Annual Report - 2007

Prepared for the California Pear Board

Project Title:	Evaluation of Postharvest Treatments for Management of Gray Mold, Blue Mold, and other Decays of Stored Pears in California
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MAIN ACHIEVEMENTS IN 2007 RESEARCH

1. Experimental packingline studies were conducted on the management of postharvest decays of pears to identify new fungicides that could be used in mixtures with registered fungicides to increase treatment efficacy and to help in the prevention of resistance development in pathogen populations.
2. The two SBI fungicides difenoconazole and propiconazole had little or no effect on preventing gray mold decay of inoculated Bartlett and Comice pears. These fungicides, however, were generally similarly effective against blue mold as compared with Scholar or Scala (Penbotec), but efficacy was rate-dependent.
3. As in previous years' trials, low-volume spray applications were generally less effective than in-line drench applications.
4. Mixtures of the SBIs with Scholar were similarly effective as single-fungicide treatments, thus, no additive effect was observed. Still, a registration of difenoconazole or propiconazole would make new mixture components available that could be used in anti-resistance management of postharvest pear pathogens.
5. IR-4 residue studies with either SBI fungicide were approved and will be conducted in 2008. Because propiconazole (Mentor 50WP) has already been registered as a postharvest treatment on stone fruits in the US, this fungicide may be given priority. In addition, for difenoconazole, two formulations (an EC and a WDG) were evaluated but a third postharvest formulation (an SC formulation) is being developed and was not available in the summer. Lastly, Elevate/Judge was federally registered for postharvest use on pears in 2007.

INTRODUCTION

Gray mold, caused by *Botrytis cinerea,* and blue mold, caused mainly by *Penicillium expansum* in addition to some less common species of *Penicillium*, are the most important storage diseases of pears in California. Other decays that may cause significant losses include Alternaria, Phomopsis, Rhizopus, and Mucor rots. Gray mold infections generally start at the stem end that is cut at harvest and becomes contaminated by the omnipresent spores of the pathogen. On Bartlett pears, calyx end-rot caused by *B. cinerea* is common that starts from infections during bloom. Additional entry points for all pathogens are wounds that are caused by abiotic or biotic agents before or during harvest. While some postharvest decay fungi like *Rhizopus* species are suppressed at storage temperatures of 0°C (32°F), *B. cinerea* and *P. expansum* will still grow, although slowly. Thus, additional chemical treatments are needed. Our preharvest studies with ziram and captan gave inconsistent and generally unsatisfactory results as stand-alone treatments for postharvest decay management. Preharvest treatments with Elevate generally significantly reduced the incidence of postharvest gray mold decay, but efficacy was not as high as when used in postharvest treatments. Until the recent registration of Penbotec and Scholar, only thiabendazole and captan (Captan 50WP) were available for postharvest use on pears. New fungicides were developed by us and others because resistance against TBZ (Mertect 340F) is wide-spread among the pome fruit pathogens *B. cinerea* and *P. expansum* and captan at the

registered postharvest rate of 2 lb/200,000 lb is not very effective in reducing the incidence of decay. In our evaluations, the biological control agent Bio-Save was inconsistent and was never as effective as Elevate/Judge for gray mold or the fungicides Scholar or Penbotec for decays caused by *Penicillium* and *Botrytis* spp. The latter two fungicides were registered in California in 2005, whereas Elevate/Judge was federally registered in 2007. Scholar and Penbotec are effective against TBZ-resistant isolates of *B. cinerea* and *P. expansum*, they belong to different classes, and they are classified as 'reduced-risk' by the US-EPA. Although five fungicides (Captan, TBZ, Scholar, Penbotec, Elevate/Judge) are now registered for postharvest use on pears, in 2007 we continued our evaluation of new materials. Our goal is to have several new fungicides with different modes of action registered for postharvest use on pear in order to be able to design resistance management strategies with fungicide mixtures and rotations. This is important due to the known risk of resistance development in the postharvest pear pathogens, similar to Penicillium decays of citrus fruit. Both commodities have in common that they are stored for extended periods of time and often receive more than one postharvest treatment, leading to an increased selection pressure in the pathogen populations. Thus, in order to prevent decay control failures, several chemical classes have to be available and applications have to be conducted using economic and efficacious techniques.

The sterol biosynthesis inhibiting fungicide propiconazole (Mentor 45WP) in recent years received emergency registrations on stone fruit for postharvest control of sour rot, and this fungicide will receive a full registration in the coming years. On stone fruit, Mentor also has some activity against gray mold and Rhizopus rot. This fungicide is also considered for registration on citrus fruit to control sour rot and Penicillium decays. Thus, because Mentor is very effective against Penicillium decays, has some activity against gray mold, and its registrant supported the evaluation for postharvest use on pears, we evaluated Mentor in our 2007 studies. Difenoconazole was suggested as an alternative SBI fungicide by the registrant of Mentor to be evaluated on pome fruits. Therefore, the comparative evaluation of these two SBI fungicides was a focus of our postharvest studies on pears in 2007. Furthermore, we conducted additional studies on the use of preharvest fungicide treatments for postharvest decay control.

Objectives

1) Evaluation of pre-harvest (fenhexamid – Elevate, cyprodinil – Vangard, pyrimethanil - Scala) and postharvest (fenhexamid – Judge/Elevate, fludioxonil - Scholar, and pyrimethanil - Penbotec) reduced-risk fungicides for postharvest management of gray mold and blue mold. TBZ-sensitive, and -resistant isolates of the pathogens will be used in inoculations and natural incidence of decay will be evaluated.
    i. Preharvest fungicide treatments (e.g., Elevate, Vangard, etc.) for postharvest decay control.
    ii. Experimental packing line treatments with postharvest fungicides.
    iii. Large-scale packinghouse studies with postharvest fungicides.
2) Evaluation of captan, chlorine, and acidified hydrogen peroxide as sanitizers of fungicide drench solutions or other water tank systems (e.g., float tanks).
3) Identify species of *Penicillium* causing decay of pears using our newly developed molecular techniques.

MATERIALS AND METHODS

Evaluate preharvest applications of new fungicides for postharvest disease management. Field trials were conducted on Shinko (2006-07) and Bosc (2007-08) pear in commercial orchards. Fungicides and their rates that were evaluated included Scala 600SC (pyrimethanil – 18 fl oz/A), Vangard 75WG (cyprodinil - 10 oz/A), Pristine 38WG (boscalid-pyraclostrobin - 0.92 lb/A), and Elevate 50WDG (fenhexamid – 1.5 lb/A. Applications were done at 7, 7 and 1, or 1 day PHI for selected treatments. Four replications of each fungicide were applied in a completely randomized design using an air-blast sprayer (100 gal/A). To evaluate the efficacy of preharvest treatments for control of natural incidence of postharvest fruit decay, ca. 100 Shinko fruit per replication were dumped into pear float tanks in a packinghouse, rinsed with water, and stored at 1 C under commercial conditions for 6 months. Bosc pear fruit were put into commercial storage without the float tank treatment.

Efficacy of postharvest treatments and application methods using single fungicides and mixtures. The efficacies of propiconazole (Mentor 45WP) and difenoconazole (Inspire 250EC or Bogard 10WG) were evaluated using different rates and in mixtures with Scholar and were compared to treatments with Scholar and Penbotec (Scala). Bartlett or Comice pears were wound-inoculated with TBZ-resistant isolates of *B. cinerea* or *P. expansum*, incubated for 12-15 h, and then treated with fungicides. Fungicides were applied on an experimental packingline at the Kearney AgCenter as aqueous solutions or in a carnauba fruit coating using a low-volume spray system (CDA). In in-line drench applications, fungicides were applied as aqueous solutions that were followed by low-volume spray applications with fruit coating. After treatment, fruit were stored at 20 C, 95% RH for 6 to 8 days and then evaluated for the incidence and severity of decay. Data were analyzed using analysis of variance and least significant difference mean separation procedures of SAS 9.1.

RESULTS AND DISCUSSION OF 2007 RESEARCH

Evaluation of preharvest fungicide applications for postharvest decay control. Preharvest treatments for control of natural incidence of postharvest decays were conducted on cv. Shinko and Bosc pears. Fruit from the cv. Shinko fruit developed a very low (<1%) incidence of gray mold and (0%) blue mold decay and thus, no efficacy data for the fungicide treatments was obtained. Fruit from the 2007 trial on Bosc pear are still being incubated and thus, data are pending. Elevate was registered as a preharvest use treatment for postharvest decay control in 2007. Due to additional feeding study requirements on apple and the preharvest registration on pears, fenhexamid will not be pursued as a postharvest treatment.

Efficacy of postharvest treatments and application methods using single fungicides and mixtures. Six experimental packingline studies were conduced to identify mixture and rotation materials for registered fungicides and to compare postharvest application methods. The SBI fungicide difenoconazole was used as an EC formulation (that was designed for field use - Inspire) and as a WG formulation (that would be more acceptable for postharvest use - Bogard), whereas the SBI propiconazole was used as a WP formulation (Mentor 50WP) that has been used for postharvest treatments of stone fruit in recent years following emergency registrations.

After in-line drench applications of inoculated Bartlett pears, rates of Inspire between 125 and 500 ppm significantly reduced gray mold decay of from that of the control, but the level of control (up to a 40% reduction of decay) was not satisfactory (Fig. 1). Mentor at a rate of 256 ppm was more effective than any rate of Inspire used and decay incidence was reduced by over 70% in this trial as compared to the untreated control. Both fungicides were highly effective against blue mold decay and Inspire at 500 ppm provided the same control level as Mentor at 256 ppm. In another in-line drench application trial on Bartlett pear, Inspire, Bogard, and Mentor were all ineffective in reducing gray mold decay, whereas Scholar at 300 ppm reduced the decay by more than 80% from the control (Fig. 2). For blue mold control, using approximately equivalent rates (250-256 ppm), Inspire was significantly more effective than Bogard, whereas Mentor was numerically, but not significantly, more effective than Inspire. Decay incidence using this rate was 61.1%, 25.0%, and 7.0% for Bogard, Inspire, and Mentor, respectively, as compared to 100% in the control. Using Scholar at 300 ppm, no blue mold decay was observed. In another comparative study on Comice pear, the SBI fungicides again were ineffective in reducing gray mold decay, in contrast to Scholar that was again highly effective (Fig. 3). For blue mold control, Inspire (2.8% decay) was significantly more effective than Bogard (27.5% decay). There was 12.5% decay incidence for Mentor as compared to the control with 100% and Scholar with 0%. Based on these three studies, gray mold cannot be managed by the SBI fungicides evaluated. For blue mold decay control, there was a trend for Mentor to be more effective than either formulation of difenoconazole. The EC formulation of difenoconazole was more effective that the WG formulation.

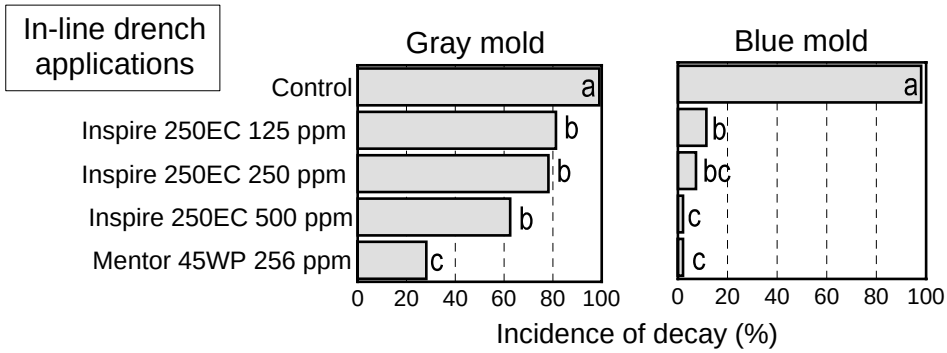
In another trial on Bartlett pear using low-volume fungicide spray applications, a mixture of Inspire with Scholar resulted in similar incidences of gray mold as when Scholar was used alone at the same rate or when Scala (Penbotec) was applied (Fig. 4). For blue mold decay control, the Inspire-Scholar mixture treatment was significantly more effective than Inspire or Scholar alone and was also more effective than Scala or Mentor. Overall, control levels for blue mold decay were not very high after these fungicide spray applications on mature fruit that ripened during incubation at 20 C for 6-7 days.

In two additional comparative postharvest studies on Bartlett and Comice pear using in-line drench and low-volume spray applications, similar results were obtained as in the first four trials. There was some reduction in gray mold incidence using Mentor at rates of 512 ppm (Figs. 5,6). In comparisons of Scholar and Scala (Penbotec), generally similar levels of gray mold control were obtained, but blue mold reduction was significantly better when using Scholar as compared to Scala. Blue mold was very effectively controlled on Comice pear by in-line drench applications with Mentor at the 512-ppm rate (Fig. 6). As we reported previously, treatments were more effective using in-line drench applications as compared to low-volume spray applications. Decay levels on the Bartlett pears (Fig. 5), however, were relatively high using all treatments and application methods because fruit used in the experiment were very mature and thus, highly susceptible to decay. Mixtures of Scholar with Mentor that were evaluated in the spray applications, did not improve the efficacy of Scholar, however, lower rates were used for each fungicide in the mixture. This may provide a more cost effective product.

In summary, in these postharvest studies we identified new, highly effective treatments for control of postharvest blue mold decay of pears. There was no consistent ranking for propiconazole and difenoconazole, although there was a trend for propiconazole (Mentor) to be the more effective material. Still, because efficacy also depends on the formulation used, extensive further studies will have to be conducted. The registrant will provide new formulations of difenoconazole for future experiments. An SC formulation of difenoconazole that is compatible with the 230SC formulation of Scholar (fludioxonil) would be ideal. In addition to efficacy, the decision on which of the two SBI fungicides will be finally pursued for a postharvest registration on pome fruits will also depend on risk cup analysis. IR-4 residue studies were approved in September 2007 for difenoconazole, but this fungicide could be switched to propiconazole if considerations regarding efficacy, formulation, and risk cup are more favorable for the latter compound. A mix partner for Scholar, Penbotec, or Elevate/Judge will be of critical importance in preventing resistance. The advantage that Syngenta Crop Protection may have is that they are the registrant for propiconazole, difenoconazole, and fludioxonil and they potentially could offer a pre-mixture for resistance management against *Penicillium* species. This is the strategy that we are developing with other crops (e.g., stone fruit – Scholar and Mentor; citrus – Graduate, Mentor, and Diploma). We originally were planning to develop azoxystrobin for postharvest use on pome fruit but phytotoxicity on some pome fruit varieties prevented us from pursuing this strategy. Thus, development of difenoconazole or propiconazole for pome fruit is an opportunity that we need to follow.

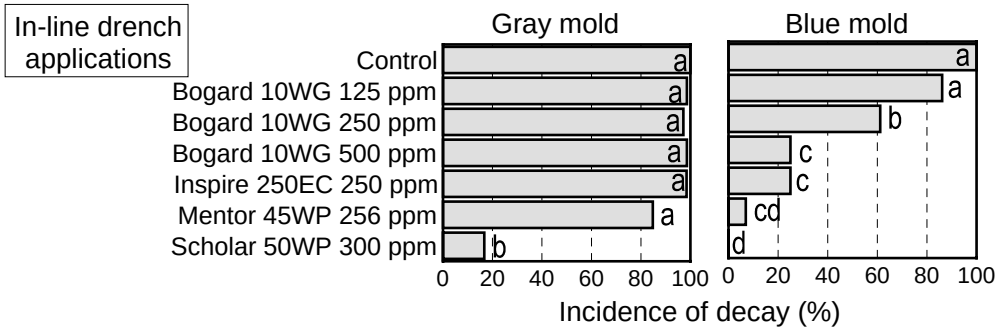
Although the SBI fungicides were poorly or not effective against gray mold and generally did not provide additive effect in blue mold control when they were used in mixtures with Scholar, as compared to using Scholar alone, registration of any one of the two SBI materials will be an important tool to decrease the risk of fungicide resistance to develop in populations of *Penicillium* spp. Currently, two new postharvest fungicides are registered on pear that effectively manage blue mold and gray mold decay caused by TBZ-resistant isolates of the pathogens. These fungicides are Scholar and Penbotec (Syngenta Crop Protection and Janssen Pharmaceutical are the registrants, respectively). Elevate/Judge (fenhexamid – Arysta LifeSciences) was registered federally in 2007 and the fungicide will be registered in California for the 2008 season. Fenhexamid, however, is only effective against gray mold. The availability of another material will provide an additional option for postharvest fruit treatment for decays caused by *Penicillium* spp. and other pathogens such as Bull's eye rot. Ideally, two active ingredients should be used or marketed in pre-mixtures so that with every application, there is a reduced pressure for resistant individuals to be selected.

Fig. 1. Evaluation of new postharvest treatments for management of postharvest decay of Bartlett pears in experimental packingline studies



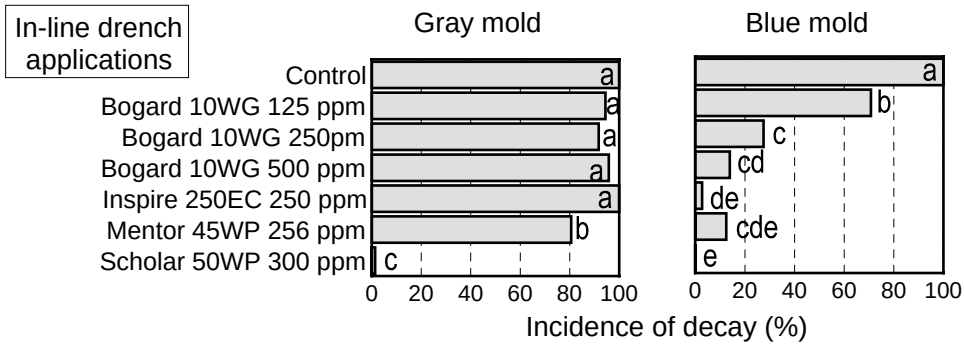
Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 13 h at 20C and treated. In-line re-circulating drench applications were done with aqueous fungicide solutions that were followed by a CDA application with carnauba fruit coating (Primafresh 45). Fruit were then incubated at 20C for 6 days.

Fig. 2. Evaluation of new postharvest treatments for management of postharvest decay of Bartlett pears in experimental packingline studies



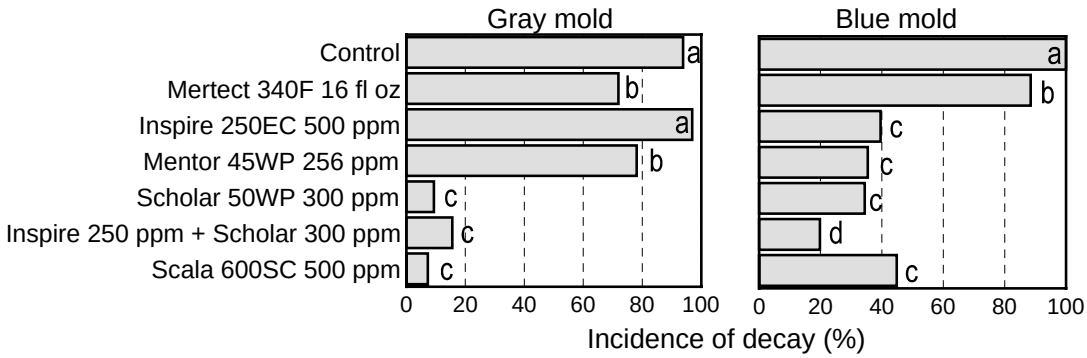
Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 12-15 h at 20C and treated. In-line re-circulating drench applications were done with aqueous fungicide solutions that were followed by a CDA application with carnauba fruit coating (Primafresh Ultra). Fruit were then incubated at 20 C for 6 days.

Fig. 3. Evaluation of new postharvest treatments for management of postharvest decay of Comice pears in experimental packingline studies



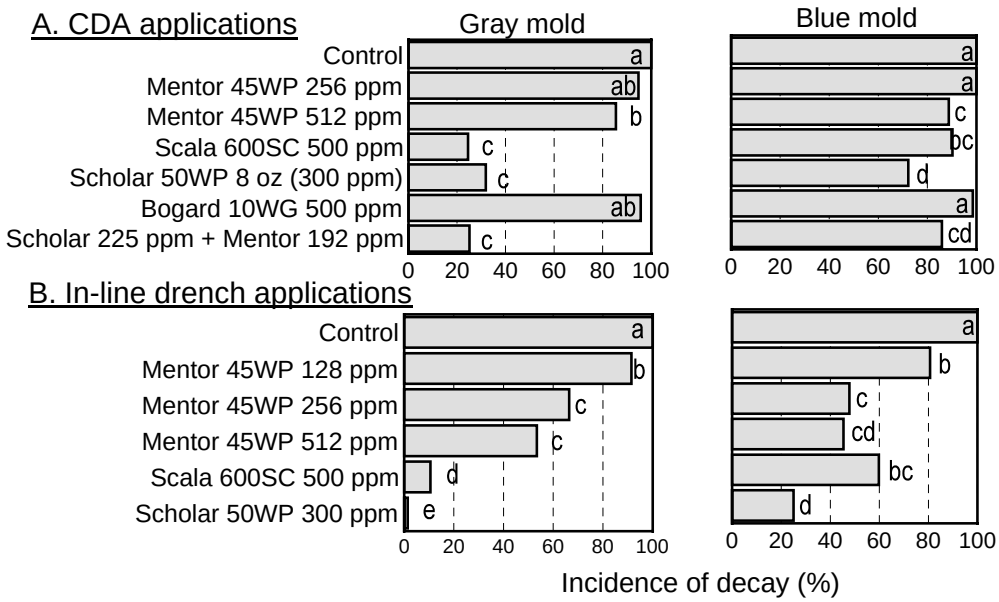
Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 12-15 h at 20C and treated. In-line re-circulating drench applications were done with aqueous fungicide solutions that were followed by a CDA application with carnauba fruit coating (Primafresh Ultra). Fruit were then incubated at 20 C for 6 days.

Fig. 4. Evaluation of new postharvest treatments for management of postharvest decay of Bartlett pears in experimental packingline studies
- CDA applications -



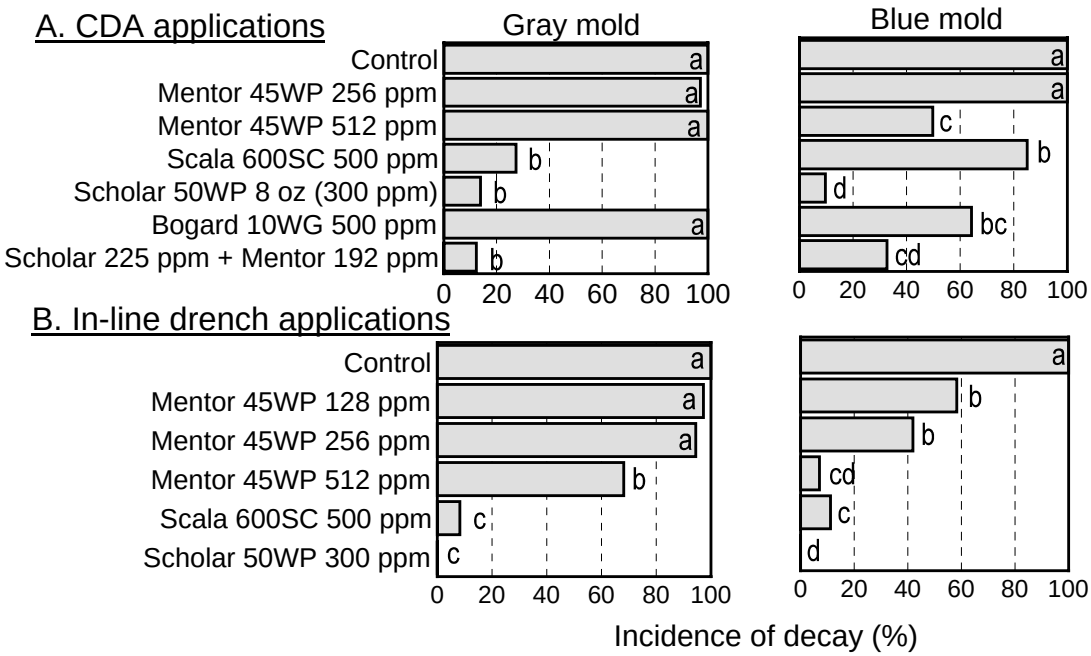
Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 13 h at 20C and treated. CDA applications at 25 gal/200,000 lb were done in a carnauba fruit coating (Primafresh 45). Fruit were then incubated at 20C for 6 days.

Fig. 5. Evaluation of new postharvest treatments for management of postharvest decay of Bartlett pears in experimental packingline studies



Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 12-15 h at 20C and treated. Fungicides for both application methods were applied as aqueous solutions that were followed by a CDA application with carnauba fruit coating. Fruit were then incubated at 20 C for 6 days.

Fig. 6. Evaluation of new postharvest treatments for management of postharvest decay of Comice pears in experimental packingline studies



Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 12-15 h at 20C and treated. Fungicides for both application methods were applied as aqueous solutions that were followed by a CDA application with carnauba fruit coating. Fruit were then incubated at 20 C for 6 days.