# Annual Report - 2003

*Prepared for the California Pear Board*

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| 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 2003 RESEARCH

1. In commercial packingline studies using inoculated and non-inoculated pear fruit, the high efficacy of two new fungicides pyrimethanil (Penbotec) and fludioxonil (Scholar) against both postharvest gray mold and blue mold and fenhexamid (Elevate) against gray mold of pear as shown in our previous studies was confirmed. Results of the new fungicide pyraclostrobin+boscalid (Pristine) are pending.
2. The high efficacy of these fungicides itemized in No. 1 above was demonstrated using TBZ-sensitive and -resistant isolates of the pathogens, thus showing no cross resistance between fungicide classes. Furthermore, collections of isolates of *P. expansum* and *B. cinerea* demonstrated the common presence of TBZ-resistance in populations of each decay fungus in California packinghouses.
3. In preharvest studies where the fungus was wound-inoculated after fungicide application, ziram had no effect on the incidence of gray mold and blue mold. Treatments with Scala (pyrimethanil) and Vangard (cyprodinil) reduced the decays from 100% in the control to 68-87%.
4. In preharvest studies with non-inoculated fruit (natural incidence), Ziram significantly reduced the natural incidence of blue mold, whereas only a trend in the reduction of gray mold was observed. Preharvest applications (8 and 0-day PHI) of Scala (pyrimethanil) and Vangard (cyprodonil), two anilinopyrimidine fungicides, were also effective in reducing gray mold decay postharvest; whereas Scala also controlled blue mold.

## INTRODUCTION

Gray mold, caused by *Botrytis cinerea,* and blue mold, caused by *Penicillium expansum*, are the most important storage diseases of pears in California. Other decays that may cause significant losses include Alternaria, Phomopsis, Rhizopus, and Mucor rots. Infections commonly 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. Currently, Ziram is registered as a preharvest treatment on pears with a 5-day preharvest interval. Thiabendazole (Mertect 340F) and captan (Captan 50WP) are the only fungicides registered for postharvest use on pears. In our postharvest studies in 2001 using ziram (8 lb/200,000 lb) or captan (2 lb/200,000 lb), both fungicides were ineffective in reducing the incidence of gray mold and Penicillium decays in wound-inoculated fruit. The lack of efficacy of captan against both decays evaluated was probably due to the low rate (2 lb/100 gal) registered. For preharvest uses, the fungicide is commonly used at 8-10 lb/100 gal. In view of the ineffectiveness of captan at the rate evaluated for the two decays, export restrictions on the fungicide in different international markets, and the visible residues of the fungicide formulation left on the fruit after treatment, captan cannot be considered a postharvest alternative. Postharvest treatments with thiabendazole can be very effective for decay control, however, resistant populations of the pathogens against the fungicide commonly occur in packinghouses, making the fungicide ineffective. We found that TBZ-resistant isolates of *P. expansum* and *B. cinerea* are common in packinghouses in California, stressing the need for postharvest alternatives to TBZ. Additional postharvest treatments recommended for postharvest decay control of pears include the biological control agent Bio-Save that was also evaluated previously by us. In these studies using different inoculum levels and TBZ-sensitive and -resistant isolates of *B. cinerea* and *P. expansum*, the efficacy of Bio-Save was inconsistent and never as good as compared to the new fungicides Elevate or Scholar. Thus, from our studies that addressed EPA concerns for the Emergency Registration of fenhexamid, we conclude that there are no registered alternatives available as effective pre- or postharvest treatments for control of postharvest decays caused by TBZ-resistant isolates of *B. cinerea* or *P. expansum*. Based on these results a Section18 registration was granted in 2000, 2001, and 2002 for postharvest use of Elevate. In 2003, we were not successful in obtaining a Section 18 for fenhexamid due to a lack of economic data for crop losses – an essential requirement for the renewal.

The efficacy of Elevate (fenhexamid) against gray mold and of Scholar (fludioxonil) and Penbotec (pyrimethanil) against gray mold and blue mold has been demonstrated in our previous studies that were summarized in our last year's Annual Report for the California Pear Board. IR-4 residue studies have been completed for Elevate and Scholar. All three fungicides belong to different classes and they are classified as 'reduced-risk' by the US-EPA. In 2003, additional commercial packingline studies were conducted on the efficacy of Scholar, Elevate, and Penbotec on Bartlett, Bosc, and Asian pears. Our goal is to have several new fungicides with different modes of action registered for postharvest use on pear to be able to design resistance management strategies with fungicide mixtures and fungicide rotations to prevent insensitive pathogen populations from developing. In addition, we also evaluated the efficacy of preharvest fungicide applications. The possible efficacy of ziram has been a concern of the EPA, because ziram is registered for this use on pears, and if effective, would minimize the need for new postharvest treatments.

**Objectives**

1) Evaluate preharvest treatments for postharvest decay control with ziram and pyrimethanil (Scala). Labeled rates will be used. Evaluations will include natural incidence and inoculation studies.
2) Evaluate postharvest treatments with the new, reduced-risk fungicides fenhexamid (Elevate), fludioxonil (Scholar), BAS516 (Pristine), and pyrimethanil (Penbotec). Studies will focus on management of gray mold and blue mold. TBZ-sensitive, and -resistant isolates of the fungi will be used.
    i. Control of natural incidence of decay in laboratory and experimental packingline studies.
    ii. Control of decay in inoculation studies in laboratory and experimental packingline studies.
    iii. Large-scale packinghouse studies with the most effective compounds.
3) Continue to conduct pathogen population studies to determine baseline fungicide resistance levels in selected commercial packinghouses.

**MATERIALS AND METHODS**

**Evaluate preharvest applications of new fungicides for postharvest disease management.** Preharvest applications were evaluated on pear fruit in a commercial orchard in 2003. Fungicides and their rates that were evaluated include Scala 40F (pyrimethanil – 27 fl oz/A at 0 day PHI), Vangard 75WG (cyprodinil - 10 oz/A at 0 day PHI), and ziram (8 lbs/A at 5 and 30 day PHI). Four replications of each fungicide were applied in a completely randomized design using an air-blast sprayer (100 gal/A). Each replication consisted of 4 trees. Preharvest sprays were applied at 14 and 7 day PHI. To evaluate the efficacy of preharvest treatments for control postharvest fruit decay, two methods were used: natural incidence and inoculation studies. For natural incidence of decay, incidence was evaluated as the number of infected fruit in a 100-fruit sample per replication after storage for 3 months at 4 C. For inoculation studies, forty fruit from each replication of each treatment were wound inoculated with 20 µl of a conidial suspension of *B. cinerea* or *P. expansum* (30,000 conidia/ml). Incidence of decay was evaluated after incubation at 0-1 C. Data were analyzed using analysis of variance and averages were separated using least significant difference mean separation procedures of SAS 6.12.

**Efficacy of new postharvest fungicides.** Fruit (Asian, Bartlett, and Bosc pears) were treated similar to commercial practices concerning harvest, handling, packing, and temperature-management of fruit. Postharvest studies included laboratory dip and spray tests and experimental packing line trials at Kearney Agricultural Center with fenhexamid (Elevate 50WG), fludioxonil (Scholar 50WP), pyrimethanil (Scala 40F, Penbotec), and the mixture of boscalid and pyraclostrobin (Pristine). These treatments were compared to the registered fungicide thiabendazole. Each treatment was evaluated using inoculated-treated and treated-inoculated procedures to determine wound protection (contact) or curative (local systemic action) properties of the evaluated fungicides. For these inoculation studies, 20-40 fruit for each of four replications were inoculated with conidial suspensions of TBZ-sensitive and -resistant isolates of *B. cinerea* and *P. expansum* (30K conidia/ml). Additionally, harvested fruit were treated and incubated to determine the natural incidence of decay (non-inoculated). For this, five boxes of fruit for each treatment were stored at 0-2 C and evaluated for decay after 3 months. Treatments were applied using either a dip tank method or a controlled droplet application (CDA) system on an experimental treater. Rates evaluated were based on registered preharvest field rates or postharvest rates used on other crops of each compound. Large-scale packinghouse studies were conducted with the most active fungicides and decay control was evaluated after inoculation and based on natural incidence of decay. Data were analyzed using analysis of variance and averages were separated using least significant difference mean separation procedures of SAS 6.12.

**Pathogen population studies to determine baseline fungicide resistance levels in selected commercial packinghouses.** Some 50-60 isolates from two packinghouses were collected after 3-4 months of storage. Isolates were evaluated for thiabendazole sensitivity in the laboratory using fungicide-amended agar media assays. Other new fungicides will also be evaluated to verify no cross resistance between fungicide classes.

## RESULTS AND DISCUSSION OF 2003 RESEARCH

**Efficacy of new postharvest fungicides for management of decays of Bartlett, Bosc, and Asian pears.** In a commercial packingline study with Bosc and Bartlett pears, CDA applications of Scholar, Penbotec, TBZ, or mixtures of TBZ and Elevate were highly effective in reducing the incidence of decay of fruit that were inoculated with TBZ-sensitive isolates of *B. cinerea* or *P. expansum* before treatment (Fig. 1). Fruit that were inoculated with TBZ-resistant isolates of *P. expansum* and treated with TBZ or TBZ+Elevate, however, developed the same amount of decay as the untreated control. Fruit that were inoculated with TBZ-resistant isolates of *B. cinerea* were also protected from decay development by TBZ. This can be explained by the fact that we used an isolate of *B. cinerea* with intermediate levels of resistance to TBZ and we obtained adequate fungicide residue on the Bartlett and Bosc fruit between 0.6 and 1.94 mg/Kg (see below). Fruit were also treated for control of natural incidence of decay, and these results are still pending. Data from our 2002 trials, that were not presented in our 2002 Annual Report, showed that the natural incidence of blue mold and gray mold was reduced from 34-36% in the control to very low levels by treatment with Penbotec or Scholar (Fig. 2). Mixtures of TBZ and Elevate reduced the incidence of gray mold only and TBZ alone had an intermediate efficacy for both decays. Residues obtained on Bosc pear were 4.09, 1.25, 3.77, and 0.59 mg/Kg; whereas on Barlett pear, residues were 4.32, 1.2, 3.23, and 1.94 mg/Kg for fenhexamid, fludioxonil, pyrimethanil, and TBZ, respectively. Evaluation of *Penicillium* isolates from decayed Bosc pears that were treated with TBZ or TBZ+Elevate indicated that 85% of the isolates were TBZ resistant. Thus, TBZ-resistance in postharvest pear pathogens were responsible for the reduced efficacy of the TBZ treatments. Thus, our large-scale commercial packingline studies confirmed decay control data from our previous laboratory studies. This indicates that our small-scale laboratory trials used in earlier research (*see* Adaskaveg, CPB Annual Reports 2001, 2002) were a good model for screening new fungicides.

In a commercial packingline study that was conducted in the fall of 2002 different rates and application systems for Scholar treatments on Asian pears were evaluated. Treatments used in this study were: drench or flooder, T-Jet, and CDA. The T-Jet and the CDA application systems were calibrated for 100 gal/200,000 lb of fruit, whereas the flooder was calibrated for 200 gal/200,000 lb of fruit. Residues obtained from these treatments were: 0.17, 0.25, 0.37, and 0.92 mg/Kg using T-Jet at 8 oz, T-Jet at 16 oz, CDA at 16 oz, and drench (or flooder) at 16 oz Scholar 50WP per 200,000 lb of fruit. Decay data that could not be presented in our 2002 Annual Report because fruit were still being stored, reflected these residue values (Fig. 3). Thus, decay was lowest following the drench application. Drench applications were again evaluated in 2003. Scholar, Penbotec, and Elevate were applied to inoculated Asian pears on an experimental packingline. Fruit is still being stored and results are pending. Currently, we are developing the in-line drenching system that re-circulates the fungicide drench. This work is being done at the Kearney AgCenter and it is proving to be a cost effective application method that provides the best coverage and highest fungicide residues of any application system in our preliminary studies (data not shown).

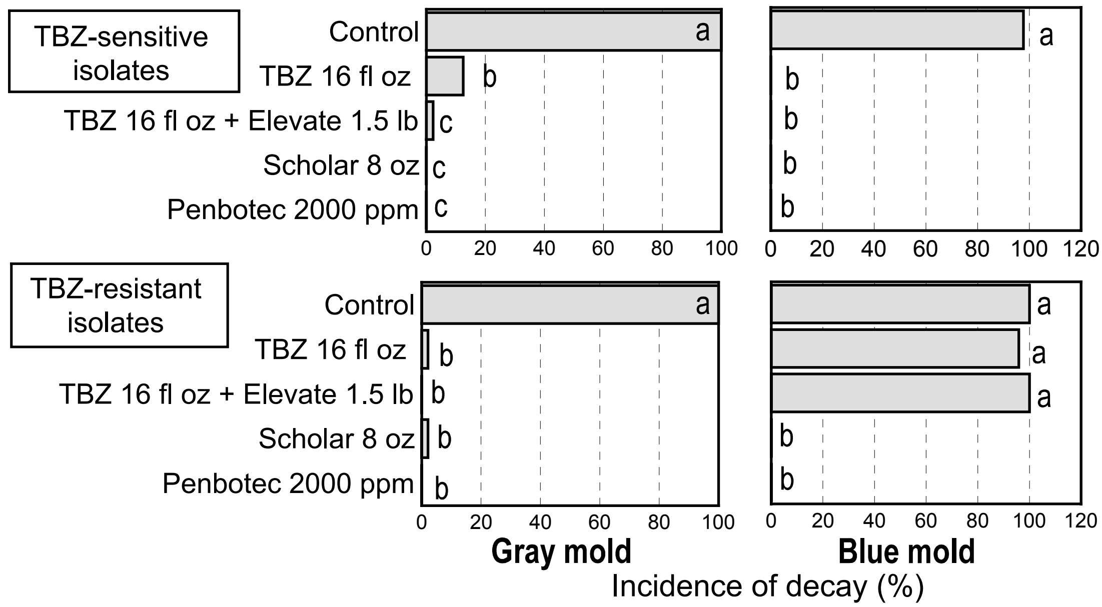
**Evaluation of preharvest fungicide applications for postharvest decay control.** In 2002, preharvest applications with ziram (Ziram – 8 lb/A) were applied 5 weeks before harvest in a commercial Bartlett and Bosc pear orchard using an air-blast sprayer (100 gal/A). Pyrimethanil (Scala 27 fl oz/A) was applied 8 and 0 days PHI on Bosc pears. To evaluate the efficacy of these preharvest treatments, fruit were harvested, dipped into a commercial float tank, re-collected prior to sanitizing washes, boxed, and put into cold storage. Efficacy data based on the natural incidence of decay were pending at the time of our 2002 Annual Report and are reported here. Ziram and Scala significantly reduced the natural incidence of blue mold in these 5-week and 8 & 0 day PHI applications, respectively (Fig. 4). Gray mold, however, was numerically, but not significantly reduced by ziram. On Bosc pears where both Ziram and Scala were evaluated, the incidence of gray mold was very low, thus data for the efficacy of Scala against gray mold could not be obtained. In 2003, preharvest treatments were evaluated for decay development after fruit inoculation and for natural incidence. Data for the effect on natural incidence of decay are still pending. For the wound-inoculated fruit, Ziram had no effect against the development of gray mold and blue mold using 5-day and 30-day preharvest application intervals (Fig. 5). Small, but significant reductions in decay were found after application of the two anilinopyrimidines Scala and Vangard for gray mold and for Scala against blue mold on Bosc pear. Thus, fungicide residues on the fruit after preharvest applications are either too low to be effective in protecting fruit after wound-inoculations, the fungicides do not penetrate the fruit sufficiently, or the fungicides were removed during the floating and extra handling on the packing line.

When preharvest-treated fruit were also postharvest treated with TBZ, significant reductions in both decays were observed on Bosc pear that were wound-inoculated with TBZ-resistant isolates of the pathogens (Fig. 6A). The 30-day PHI Ziram and the Scala treatments had significantly less gray mold decay than the postharvest TBZ treatments alone. For blue mold, the 30-day PHI Ziram and Vangard treatments were most effective. On Bartlett pear, only 5-day PHI Ziram treatments were evaluated. No additional benefit was found of this preharvest treatment as compared to the postharvest TBZ treatment alone (Fig. 6B). Thus, preharvest treatments of pears with Ziram or other fungicides in some cases have some additional benefit to postharvest treatments with TBZ when TBZ-resistant pathogen populations have to be controlled. Still, the efficacy is inconsistent and generally much lower with higher levels of decay than when fruit is postharvest treated with the new ‘reduced-risk’ fungicides that we are developing in our studies.

Fig. 1. Evaluation of postharvest fungicides for management of gray mold and blue mold decay of pears

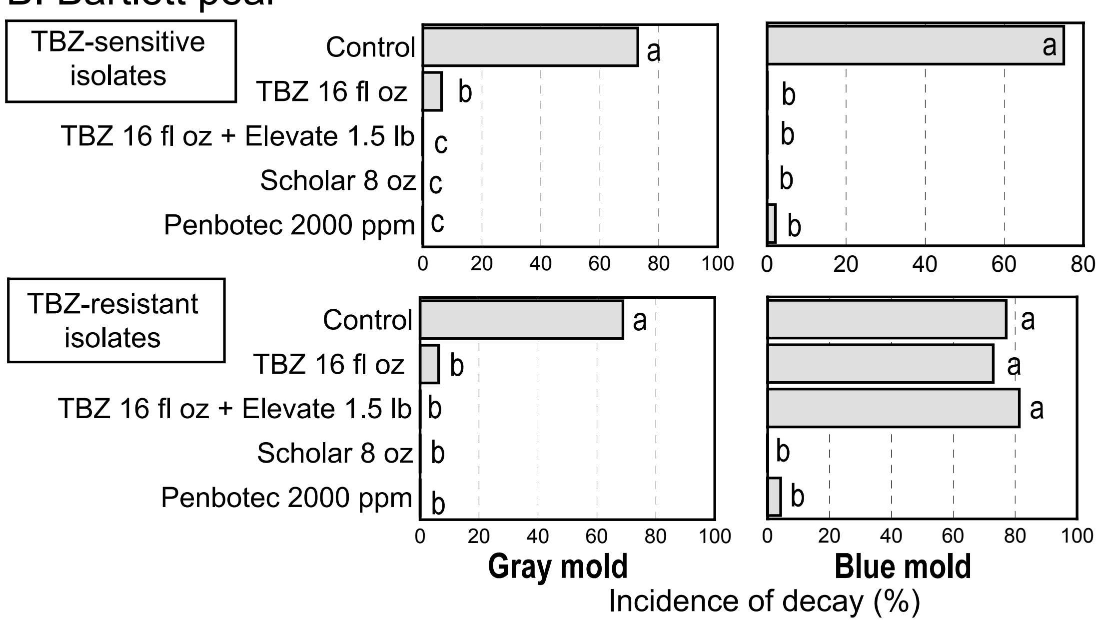
Commercial packingline study 2003

## A. Bosc pear



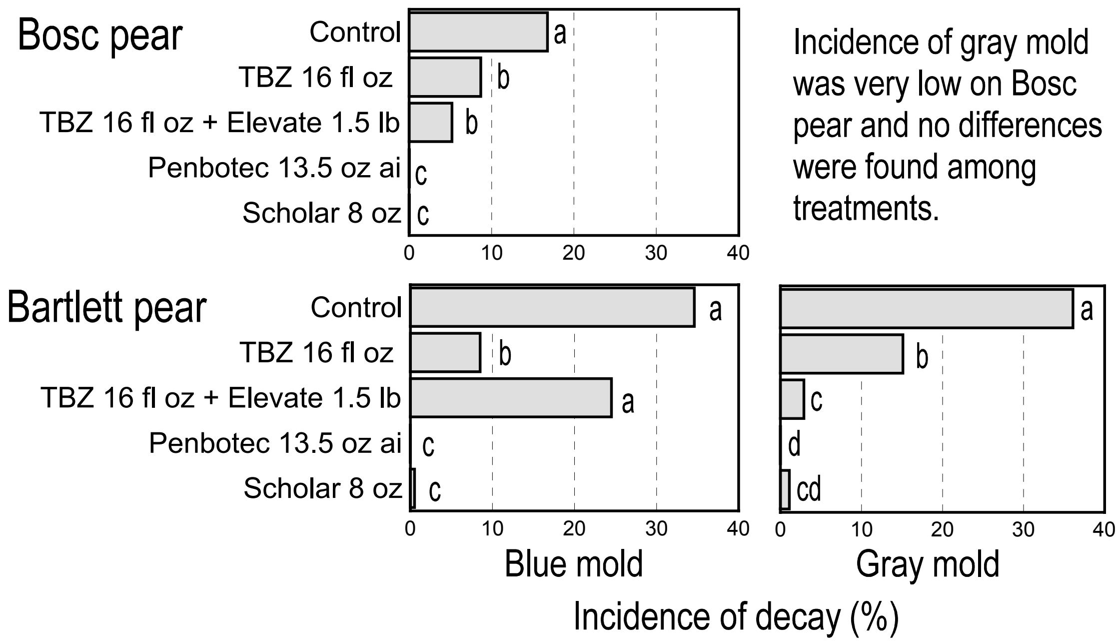

Fruit were wound-inoculated with conidia of *Botrytis cinerea* or *Penicillium expansum* (30,000 conidia/ml). Treatments were applied as aqueous suspensions using a CDA applicator calibrated to deliver 62.3 gal/200,000 lb of fruit. Fruit were stored under commercial conditions at 0-1C.

## B. Bartlett pear



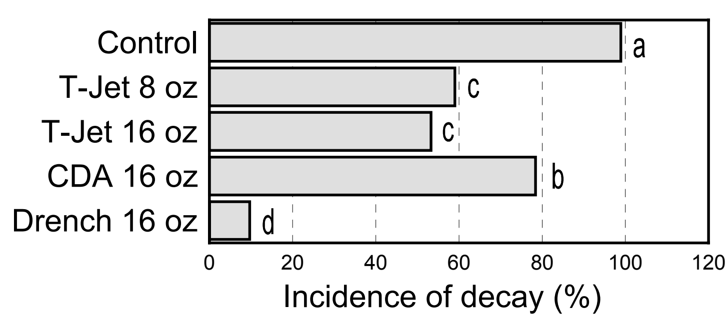

Fruit were wound-inoculated with conidia of *Botrytis cinerea* or *Penicillium expansum* (30,000 conidia/ml). Treatments were applied as aqueous suspensions using a CDA applicator calibrated to deliver 62.3 gal/200,000 lb of fruit. Fruit were stored under commercial conditions at 0-1C.

Fig. 2. Evaluation of postharvest fungicides for management of natural incidence of postharvest decays of pears
Commercial packingline study 2002




Treatments were applied as aqueous suspensions using a CDA applicator calibrated to deliver 25 gal/200,000 lb of fruit. Fruit were stored under commercial conditions at 0-1C for 5 months. Eighty percent of the isolates collected from decayed fruit were resistant to TBZ in our assays.

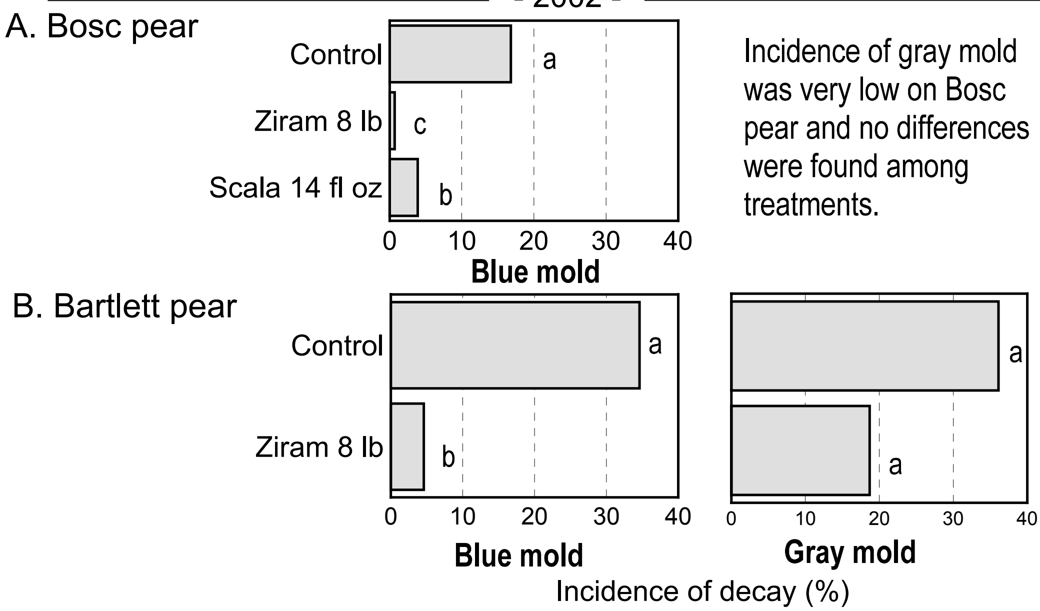
Fig. 3. Evaluation of Scholar treatments for management of gray mold of Asian pears using different application methods in a commercial packingline study 2002




Fruit were wound-inoculated with conidia of *Botrytis cinerea*. Scholar was applied as drench, T-Jet, or CDA. The T-Jet and the CDA application systems were calibrated for 100 gal/200,000 lb of fruit, whereas the flooder was calibrated for 200 gal/200,000 lb of fruit. Fruit were stored at 0-1C for 5 months.

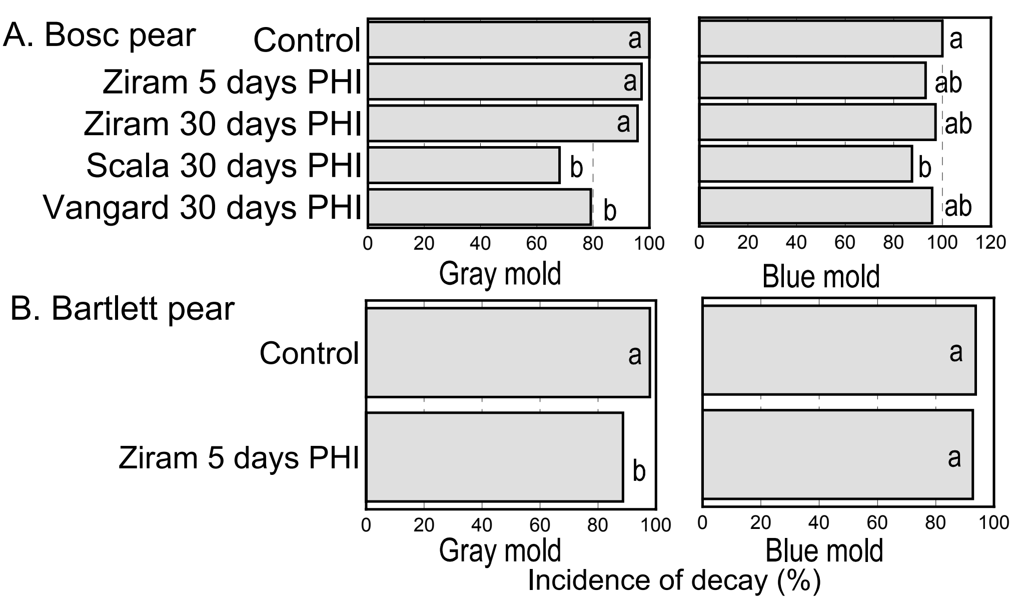
Fig. 4. Efficacy of preharvest treatments on the natural incidence of postharvest decays of pears

\- 2002 -



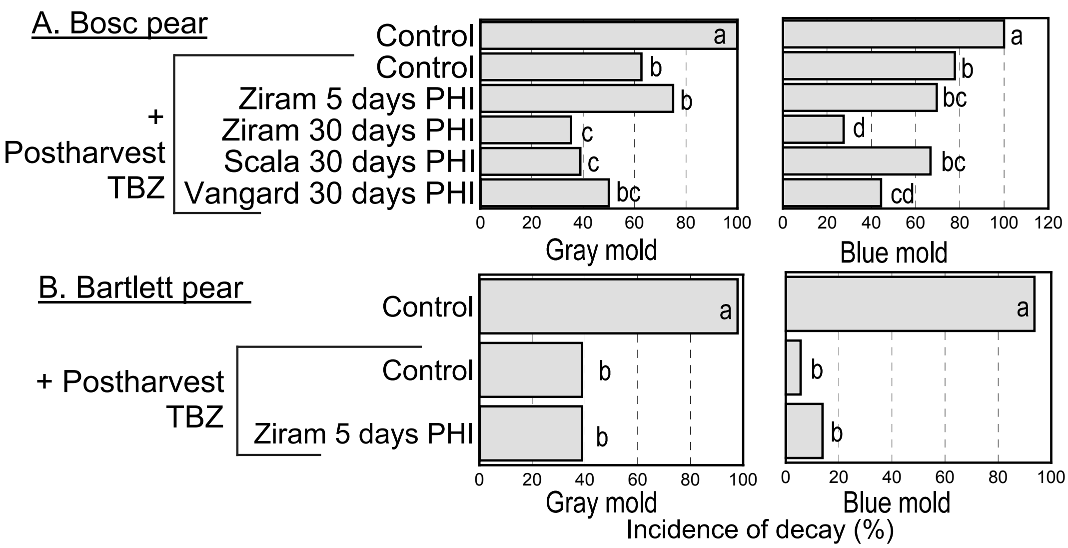

Preharvest treatments were applied using an airblast sprayer at 100 gal/A. Ziram was applied at 5 wk before harvest and Scala at 8 & 0 days PHI. Fruit were harvested, wound-inoculated with conidia of *Botrytis cinerea* or *Penicillium expansum* (30,000 conidia/ml) and stored under commercial conditions at 0-1C.

Fig. 5. Efficacy of preharvest treatments on incidence of postharvest decays of pears after wound-inoculation with TBZ-resistant isolates of the pathogens




Preharvest treatments were applied using an airblast sprayer at 100 gal/A. Rates were 8 lb for Ziram, 14 fl oz for Scala, and 10 oz for Vangard. Fruit were harvested, wound-inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum* (30,000 conidia/ml) and stored under commercial conditions at 0-1C.

Fig. 6. Efficacy of pre- and postharvest treatments on incidence of postharvest decays of pears after wound-inoculation with TBZ-resistant isolates of the pathogens




Preharvest treatments were applied using an airblast sprayer at 100 gal/A. Rates were 8 lb for Ziram, 14 fl oz for Scala, and 10 oz for Vangard. Fruit were harvested, and treated posthravest with TBZ with a CDA application system at 62.3 gal/200,000 lb. Fruit were then wound-inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum* (30,000 conidia/ml) and stored under commercial conditions at 0-1C.