Annual Report - 2004

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 2004 RESEARCH

1. In laboratory postharvest studies, Scholar, Pristine, and Scala were highly effective against blue mold and gray mold caused by TBZ-resistant isolates of the pathogens. Aqueous applications were more effective than applications in a diluted carnauba fruit coating. Pristine, but not Scholar, reduced decay caused by *Mucor pyriformis*.
2. In an experimental packingline study using inoculated fruit, Scholar reduced the incidence of blue and gray molds to very low levels. In-line drench applications were more effective than CDA applications.
3. In a 2003 commercial packingline study conducted to evaluate treatments against natural incidence of decay after several months of storage, Scholar, Elevate, and Penbotec were very effective in reducing gray mold, whereas Scholar and Penbotec were effective against blue mold (not included in last years report).
4. In a commercial packingline study done in 2004, Scholar, Pristine, and Scala were very effective in reducing the natural incidence of blue mold. Natural incidence of gray mold was less than 1% in the control and efficacy against this decay could not be evaluated. In addition, decay caused by a *Phomopsis* species was also significantly reduced as compared to the control. Pristine (only on Bartlett pear, not on Bosc pear), Scholar, Penbotec, and a mixture of Scholar and Penbotec also significantly reduced the incidence of decay after inoculation of fruit with TBZ-resistant isolates of *B. cinerea* and *P. expansum*. The efficacy of Pristine was lower than for the other two fungicides and the mixture, and all fungicides were more effective in reducing decay on Bartlett than on Bosc pear.
5. In a preharvest study with Bosc pears that was conducted in 2003, Ziram applied at 5 or 30 days PHI reduced the natural incidence of blue mold and gray mold. Preharvest applications (0 day PHI) of Scala (pyrimethanil) and Vangard (cyprodonil), two anilinopyrimidine fungicides, were more effective than Ziram in reducing postharvest gray mold and blue mold decay (not included in last years report).
6. Collections of isolates of *Penicillium* spp. and *B. cinerea* from fruit stored in one packinghouse demonstrated that among the *Penicillium* isolates 75% were highly resistant to TBZ (EC_{50} >20 ppm), 5% had intermediate sensitivity (EC_{50} values between 2.6 and 4.8 ppm), and 20% were TBZ-sensitive (EC_{50} values between 0.17 and 0.33 ppm). All of the isolates of *B. cinerea* were TBZ-sensitive (EC_{50} values between 0.16 and 0.35 ppm). Baseline sensitivities were established for *Penicillium* spp. for fludioxonil and pyrimethanil and for *B. cinerea* for fenhexamid, fludioxonil, and pyrimethanil.

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. Gray mold 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 captan (2 lb/200,000 lb), the fungicide was 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. Preharvest studies that were conducted with ziram in two years gave inconsistent, and generally unsatisfactory results. 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 in our evaluations was inconsistent and never as effective as the 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*.

The efficacy of Elevate (fenhexamid) against gray mold and of Scholar (fludioxonil), Pristine (boscalid/pyraclostrobin) and Penbotec (pyrimethanil) against gray mold and blue mold has been demonstrated in our previous studies that were summarized in our previous years' Annual Reports for the California Pear Board. IR-4 residue studies have been completed for Elevate and Scholar. All fungicides belong to different classes and they are classified as 'reduced-risk' by the US-EPA. In 2004, additional commercial packingline studies were conducted on the efficacy of Scholar, Elevate, and Penbotec on Bartlett and Bosc 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.

Objectives

1) Evaluate application methods of postharvest treatments with the new, reduced-risk fungicides fenhexamid (Elevate), fludioxonil (Scholar), BAS516 (Pristine), and pyrimethanil (Scala/Penbotec). Studies will focus on management of gray mold and blue mold. TBZ-sensitive, and -resistant isolates of the fungi will be used.
    i. Experimental packing line studies to evaluate the management of blue and gray mold decays of pear using natural incidence and wound-inoculation studies.
    ii. Large-scale packinghouse studies with registered materials.
2) Continue to conduct pathogen population studies to determine baseline fungicide resistance levels in selected commercial packinghouses.
3) Develop improved identification methods using selective media and molecular techniques for *P. expansum* and other *Penicillium* spp. on pear.
4) Evaluate a new biocontrol, Arabesque, used as a biofumigant of edible horticultural crops.

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 400SC (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. 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 *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. Fungicides evaluated included Elevate 50WG, Scholar 50WP, Penbotec 400SC, and Pristine 38WG. In laboratory studies fruit (Bartlett or Bosc pears) were wound-inoculated with TBZ-resistant isolates of *B. cinerea* or *P. expansum*, incubated for 9 to 16 h, and then spray treated with fungicides. Fungicides were applied as aqueous solutions or in a diluted carnauba-based fruit coating. In an experimental packing line trial at Kearney Agricultural Center with wound-inoculated Bartlett pear fruit, CDA applications of Scholar were compared to in-line drench applications. Fruit were stored at 20C until evaluation of decay. In a commercial packingline study, the efficacy of new fungicides was evaluated on wound-inoculated and non-inoculated (natural incidence) fruit. Fruit were stored under commercial conditions at 0-1 C for 14 weeks. Data were analyzed using analysis of variance and averages were separated using least significant difference mean separation procedures of SAS 6.12.

Pathogen sensitivities to TBZ, baseline fungicide sensitivity levels to new postharvest fungicides, and molecular characterization of *Penicillium* spp. A total of 37 isolates of *B. cinerea* and of 56 isolates of *Penicillium* spp. were collected from decayed Bartlett and Bosc pear fruit in a packinghouse. Fungicide sensitivity was determined using the spiral gradient dilution method. A conidial suspension of the fungus was streaked along the radial fungicide gradient in the agar Petri dish and the 50% inhibitory concentrations for mycelial growth were determined subsequently. For *Penicillium* spp., sensitivities to TBZ, fludioxonil, and pyrimethanil were determined, whereas for *B. cinerea* sensitivities to fenhexamid were also evaluated. For a preliminary molecular characterization of *Penicillium* isolates from pear fruit, fungal DNA was extracted and amplified with a PCR primer that has been developed by others for the specific detection of *P. expansum*. In addition, amplified DNA regions of ribosomal DNA and the beta-tubulin gene were subjected to restriction fragment analysis. DNA fragments were separated in agarose gels and visualized under UV light after ethidium bromide staining.

RESULTS AND DISCUSSION OF 2004 RESEARCH

Efficacy of new postharvest fungicides for management of decays of Bartlett and Bosc pears. In laboratory postharvest studies, Scholar, Pristine, and Penbotec (Scala) were highly effective against blue mold and gray mold caused by TBZ-resistant isolates of the pathogens after wound-inoculations (Fig. 1A). The rates evaluated (300 and 600 ppm for Scholar; 1000, 1500, and 2000 ppm for Pristine; and 500, 1000,and 1500 ppm for Penbotec) were equally effective. In addition, mixtures of Scholar and Penbotec at reduced rates of each fungicide were also highly effective. Aqueous applications were more effective than applications in a diluted carnauba fruit coating (Fig. 1B). In another study, the efficacy of new fungicides against Mucor decay caused by *M. piriformis* was evaluated. Pristine, but not Scholar, reduced this decay (Fig. 2). Mixtures of the anti-scald agent ethoxyquin did not significantly affect fungicide efficacy. Previously, we demonstrated that Penbotec was also ineffective against *M. piriformis*.

In a 2003 commercial packingline study conducted to evaluate treatments against natural incidence of decay after several months of storage, Scholar, Elevate, and Penbotec were very effective in reducing gray mold, whereas Scholar and Penbotec were effective against blue mold (not included in last years report) (Fig. 3). In a 2004 commercial packingline study, Scholar, Pristine, Scala, and a mixture of Scholar and Penbotec (both at reduced rates) were very effective in reducing the natural incidence of blue mold on Bartlett and Bosc pears (Fig. 4B). Natural incidence of gray mold was less than 1% in the control in this trial and efficacy against this decay could not be evaluated. In addition, decay caused by a *Phomopsis* sp. (probably *P. mali*) was also significantly reduced by all fungicides as compared to the control. Scholar, Penbotec, Pristine (only on Bartlett pear, not on Bosc pear), and the mixture of Scholar and Penbotec also significantly reduced the incidence of decay after wound-inoculation of fruit with TBZ-resistant isolates of *B. cinerea* and *P. expansum* (Fig. 4A). The efficacy of all single-fungicide treatments was higher on Bartlett than on Bosc pear.

In contrast to the laboratory experiments, Pristine was less effective than the other two fungicides, especially on Bosc pear where decay incidence of gray and blue mold was only slightly reduced as compared to the control (Fig. 4A). This reduced efficacy could be explained by the long storage of the fruit in this experiment (14 weeks) as compared to the laboratory test and previous packingline trials. Thus, decay development may resume after applications of fungicides with only fungistatic action when the materials break down during prolonged storage. Overall, in this commercial packinghouse trial, numerically the Penbotec-Scholar mixture was the most effective treatment. This indicates that mixtures may be the most effective strategy not only for decay management, but also for resistance management.

In an experimental packingline study using Scholar on inoculated fruit, CDA and in-line, recirculating drench applications were compared for their efficacy. For both, gray mold and blue mold control, drench applications as wound-protection treatments were more effective than low-volume CDA spray applications (Fig. 5). Thus, this new application method is proving to be a cost effective application method that provides the best coverage and highest fungicide residues (as previously shown) of any application system in our studies. Ethoxyquin again did not negatively effect fungicide efficacy. In addition, pre-infection treatments where fruit were wound-inoculated after treatment (Treated-Inoculated) were much less effective than the post-infection treatments.

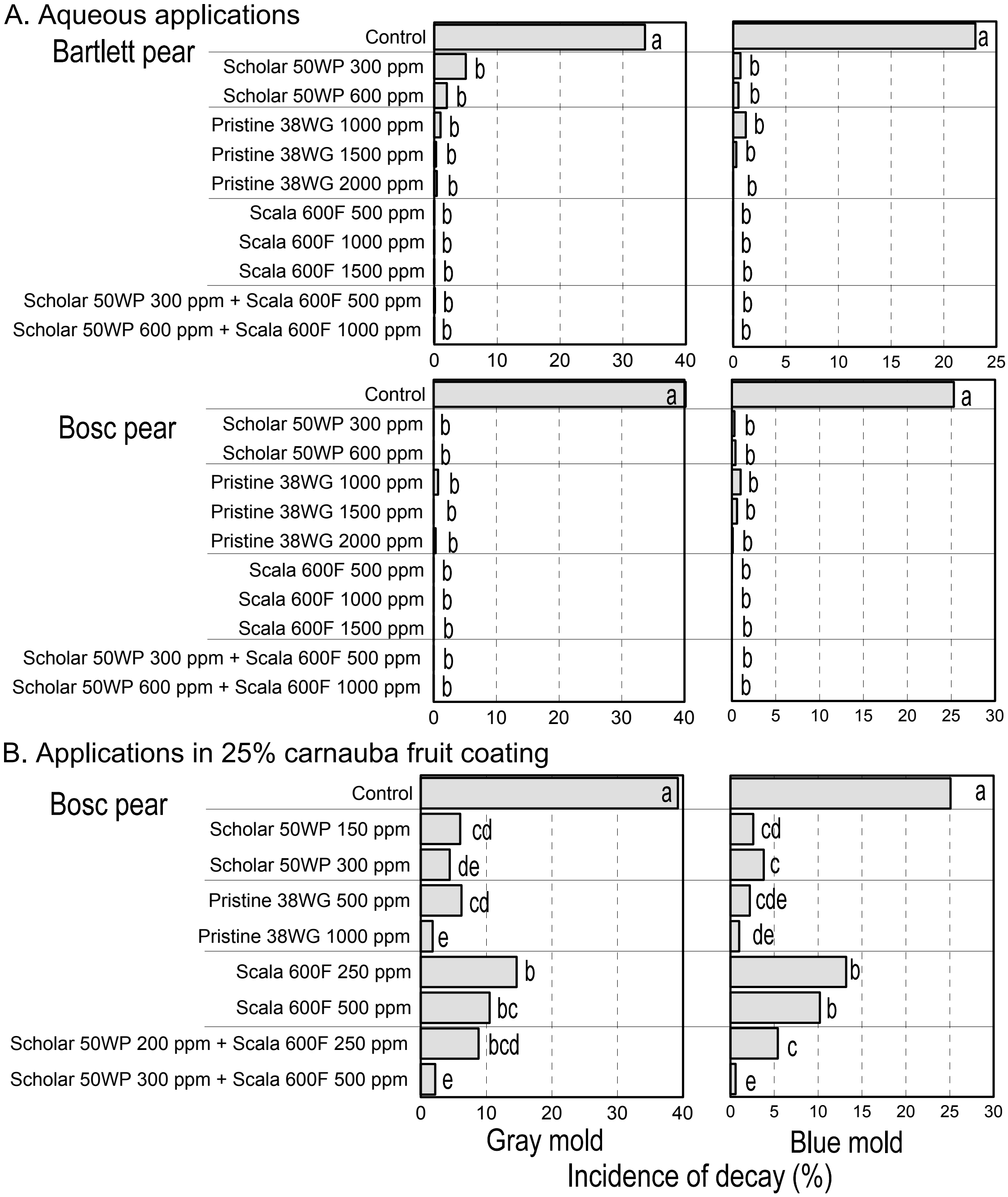
Evaluation of preharvest fungicide applications for postharvest decay control. Preharvest treatments for postharvest decay control of pears were not conducted again in 2004. Data on management of natural incidence of decay on Bosc pears after preharvest treatments with Ziram, Scala, or Vangard that are presented here were pending from our 2003 research. In this trial Ziram applied at 5 or 30 days PHI reduced the natural incidence of blue mold and gray mold (Fig. 6). There was a high variability among replications of each treatment; thus, differences between treatments mostly could not be statistically substantiated. Preharvest applications (0 day PHI) of Scala (pyrimethanil) and Vangard (cyprodonil), two anilinopyrimidine fungicides, were more effective than Ziram in reducing postharvest gray mold and blue mold decay. Overall, our studies on the evaluation of preharvest treatments indicate, that the efficacy of Ziram was inconsistent over the years, whereas the anilinopyrimidines Scala and Vangard generally were more effective. Preharvest treatments of pears with Ziram or other fungicides can sometimes have additional benefits 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.

Pathogen sensitivities to TBZ, baseline fungicide sensitivity levels to new postharvest fungicides, and molecular characterization of *Penicillium* spp. isolated from pear. Collections of isolates of *Penicillium* spp. and *B. cinerea* from fruit stored in one packinghouse demonstrated that among the *Penicillium* isolates 75% were highly resistant to TBZ (EC_{50} >20 ppm), 5% had intermediate sensitivity (EC_{50} values between 2.6 and 4.8 ppm), and 20% were TBZ-sensitive (EC_{50} values between 0.17 and 0.33 ppm) (Fig. 7). In contrast to previous years, all of the isolates of *B. cinerea* were TBZ-sensitive (EC_{50} values between 0.16 and 0.35 ppm) (Fig. 8). Thus, pathogen populations resistant to TBZ are consistently identified in California packinghouses over the years, emphasizing the need for new postharvest treatments.

Baseline sensitivities were established for *Penicillium* spp. for fludioxonil and pyrimethanil (Fig. 7) and for *B. cinerea* for fenhexamid, fludioxonil, and pyrimethanil (Fig. 8). Baseline sensitivities for *Penicillium* spp. were 0.010 to 0.032 ppm for fludioxonil and 0.105 and 0.669 ppm for pyrimethanil. Thus, there was no cross resistance of *Penicillium* isolates resistant to TBZ with any of the new fungicides (Fig. 7). Baseline sensitivities for *B. cinerea* were 0.050 to 0.013 ppm for fludioxonil, 0.109 and 0.389 ppm for pyrimethanil, and 0.008 to 0.031 ppm for fenhexamid.

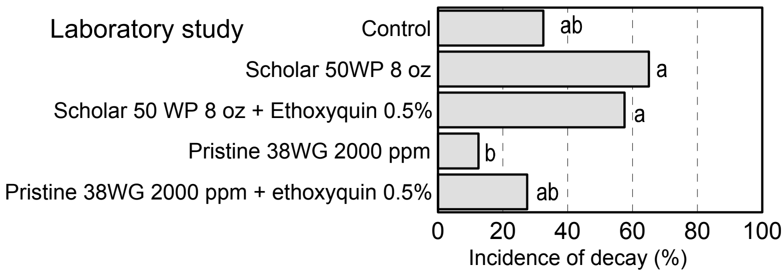
In these studies on fungicide sensitivity we observed *Penicillium* cultures with several cultural appearances. Although similar these cultures had slightly different growth patterns and colony colors. Preliminary molecular studies using restriction fragment analyses of PCR amplified ribosomal DNA and random amplified polymorphic DNA were conducted that supported the presence of different taxa of *Penicillium*. Thus, several species in addition to *P. expansum* may cause postharvest decay of pears in California. These studies will be continued in 2005. California isolates of *Penicillium* spp. from pear will be compared to reference cultures and identification methods for the different pathogens will be developed.

Fig. 1. Evaluation of postharvest fungicides for management of postharvest decays of pears in a laboratory study



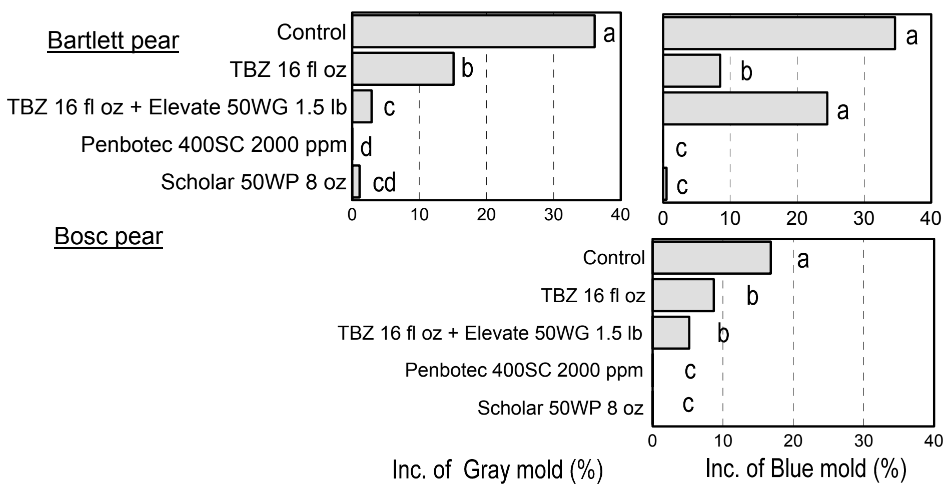
Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, incubated for 16 h at 20C, treated using an air-nozzle sprayer and incubated again at 20C.

Fig. 2. Efficacy of postharvest fungicides on decay caused by *Mucor pyriformis*



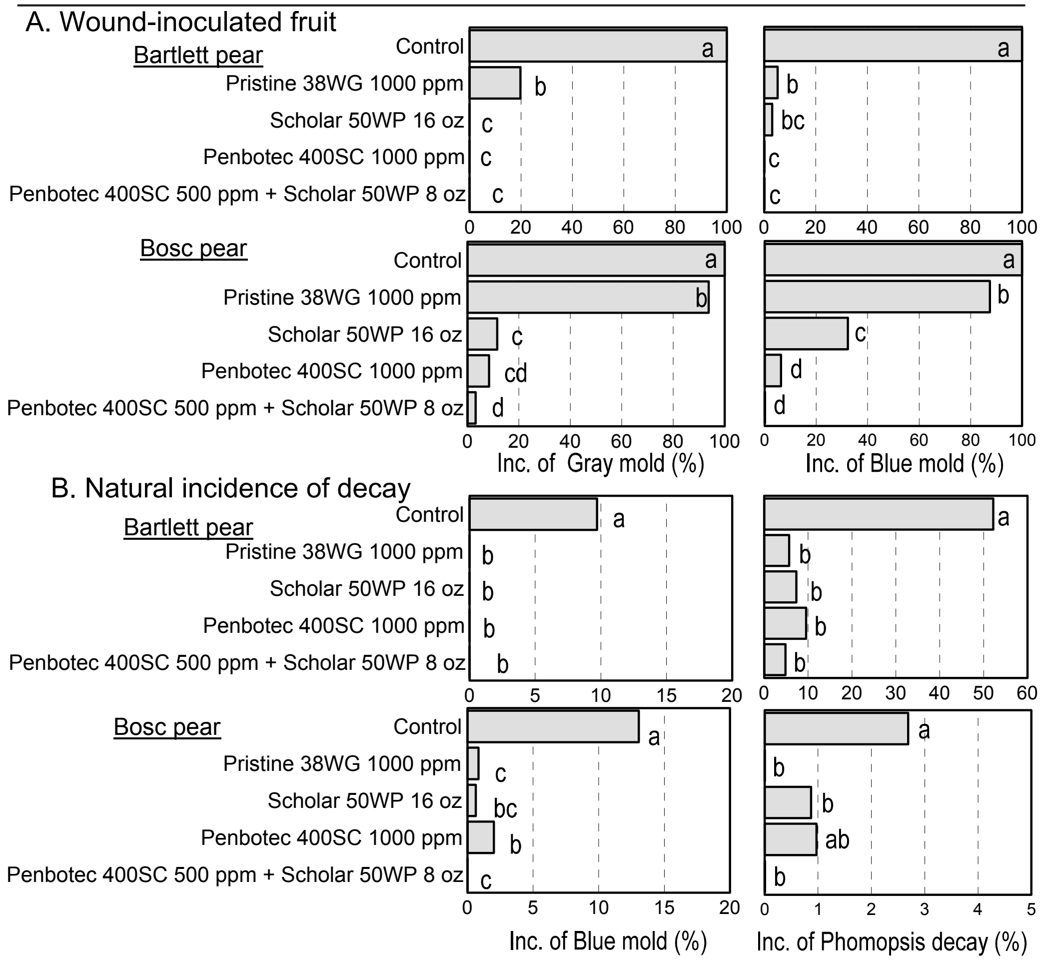
Fruit were inoculated with spores of *M. pyriformis* (30,000 spores/ml), incubated for 9 h at 20C, treated using an air-nozzle sprayer and incubated again at 20C.

Fig. 3. Evaluation of pre- and postharvest treatments with new fungicides for management of natural incidence of decays of pears in a commercial packingline study 2003



After harvest, fruit were moved through a floater and then treated with fungicides using a CDA application system (62 gal/200,000 lb fruit). Fruit were then stored under commercial conditions at 0-1C for 4 months. Natural incidence of gray mold on Bosc pears was less than 1%.

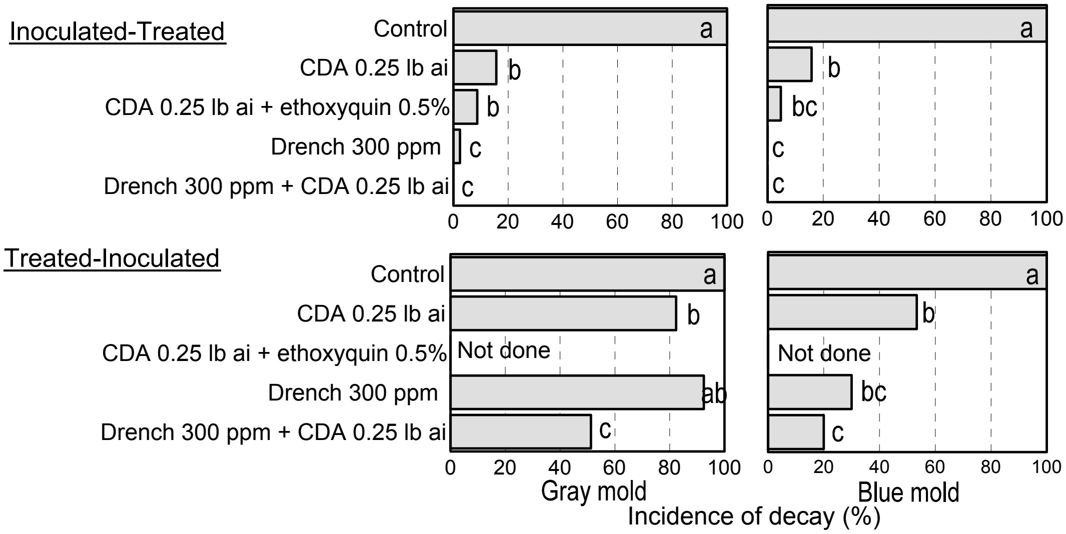
Fig. 4. Evaluation of postharvest treatments with new fungicides for management of gray mold and blue mold decay of pears in a commercial packingline study 2004



For the study using inoculated fruit, fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum* and incubated for 4 h. Fruit were treated with fungicides using a CDA application system (62 gal/200,000 lb fruit), and stored under commercial conditions at 0-1C for 14 weeks. For the evaluation of fungicide efficacy against natural incidence of decay fruit were run through a floater before fungicide treatment.

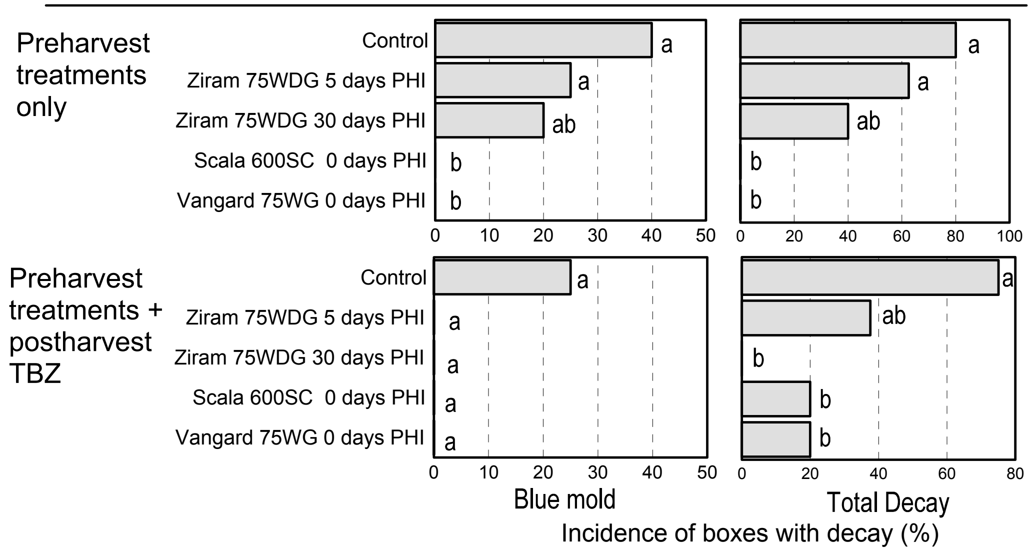
Fig. 5. Evaluation of postharvest treatments with Scholar 230SC for management of gray mold and blue mold decay of Bartlett pears

Experimental packingline study 2004



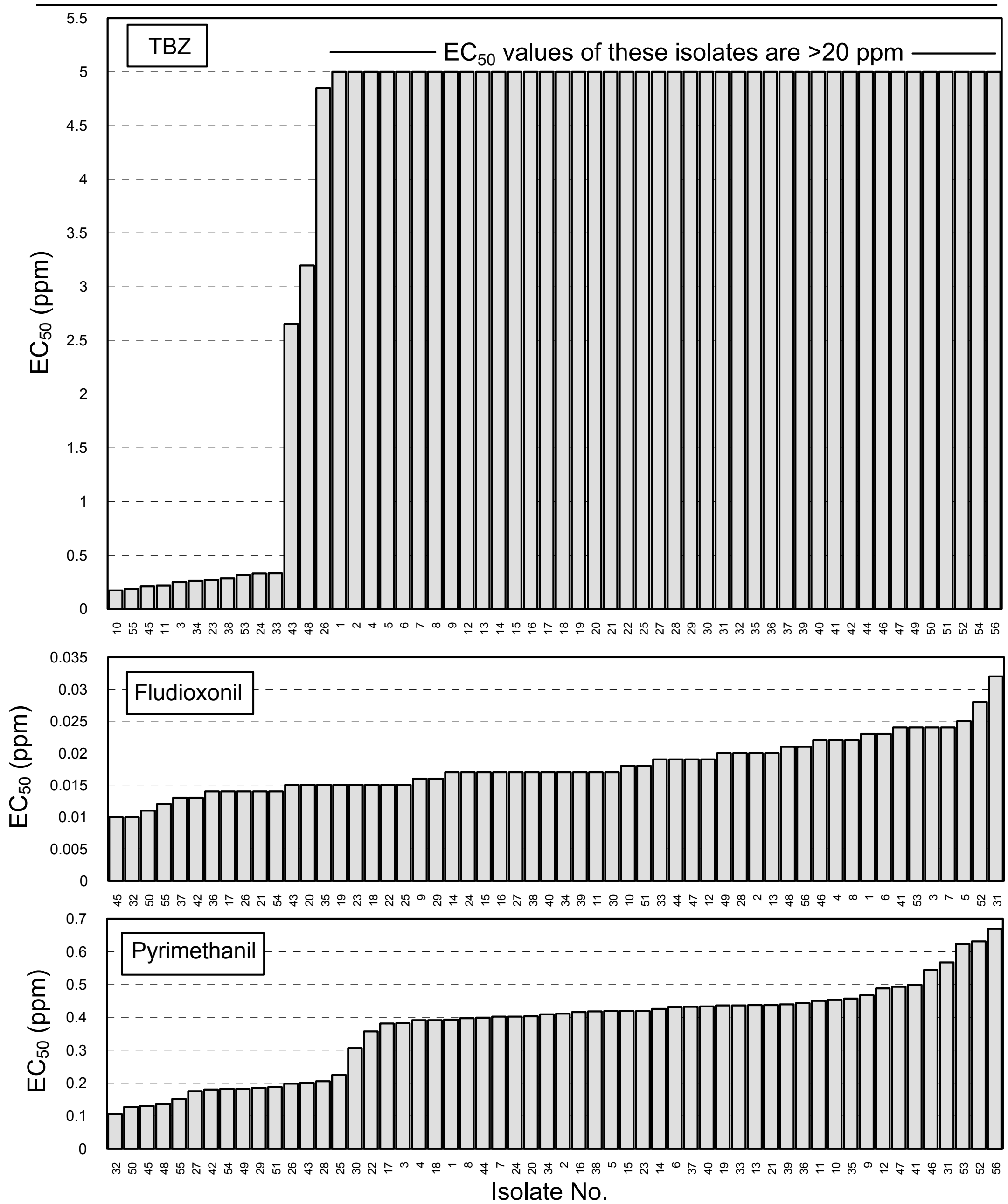
For the Inoculated-Treated method, fruit were wound-inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* ($5x10^4$ conidia/ml) or *Penicillium expansum* (10^5 conidia/ml). Treatments were applied 4 h after inoculation. For the Treated-Inoculated method, fruit were first treated, air-dried and then wound-inoculated. CDA aplications were in a diluted carnauba-based fruit coating at a rate of 16.6 gal,200,000 lb fruit. For the combination drench-CDA application, fruit were briefly air-dried after drench treatments, dip-washed in water, and CDA-treated. Treatments were applied as aqueous suspensions using a CDA applicator calibrated to deliver 16.6 gal/200,000 lb of fruit. Fruit were stored at 20C.

Fig. 6. Efficacy of pre- and postharvest treatments for management of natural incidence of postharvest decays of Bosc pears - 2003



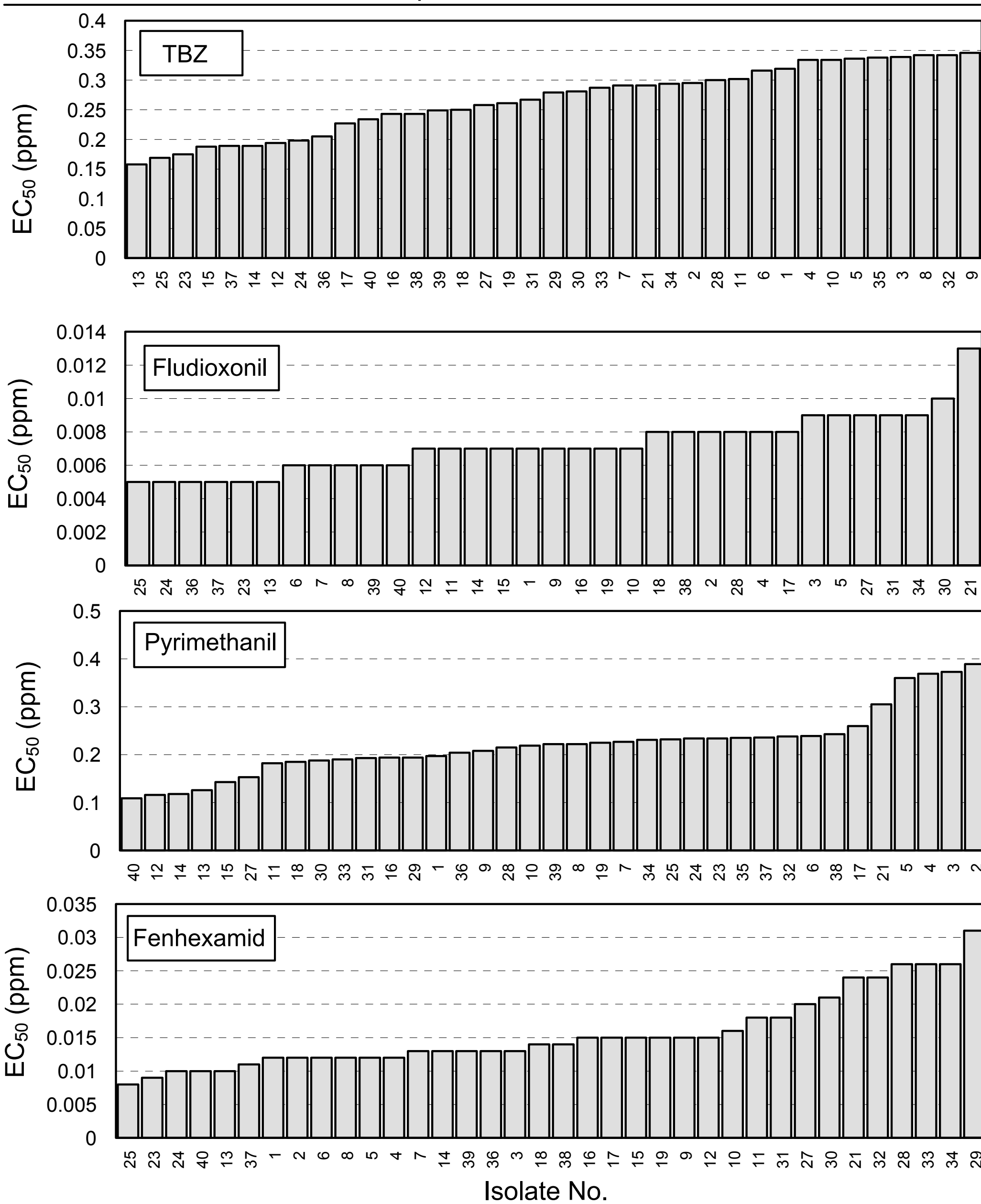
Preharvest treatments were applied using an airblast sprayer at 100 gal/A. Fruit were harvested, run through a commercial floater, treated with TBZ or not, and stored under commercial conditions at 0-1C. Total decay was comprised of blue and gray mold.

Fig. 7. Fungicide sensitivities for TBZ and baseline sensitivities for fludioxonil and pyrimethanil for isolates of *Penicillium* spp. from pear in California - 2003



Isolates of *Penicillium* spp. were collected from decayed Bartlett and Bosc pear fruit in a packinghouse in December of 2003. Fungicide sensitivities were determined using the spiral gradient dilution method. TBZ was supplied as Mertect 340SC, fludioxonil as Scholar 50WP, and pyrimethanil as Penbotec 400SC.

Fig. 8. Fungicide sensitivities for TBZ and baseline sensitivities for fludioxonil, pyrimethanil, and fenhexamid for isolates of *Botrytis cinerea* from pear in California - 2003



Isolates of *B. cinerea* were collected from decayed Bartlett and Bosc pear fruit in a packinghouse in December of 2003. Fungicide sensitivities were determined using the spiral gradient dilution method. TBZ was supplied as Mertect 340SC, fludioxonil as Scholar 50WP, pyrimethanil as Penbotec 400SC, and fenhexamid as Elevate 50WG.