Control of Bull's-Eye Rot of Apple Caused by *Neofabraea perennans* and *Neofabraea kienholzii* Using Pre- and Postharvest Fungicides

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Abstract

Bull's-eye rot is a major postharvest disease of apple caused by several fungi belonging to the *Neofabraea* and *Phlyctema* genera. Chemical control of these fungi is a crucial component of disease management for apples that are conventionally grown. The efficacy of several preharvest and postharvest applied fungicides were evaluated to identify effective chemistries that can control bull's-eye rot incited by *Neofabraea perennans* and *N. kienholzii* on apples. In general, the preharvest fungicide thiophanate-methyl was found to be effective at reducing disease caused by *N. perennans* and *N. kienholzii*.

Two postharvest fungicides, thiabendazole and pyrimethanil, also provided disease control that was far superior to other chemical compounds evaluated in this study. The efficacy of thiabendazole and pyrimethanil was unaffected by application method (fungicide dip compared with thermofog). Despite providing satisfactory control of bull's-eye rot, integration of these three chemicals into disease management programs should proceed judiciously with consideration of their impact on the development of fungicide resistance and influence on diversity in populations of apple postharvest pathogens.

The Pacific Northwest (PNW) region of the United States is a major area for pome fruit production in part due to the arid climate and well-developed irrigation network distinctive of the area (Schoetzko and Granatstein 2005). For many decades, Washington State has remained at the forefront of apple production, accounting for over half of the total annual U.S. crop (Slattery et al. 2011). As a multibillion dollar industry, apples constitute a major component of the agricultural economy for Washington State. About one third of the total apple crop produced in Washington is exported, with major consumers including Mexico, Canada, and India (Geisler 2013).

Recently, efforts to expand existing trade relations between the U.S. and The People's Republic of China were curtailed for a 2-year period due to the detection of quarantine pathogens on apple shipments originating from Washington State (WSDA 2014). Identification of postharvest pathogens causing Sphaeropsis rot (*Sphaeropsis* spp.), speck rot (*Phacidiopycnis washingtonensis*), and bull's-eye rot (*Neofabraea* spp., *Phlyctema* sp.) during the summer of 2012 resulted in a temporary suspension of apple fruit trade between the two nations (Wheat 2013). By 2014, a bilateral trade agreement was reached and access to the Chinese market was reinstated with the stipulation that Washington growers and packers abide by phytosanitary guidelines and shipment inspections to prevent further spread of postharvest pathogens and other important pests (Heping 2015; Warner 2014).

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In addition to posing a quarantine concern, bull's-eye rot has been a major postharvest disease of apples and pears grown in the PNW for over a century (Cordley 1900). The disease is attributed to fungal infections initiated by *Phlyctema vagabunda*, *Neofabraea kienholzii*, *N. malicorticis*, and *N. perennans*. While all four fungi commonly occur in the PNW, only *P. vagabunda*, *N. kienholzii*, and *N. perennans* have been documented in the major pome fruit production districts of central and eastern Washington (Spotts et al. 2009).

Bull's-eye rot is characterized by the appearance of circular, dark-brown to golden-brown lesions originating at open lenticels within the fruit cuticle. These lesions are generally firm, and display a concentric or "bull's-eye-like" pattern (Kienholz 1939; Pierson et al. 1971). Although fruit infection by *Phlyctema* and *Neofabraea* spp. is initiated in the orchard, the disease is asymptomatic at harvest and if fruit are kept under reduced temperatures, symptoms do not appear until at least 3 months into the postharvest cold storage season (Spotts 1990). Year-round orchard survival by these fungi is achieved through the initiation of cankers on pome fruit trees (Lawrence 1904; Zeller and Childs 1925). *N. malicorticis* and *N. perennans* cause anthracnose canker and perennial canker of apple trees, respectively. While *P. vagabunda* has been shown to induce superficial cankers following artificial wound inoculations of pear trees (Henriquez et al. 2006), the fungus more commonly behaves as a saprophyte of pruning stubs and dead tree branches (Bompeix 1988; Henriquez et al. 2004). By forming these associations with pome fruit trees, bull's-eye rot-causing fungi can withstand adverse environmental conditions and disseminate primary inoculum throughout the year.

Current management practices to control *Phlyctema* sp. and *Neofabraea* spp. in the orchard include pruning cankers from infected trees to minimize the buildup of inoculum during the fruit growing season, removal of thinned or fallen fruit and dead tree branches from the orchard floor, and reduced use of over-tree irrigation systems that may otherwise promote splash dispersal of conidia from sporulating cankers onto developing fruit (Creemers 2014). For apples grown under a conventional orchard production system, fungicide application is a significant component of bull's-eye rot management. Fungicides registered for pome fruit use can either be applied during the fruit growing season or after harvest prior to prolonged cold storage under a modified atmosphere. Despite the importance of bull's-eye rot as a major postharvest disease, little has been published relating to effective control of the causal fungi through application of chemical fungicides registered for pome fruit production in the U.S. (Grantina-Ilevina 2015; Henriquez et al. 2006; Spotts et al. 2009; Weber

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and Palm 2010). Therefore, the primary objectives of this study were to: (i) evaluate the efficacy of various preharvest applied fungicides for controlling bull's-eye rot of apple fruit initiated by *N. perennans* and *N. kienholzii* during specific inoculation events; (ii) evaluate the efficacy of various postharvest fungicides for controlling bull's-eye rot fruit infections caused by *N. perennans* and *N. kienholzii* initiated during specific inoculation events; and (iii) compare the efficacy of specific postharvest fungicides applied using either a dip/drench or thermofogging system for control of *N. perennans* apple fruit infections initiated late during the fruit growing season.

Materials and Methods

Orchard study sites. This study was conducted during the 2013 and 2014 fruit growing seasons on cv. Fuji apple trees planted in 2007 on a 0.4-ha plot at the Washington State University Sunrise Research Orchard located southwest of Palisades, WA (47°25'08"N, 119°54'52"W). An under-tree irrigation system was employed, and trees were pruned, thinned, and maintained for weed and insect control as per conventional commercial orchard standards typical for the PNW region (Bush et al. 2008).

Inoculum preparation. *N. perennans* isolate CLX5396 and *N. kienholzii* isolate CLX4426 were selected for use in fruit inoculations. Isolate CLX5396 was originally collected in 2007 from decayed 'Gala' apple fruit grown in East Wenatchee, WA. Isolate CLX4426 was initially cultured from decayed 'Fuji' apple fruit originating in Tonasket, WA, in 2005. The speciation of these two isolates was determined using both morphological (Dugan et al. 1993; Spotts et al. 2009; Verkley 1999) and molecular diagnostic methods (Gariépy et al. 2003; Soto-Alvear et al. 2013). Isolates were revived from 15% glycerol stocks stored at -80°C and cultured on potato dextrose agar (PDA; Difco, Becton, Dickinson and Company, Sparks, MD). Cultures were incubated at 20°C in total darkness for 14 days, transferred to fresh PDA, and incubated at 20°C without light for an additional 21 to 30 days. To prepare conidial suspensions of each isolate, 10 ml of sterile distilled water was flushed over the fungal culture and a sterile inoculation loop was applied over the mycelium to detach conidiospores into the solution. In an effort to separate conidia from agar debris and mycelial fragments, the suspension was passed through two layers of sterile cheesecloth and collected into a sterile beaker. Spore concentration of the suspension was adjusted to 5×10^5 spores/ml using a hemocytometer. The suspension was transferred into plastic spray bottles and kept on ice for no longer than 5 h.

Inoculation procedure. Due to the large volume of fruit used in this study and the amount of time needed to properly inoculate fruit, pathogen inoculations were completed on two separate days. As such, fruit were split into two groups – those to be treated with a preharvest applied fungicide and those to be treated with a postharvest applied fungicide. In general, preharvest-treated fruit were inoculated one day prior to postharvest-treated fruit.

During the 2013 growing season, two inoculation events were used: a midseason inoculation occurring 5 weeks before harvest (wbh), and a late season inoculation occurring 2 wbh. For the 2014 fruit growing season, an additional inoculation event occurring 18 wbh was included to represent early season fruit infections. In 2013, midseason fruit inoculations were conducted on 18/19 September, and late season inoculations were done on 8/9 October. For the 2014 season, the early season fruit inoculation event was completed on 9/10 June, while midseason and late season inoculations were conducted on 10/11 September and 30 September/1 October, respectively.

Inoculations were conducted within 3 h of sunset to prevent excess exposure of the spore suspension to sunlight and to avoid high daytime temperatures. Trees were selected according to a completely randomized design, with a final sample size of 20 fruit per tree (an excess of fruit were inoculated to account for incidental losses experienced throughout the growing season) and a total of four replicate trees for each treatment combination (pathogen by inoculation period by fungicide). Attached fruit were sprayed with a conidiospore suspension of either *N. perennans* or *N. kienholzii* until run-off. After the

spore suspension was applied, premoistened white plastic trash bags of different sizes (15, 30, and 49 liters depending on tree limb length) were used to cover fruit in order to shield spores from direct sunlight and to maintain a favorable microclimate for spore germination and hyphal penetration (Edney 1958). Information pertaining to temperature and relative humidity during the inoculation process was recorded by placing a WatchDog data logger (Spectrum Technologies, Inc., Aurora, IL) inside one of the plastic bags containing inoculated fruit. Bags were removed from trees after a 15-h incubation period and fruit were left attached to trees for the remainder of the growing season.

Fungicide application. Ziram 76DF (zinc dimethyldithiocarbamate) at 3.59 g per liter of water (United Phosphorus, Inc, King of Prussia, PA), thiophanate-methyl as Topsin-M WSB at 0.61 g per liter of water (United Phosphorus Inc.), and a pyraclostrobin plus boscalid mixture as Pristine at 0.55 g per liter of water (BASF Corporation, Research Triangle Park, NC) were independently applied to runoff on fruit surfaces using a powered handgun sprayer at 14 (zinc) and 2 (thiophanate-methyl; pyraclostrobin plus boscalid) days before harvest. During the 2013 trial, preharvest fungicide application dates were completed on 10 October for zinc and 21 October for both thiophanate-methyl and pyraclostrobin plus boscalid. In 2014, preharvest fungicides were applied on 3 October for zinc and 14 October for both thiophanate-methyl and pyraclostrobin plus boscalid. Application dates were determined by preharvest interval established for the respective chemistries.

The fungicides fludioxonil as Scholar SC at 1.24 ml per liter of water (Syngenta, Greensboro, NC), thiabendazole as Mertect 340-F at 1.24 ml per liter of water (Syngenta), pyrimethanil as Penbotec 400SC at 1.24 ml per liter of water (Pace International, Seattle, WA), and a difenoconazole plus fludioxonil mixture as Academy at 1.24 ml per liter of water (Syngenta) were applied to selected fruit as a postharvest drench treatment by dipping the fruit in 40 liters of a fungicide solution for 30 s within 4 h of harvest. Additionally, the postharvest fungicides fludioxonil as ecoFOG 80 at 60 g per ton of apples (Pace, International), pyrimethanil as ecoFOG 160 at 40 g per ton of apples (Pace, International), thiabendazole as ecoFOG TBZ at 80 g per ton of apples (Pace, International), and difenoconazole as an experimental formulation at 40 g per ton of apples (Pace, International) were applied as a thermofog treatment by placing fruit in a plastic crate (L60 × W40 × H29 cm) and exposing to each designated chemical for 4 h. After fogging, fruit were packed into apple boxes and held in cold storage as described for other treatments below. The difenoconazole plus fludioxonil drench and difenoconazole thermofog treatments were incorporated into this study for only a single year while all other chemical formulations were tested for two growing seasons. All preharvest- and postharvest-treated fruit were compared against control fruit that were pathogen-inoculated yet lacking fungicide treatment.

Fruit harvest and storage. In 2013, postharvest fungicide-treated fruit were harvested on 23 October while preharvest fungicide-treated fruit were harvested on 24 October. In 2014, postharvest fungicide-treated fruit were harvested on 16 October while preharvest fungicide-treated fruit were harvested on 17 October.

Preharvest fungicide-treated fruit were sorted immediately after harvest and any fruit with abnormalities or wounds were discarded so that a minimum sample size of 20 fruit per replicate was achieved. Fruit were then transferred to sterilized fiberboard apple trays and placed inside cardboard apple boxes lined with perforated polyethylene bags. Fruit destined for postharvest treatment were sorted immediately after harvest and treated with postharvest fungicides following sorting. After treating fruit with the assigned drench or thermofog chemical, fruit were packed onto fiberboard trays in cardboard boxes as described above. All fruit were stored at 0°C under regular atmosphere for up to 9 months.

Fruit evaluations. Fruit were visually inspected for symptoms of bull's-eye rot beginning on the third month of cold storage and continuing monthly until the ninth month of storage. Tissue isolations were conducted from decayed fruit to identify the causal pathogen inducing these symptoms. To achieve this, symptomatic tissue was

removed from the margin of lesions using a sterile scalpel and incubated on PDA at 20°C for approximately 10 days. Typical morphological characteristics of *N. perennans* and *N. kienholzii* colonies growing in culture were used to identify whether symptomatic fruit were due to bull's-eye rot (Dugan et al. 1993; Spotts et al. 2009). Incidence of bull's-eye rot was recorded for each treatment.

Data analysis. Disease incidence data were analyzed according to a generalized linear model using SAS PROC GENMOD (version 9.4). Separate analyses were conducted for each treatment combination (pathogen inoculum by fungicide application type by inoculation event). Means separation was conducted using the Tukey-Kramer method ($P \leq 0.05$).

Results

Preharvest fungicides. During the 2013 trial, average disease incidence for *N. perennans* midseason-inoculated fruit was 63%, and 78% for the no-treatment control following late season fruit inoculations (Fig. 1A). Only fruit treated with Topsin-M demonstrated a significant reduction in disease relative to the control

($P < 0.0001$), Pristine ($P < 0.0001$), or Ziram ($P < 0.0001$) treatments, irrespective of when in the growing season inoculations were conducted.

When fruit were inoculated with *N. kienholzii*, average disease incidence for control fruit inoculated midseason was 53%, which increased to 88% when inoculations were conducted later in the season (Fig. 1A). Disease incidence was significantly reduced when fruit inoculated during the middle ($P \leq 0.0002$) and end ($P < 0.0001$) of the growing season were treated with Topsin-M compared with the remaining fungicide treatments. Additionally, late season-inoculated fruit treated with Ziram exhibited a significant reduction in disease incidence relative to the control ($P = 0.0037$) or Pristine ($P = 0.0019$) treatments.

During the 2014 trial, average disease incidence for early, mid-, and late season *N. perennans* inoculations was 17, 31, and 68%, respectively, for the no-treatment control (Fig. 1B). There was no significant difference in disease incidence for any of the fungicide treatments on fruit representing early or midseason fruit infections. However, when fruit were inoculated late in the growing season,

Fig 1. Influence of preharvest fungicide application on incidence of bull's-eye rot detected on fruit when inoculated with *Neofabraea perennans* and *N. kienholzii* at 5 and 2 weeks before harvest during the 2013 (A) and 2014 (B) fruit growing seasons. The Tukey-Kramer method was utilized to identify significant differences among fungicide treatments. Means designated with the same letter are not significantly different ($P < 0.05$). Error bars represent the standard error of the mean.

Topsin-M significantly reduced disease by 40% relative to the control treatment ($P < 0.0001$). Additionally, disease incidence for fruit treated with Topsin-M was significantly lower than those treated with Pristine ($P < 0.0001$) or Ziram ($P = 0.0101$). Compared with the control, a significant reduction in disease incidence was also observed when fruit were treated with Ziram ($P = 0.0010$). Disease incidence following Ziram treatment, however, was not significantly different from the Pristine treatment ($P = 0.1219$).

Average disease incidence for the no-treatment control when fruit were inoculated with *N. kienholzii* was 35% following early season inoculations, 13% for midseason inoculations, and 50% for late season inoculations (Fig. 1B). Compared with the control from the early season fruit inoculation event, disease incidence was significantly reduced following treatment with Ziram ($P = 0.0019$) and Topsin-M ($P = 0.0069$). However, none of the fungicides significantly reduced bull's-eye rot following the midseason fruit inoculation event. When disease incidence was compared for fruit inoculated late in the growing season, only Topsin-M significantly reduced disease relative to the control ($P < 0.0001$) and relative to all other fungicide treatments ($P \leq 0.0007$).

Postharvest fungicides. During the 2013 trial, *N. perennans* disease incidence for the control was measured at 61% for fruit inoculated midseason, and 60% when fruit were inoculated late in the growing season (Fig. 2A). Fungicide applications of Penbotec on fruit inoculated during the middle of the growing season exhibited a significant reduction in disease relative to the control ($P < 0.0001$), but this reduction was not observed on fruit inoculated late in the

season ($P = 1.0$). Additionally, Mertect application resulted in a significant reduction in disease relative to the control for both mid ($P < 0.0001$) and late season ($P < 0.0001$) fruit inoculations. Thermofog applications of pyrimethanil ($P = 0.0060$) and thiabendazole ($P < 0.0001$) were equally effective at reducing disease initiated during the late season inoculation event.

In the case of *N. kienholzii*, disease symptoms developed on 51% of control fruit inoculated during the middle of the season, whereas disease incidence was 88% when inoculations were conducted late in the season (Fig. 2A). Both Penbotec ($P < 0.0001$) and Mertect ($P < 0.0001$) significantly reduced disease incidence irrespective of the inoculation event.

During the 2014 trial, disease incidence due to *N. perennans* inoculations conducted early in the season was 17% for the no-treatment control. When inoculations were conducted midseason, incidence was 48%, and 70% following late season inoculations (Fig. 2B). None of the fungicides significantly reduced disease incidence on fruit that were inoculated early in the growing season. However, a significant reduction in disease incidence was observed in fruit treated with Penbotec and Mertect relative to the midseason (Penbotec, $P = 0.0005$; Mertect, $P < 0.0001$) and late-season inoculation controls (Penbotec, $P < 0.0001$; Mertect, $P < 0.0001$). Additionally, Academy significantly reduced disease when fruit were inoculated during midseason ($P = 0.0183$) but not when fruit were inoculated late in the season ($P = 0.4178$). Scholar also significantly reduced disease incidence on fruit inoculated late in the season ($P < 0.0001$), but was ineffective on fruit inoculated midseason ($P = 0.3994$).

Fig 2. Influence of postharvest fungicide application on incidence of bull's-eye rot detected on fruit when inoculated with *Neofabraea perennans* and *N. kienholzii* at 5 and 2 weeks before harvest during the 2013 (A) and 2014 (B) growing seasons. The Tukey-Kramer method was utilized to identify significant differences among fungicide treatments. Means designated with the same letter are not significantly different ($P < 0.05$). Error bars represent the standard error of the mean.

Thermofog applications of pyrimethanil ($P < 0.0001$), thiabendazole ($P < 0.0001$), and difenoconazole ($P = 0.0196$) also significantly reduced the level of disease initiated at the late season inoculation event.

Average disease incidence due to *N. kienholzii* inoculations early, mid-, and late season was 26, 21, and 60%, respectively, for the no-treatment control. Regardless of inoculation event, only thiabendazole ($P \leq 0.0114$) and pyrimethanil ($P \leq 0.0277$) treatments were effective at significantly reducing disease incidence relative to the controls (Fig. 2B).

Discussion

Neofabraea spp. induced development of postharvest bull's-eye rot when fruit were artificially inoculated at three different inoculation events representing early, mid-, and late season fruit infection. In general, disease incidence increased as inoculations were conducted closer to the fruit harvest period, possibly reflecting fluctuations in host susceptibility as a factor of apple fruit maturity (Xu and Robinson 2010). While the efficacy of the different fungicides tested in this study varied slightly depending on the influence of certain experimental variables and the year the study was conducted, in general, when disease pressure was high, Topsin-M (thiophanate-methyl) was the most effective preharvest fungicide for control of bull's-eye rot. Mean disease incidence for fruit treated with this fungicide ranged from 7.5 to 27.5%, compared with 14.9 to 88.5% in the preharvest untreated controls. Additionally, Mertect (thiabendazole) and Penbotec (pyrimethanil) were the most effective postharvest fungicides in reducing the incidence of bull's-eye rot. When applied as a drench, disease incidence ranged from 0 to 28.0% and 1.0 to 61.3% on fruit treated with Mertect and Penbotec, respectively, compared with 17.5 to 89.0% in the postharvest untreated controls. Fruit treated with Pristine (pyraclostrobin plus boscalid), Scholar (fludioxonil), and Academy (difenoconazole plus fludioxonil) possessed the highest disease incidence ratings, occasionally having levels of disease that were greater than the untreated control. Typically, disease incidence following Ziram (zinc) application was as high as the control, but on certain rare occasions, bull's-eye rot was significantly reduced by this preharvest fungicide (for example, *N. kienholzii* late season fruit inoculations for the 2013 growing season and fruit inoculated with *N. perennans* at 2 whb during the 2014 growing season). However, the lack of year-to-year or pathogen-to-pathogen consistency in these trials demonstrates that Ziram may not be reliable for use in the control of bull's-eye rot.

While Topsin-M and Mertect provided significant control of bull's-eye rot, both products belong to the methyl benzimidazole carbamate (MBC) group of fungicides. These chemistries target a single mode of action involved with interference of beta-tubulin assembly during mitosis. As such, these fungicides have been deemed high risk for development of resistance in fungal populations by the Fungicide Resistance Action Committee (FRAC). In pome fruit production districts of northern Germany where bull's-eye rot is common, multiple isolates of *N. perennans* and *P. vagabunda* were found to demonstrate varying degrees of resistance to thiophanate-methyl (Weber and Palm 2010). In order to ensure the long-term efficacy of these fungicides in the PNW, integration of these fungicides into pest management programs should continue with judicious consideration of fungicide resistance. To accomplish this, one must ensure that fungicides are applied according to manufacturer recommendations, that label rates are being adhered to, and that spray coverage is optimal. Furthermore, fungicides sharing the same mode of action should not be applied to the same fruit prior to or after harvest during the same growing season. Alternating between fungicides targeting different modes of action can help reduce the risk of resistance development. In pome fruit packinghouses of the PNW, repeated and excessive use of benzimidazole and anilino-pyrimidine fungicides in the past has favored selection of fungicide resistant strains within populations of *Penicillium expansum* (Caiazzo et al. 2014; Li and Xiao 2008) and *Botrytis cinerea* (Zhao et al. 2010). While these fungi exhibit shorter life cycles and higher rates of reproduction and sporulation in comparison with *Neofabraea* spp., the potential for

fungicide resistance in pathogen populations causing bull's-eye rot exists. Fortunately, implementation of fungicide rotation programs in recent years has helped alleviate selection pressure on resistant strains of *Penicillium* and *Botrytis* and presumably could also be applied to minimize the risk of fungicide resistance development in *Neofabraea* species as well.

During the course of this study, the fungicides Luna Sensation (fluopyram plus trifloxystrobin; Bayer CropScience), Aprovia (benzovindiflupyr; Syngenta), and Inspire Super (difenoconazole plus cyprodinil; Syngenta) were registered for use in pome fruit production. Trifloxystrobin and fluopyram belong to the quinone outside inhibitor (QoI) and succinate dehydrogenase inhibitors (SDHI) group of fungicides, respectively. In the present study, the premixture pyraclostrobin plus boscalid was shown to provide inadequate control of bull's-eye rot. Pyraclostrobin and boscalid also belong to the QoI and SDHI group of fungicides, respectively. While it would be unwise to make predictions as to the efficacy of this newly registered fungicide, cross resistance between fungicides belonging to the QoI group has been demonstrated (Gisi et al. 2002). Given that little information exists regarding bull's-eye rot control with these newly registered fungicides, studies evaluating baseline sensitivities of *Phlyctema* and *Neofabraea* spp. to these chemistries should serve the apple industry well, and improve spray programs currently implemented by pome fruit growers of the PNW.

Results from the present study indicate that a single application of thiophanate-methyl during the final weeks prior to harvest, or a single application of thiabendazole or pyrimethanil immediately following harvest, may be sufficient to mitigate early, mid-, and late season fruit infections. However, since *Neofabraea* spp. are considered quarantine pests in certain countries abroad, this type of spray program may not be sufficiently stringent for fruit intended for international export. Thus, integrated pest management strategies that incorporate fungicide spray programs are highly encouraged in order to minimize disease risk caused by bull's-eye rot organisms. This entails pruning and destroying twig and branch cankers harboring *Neofabraea* spp. from fruit trees so as to minimize primary inoculum available in the orchard, practicing proper orchard sanitation techniques that minimize the amount of plant debris left decomposing on the orchard floor (Grove et al. 1992), and reducing the use of overhead sprinklers or evaporative cooling, especially during the final weeks prior to harvest, as this may create a favorable microclimate encouraging infection by *Neofabraea* spp. (Henriquez et al. 2008).

Thermofog application of fungicides were shown to provide similar efficacy against bull's-eye rot compared with drench applications of the same compound. Fog application of postharvest fungicides may be advantageous over conventional drench systems in that less chemical waste is accumulated. However, it is important to note that fungicide distribution using thermofog application can be affected by many factors including air circulation and temperature within the cold storage room at the time of fungicide application (Delele et al. 2012), and in the past, this has been shown to affect fungicide residue levels on fruit (Bertolini et al. 1995). While many improvements have been made in recent years to help perfect chemical application via thermofogging, packers should be cognizant of potential issues that can be encountered during the application process should storage conditions in cold rooms not be precisely monitored.

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