originates at small wounds that occur during harvest and handling. Experiments were conducted to characterize the effect of timing of application of postharvest decay control materials, and to evaluate sequential postharvest applications of fungicides or fungicides and biocontrol agents. Fungicides and biocontrol agents were increasingly less effective when the period between harvest and application was prolonged. Thiabendazole (TBZ) applied to fruit without artificial wounding or inoculation effectively reduced decay when applied within 3 weeks or 6 weeks in 2 years of study. TBZ, fludioxonil, and pyrimethanil were effective in controlling decay at artificial wounds inoculated with *Penicillium expansum* up to 14 d after inoculation. Application of TBZ at harvest followed 3 weeks later by application of fludioxonil was superior to application of TBZ at harvest alone. Three yeast and one bacterial biocontrol agents reduced decay at pear wounds inoculated with *P. expansum* up to 14 d after inoculation with *P. expansum*, but were ineffective when applied at 28 d after inoculation. Of possible sequential arrangements of fungicide and biocontrol treatments, application of the most effective material promptly after harvest generally resulted in the highest level of decay control.

Keywords: Postharvest management; *Pyrus communis*; *Rodotorula glutinis*; *Cryptococcus infirmo-miniatum*; *Cryptococcus laurentii*; *Pseudomonas syringae*; Bio-Save 110

1. Introduction

Decay of pear fruit during long-term storage, caused by any of several fungal pathogens, can result in significant economic losses for pear producers (Kupferman, 1998). In order to reduce incidence of postharvest decay, fungicides or biocontrol agents may be applied to the fruit after harvest (Chand-Goyal and Spotts, 1997; Eckert and Sommer, 1967; Roberts, 1994). For most of the past three decades, most pears packed for long-term storage in the United States were treated with a benzimidazole fungicide (benomyl or thiabendazole [TBZ]). Recently, pyrimethanil and fludioxonil have been registered for postharvest application to pears in the United States and elsewhere, and have been shown to be effective against pear decay (Errampalli, 2003; Vostermans et al., 2005).

Harvest of pears takes place during a relatively narrow range of fruit maturity, followed by prompt cooling to remove field heat (Hansen and Mellenthin, 1979). Among the varying methods of postharvest handling employed by commercial operators, opportunities for application of decay control treatments typically occur (1) before fruit are placed in long-term storage, either as high-volume recirculating "drenches" while the fruit are in field bins or as in-line spray treatments during pre-storage sorting and sizing and (2) as in-line spray treatments immediately before fruit are packed into the boxes in which they are marketed. Many commercial pear operations store pears for extended periods in field bins because the large volume of pears harvested in the maturity period may require several months for sorting and packing to be completed, and because of uncertainty regarding the type of packaging that will be needed to fill specific market demands at the time of sale (E.A. Kupferman, personal communication). For various reasons, pears are often stored in field bins without postharvest fungicide treatment, receiving decay control treatment only as an in-line spray before final packing. These reasons include avoiding drench applications to minimize risk of accumulating spores of pathogens, especially of *Penicillium expansum*, washed from the surface of the fruit (Fidler et al., 1973), and avoiding pre-storage sizing to minimize risk of bruis-

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ing and surface injury during handling. Increased incidence of decay caused by *P. expansum* and by *Mucor piriformis* have been associated with pre-storage drenching in field bins (Sanderson et al., 1998; Xiao et al., 2004).

Since decay is often initiated at small wounds that occur during harvest and handling (Spotts et al., 1998), the timing of applications of postharvest decay control materials should be critical to successful decay control. The objectives of this study were to: (1) characterize the effects of delay in application of fungicides and biocontrol agents after harvest or after pathogen inoculation on pear postharvest decay and (2) to compare possible sequential arrangements of postharvest applications of two fungicides or a fungicide and a biocontrol agent for decay control.

2. Materials and methods

2.1. Timing of postharvest fungicide applications

2.1.1. TBZ timing with natural inoculum

At normal harvest maturity (71–62 N firmness) in 1996 and 1997, approximately 500 pears were harvested from each of five randomly arrayed mature 'Bosc' pear trees in an orchard at the Southern Oregon Research and Extension Center near Medford, and brought to the laboratory. One hundred fruit from each replicate tree were immediately treated with TBZ, applied as Mertect 340F (Syngenta Crop Protection, Greensboro, NC) at 1.25 mL L^{-1} (0.6 g active ingredient per litre applied) by spraying the fruit while traveling across a series of rotating brushes, simulating the common packinghouse treatment method. Fruit were then stored in polyethylene-lined fiberboard boxes in air at 0 °C. The remaining pears were stored at 0 °C. One hundred fruit from each replicate tree were removed from storage after 3, 6, and 9 weeks and treated with TBZ as described above. After 5 months of storage, all fruit were evaluated for incidence of decay lesions, and types of decay were identified.

2.1.2. TBZ, fludioxonil and pyrimethanil timing with artificial inoculation

In 1999, 2003 and 2004, 'Bosc' pears were harvested as described above and stored at 0 °C for 1 week. All fruit were then removed from storage and surface-disinfested by immersion in a 0.5% sodium hypochlorite solution for 2 min, then rinsed in fresh water. Each fruit was then wounded in three locations with a sterile finishing nail (2 mm diameter × 3 mm depth) and dipped in a spore suspension (1×10^7 conidia L^{-1}) of *P. expansum*. Conidia of *P. expansum* were obtained by washing the surfaces of 2-week-old colonies growing on potato dextrose agar at 20 °C, and adjusting conidial concentration in water with the aid of a hemacytometer. In 2004 the strain of *P. expansum* used was resistant to TBZ. From each of the five replicate trees, 25 fruit were wounded and treated with TBZ as described above, or with fludioxonil, applied as Scholar 50W (Syngenta Crop Protection, Greensboro, NC) at 0.6 g L^{-1} (0.3 g active ingredient per litre applied), or pyrimethanil, applied as Penbotec 400SC (Janssen Pharmaceutica, Titusville, NJ) at 2.5 mL L^{-1} (1.0 g active ingredient per litre applied). Treatments were applied to the fruit either shortly after inoculation (day 0) or at 1, 2, 7, 14 and 21 d after inoculation. All equipment was thoroughly cleaned with water between fungicide treatments. After treatment, fruit were stored in polyethylene-lined fiberboard boxes in air at 0 °C, and incidence of decay lesions at wounds was evaluated 2 months after inoculation.

2.2. Sequence of postharvest fungicide applications

The efficacy of various sequences of two postharvest treatments was compared by applying either water (control), TBZ (Mertect 340F) at 1.25 mL L^{-1}, or fludioxonil (Scholar 50W) at 0.6 g L^{-1} to 'Bosc' pears immediately after harvest followed by a second treatment with a different material at 3 weeks after harvest. In 1999 and 2000, treatments were applied to non-wounded fruit without artificial inoculation; in 2001 and 2003, each fruit was wounded immediately after harvest in three locations with a finishing nail (2 mm diameter × 3 mm depth) and dipped in a spore suspension of *P. expansum* (1×10^7 conidia L^{-1}) prior to initial treatment. All treatments were applied by spraying the fruit while traveling across a series of rotating brushes. Fruit were stored as described previously at 0 °C, and incidence of decay was evaluated after 2 months (wound-inoculated fruit) or 5 months (non-wounded fruit).

2.3. Timing of postharvest biocontrol agent applications

The yeasts *Rodotorula glutinis* and *Cryptococcus infirmo-miniatum* were obtained from R.A. Spotts, Oregon State University (Chand-Goyal and Spotts, 1997), and *Cryptococcus laurentii* was obtained from R.G. Roberts, USDA-ARS, Wenatchee WA (Roberts, 1990). The yeasts were grown on yeast malt dextrose agar (Difco) for 2 d at 20 °C, then suspended in water at concentrations adjusted to approximately 1–3 × 10^{11} cfu L^{-1} using a spectrophotometer (Sugar and Spotts, 1999). The formulated biocontrol product Bio-Save 110 (formerly Bio-Save 11) was obtained from EcoScience Corp., Worcester MA (subsequently produced by Village Farms LP, Longwood FL). This product contains the bacterium *Pseudomonas syringae* strain ESC-11 at a concentration of 9×10^{13} cfu kg^{-1} (Janisiewicz and Marchi, 1992; Jeffers and Hankinson, 1995), and was applied at 1.65 g L^{-1}.

'Bosc' pear fruit from five replicate orchard trees were surface-disinfested by immersion in a 0.5% sodium hypochlorite solution for 2 min, then rinsed in fresh water. Each fruit was then wounded in five locations with a sterile finishing nail (6 mm diameter × 3 mm depth) and inoculated by delivering 40 μL of a spore suspension of *P. expansum* (1×10^6 conidia L^{-1}) into each wound by micropipette. The conidial suspension of *P. expansum* was prepared as described above.

Immediately after inoculation with *P. expansum*, or 1, 7, 14, or 28 d after inoculation, suspensions of biocontrol agents (40 μL), at the concentrations described above, were added to the inoculated wounds. Ten fruit from each replicate (a total of 50 wounds) were used for each biocontrol agent at each application timing. Between pathogen inoculation and biocontrol treatment, and following biocontrol treatment, the fruit were stored in