Annual Report - 2005

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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Acknowledgements:	Special thanks to Naumes Packing, Marysville, CA for their cooperation in both pre- and postharvest research and for donation of fruit used in these trials.

MAIN ACHIEVEMENTS IN 2005 RESEARCH

1. In laboratory postharvest studies, Scholar, Pristine, and Scala (Penbotec) were highly effective against blue mold caused by *P. expansum, P. solitum,* or *P. commune*. Phytotoxicity was observed on Bartlett pears after treatments with pyraclostrobin-boscalid (e.g., Pristine) or the new fungicide fluoxystrobin (both strobilurin fungicides), especially when treatments were applied in a carnauba-based fruit coating.
2. In an experimental packingline study using inoculated fruit, in-line drench applications with Scholar at 150 to 300 ppm ai. were highly effective in reducing the incidence of blue and gray molds although low residue levels ($\leq$0.1 ppm) were found on the treated fruit. Penbotec and mixtures of Scholar and TBZ were also highly effective.
3. In a commercial packingline study using inoculated fruit, Scala (Penbotec) and mixtures of Scholar with Scala were more effective than Pristine or a mixture of Elevate with fluoxystrobin. Results on the efficacy on the natural incidence of decay after three months of cold storage are pending at this time.
4. Results on the efficacy of preharvest treatments with Elevate, Vangard, Scala, Pristine, and fluoxystrobin against the natural incidence of postharvest decays of Bartlett, Bosc, and Asian pears after three months of cold storage are pending.
5. Using relatively simple molecular methods we were able to identify four species of *Penicillium* that cause blue mold decay of pears. *P. expansum* was the most common and most virulent species. *P. solitum*, *P. commune*, and *P. roquefortii* were isolated at lower frequencies in 2004 than in 2003.
6. Of the 60 isolates of *Penicillium* spp. obtained from fruit stored in one packinghouse in 2004, 36.7% were highly resistant to TBZ (EC_{50} >18 ppm). Sensitivities for fludioxonil were similar as in 2003 (EC_{50} values between 0.008 and 0.021 ppm). Sensitivities for pyrimethanil ranged from 0.07 to 1.21 ppm. Implications of baseline sensitivities on resistance management are discussed.

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. Our preharvest studies with ziram, that is registered on pears with a 5-day preharvest interval, gave inconsistent, and generally unsatisfactory results as a stand-alone treatment for postharvest decay management. Thiabendazole (Mertect 340F) and captan (Captan 50WP) are registered for postharvest use on pears. In our postharvest studies in 2001 captan applied at the registered rate of 2 lb/200,000 lb was ineffective in reducing the incidence of gray mold and Penicillium decays on wound-inoculated fruit that was incubated for 14-18 h prior to treatment+. For preharvest uses, the fungicide is commonly used at 8-10

lb/100 gal. In view of the ineffectiveness of captan as a post-infection treatment at the rate registered, export restrictions on the fungicide in different international markets, and the visible residues of the fungicide formulation left on the fruit after treatment, captan also cannot be considered an alternative stand-alone fungicide to fungicides with post-infection activity. The fungicide, however, may be effective as a direct contact fungicide. Postharvest treatments with thiabendazole can be very effective for decay control, however, resistant populations of the pathogens *P. expansum* and *B. cinerea* against the fungicide commonly occur in packinghouses, making the efficacy of the fungicide inconsistent or economically ineffective. Thus, postharvest alternatives to TBZ are needed. In our evaluations the biological control agent Bio-Save was inconsistent and was never as effective as the fungicides Elevate, Scholar, or Penbotec. The latter two fungicides are registered in California as of July and August, 2005, respectively. Both fungicides are effective against TBZ-resistant isolates of *B. cinerea* and *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 studies that were summarized in our previous years' Annual Reports for the California Pear Board. All fungicides belong to different classes and they are classified as 'reduced-risk' by the US-EPA. In 2005, additional studies were conducted on the efficacy of Scholar, Elevate, and Penbotec on Bartlett and Bosc pears. Furthermore, we evaluated the efficacy of a new fungicide (fluoxystrobin). 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 evaluated application methods to optimize fungicide efficacy and efficiency. We also started to characterize the etiology of blue mold of pears in California. In addition to *P. expansum,* three other species of *Penicillium* were found to cause blue mold of pears.

Objectives

1) Evaluate application methods of postharvest treatments with the new reduced-risk fungicides fenhexamid (Elevate), fludioxonil (Scholar), and pyrimethanil (Penbotec). Studies will focus on 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. Experimental packing line studies.
    ii. Large-scale packinghouse studies.
2) Continue to conduct pathogen population studies to determine baseline fungicide sensitivity levels in selected commercial packinghouses and monitor the presence of TBZ-resistance.
3) Develop identification methods for species of *Penicillium* causing decay of pears using selective media and molecular techniques.
4) Evaluate the new biocontrol Arabesque as a biofumigant for management of postharvest decays.

MATERIALS AND METHODS

Evaluate preharvest applications of new fungicides for postharvest disease management.
Preharvest applications (7 and 1 day PHI) were evaluated on Bartlett, Bosc, and Asian pear fruit in commercial orchards in 2005. Fungicides and their rates that were evaluated include Scala 600SC (pyrimethanil – 18 fl oz/A), Vangard 75WG (cyprodinil - 10 oz/A), Pristine 38WG (boscalid-pyraclostrobin - 0.92 lb/A), Elevate 50WDG (fenhexamid – 1.5 lb/A), and Crown 480SC (fluoxystrobin 10 fl oz/A). 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 postharvest fruit decay, fruit were either wound-inoculated and then incubated or not inoculated but commercially handled (except for postharvest treatments), and then incubated and evaluated for natural incidence of decay. For inoculation studies, 25 fruit from each replication of each treatment were wound-inoculated with *B. cinerea* or *P. expansum* (30,000 conidia/ml). For natural incidence of decay, ca. 100 fruit per replication were dumped into pear float tanks in a packinghouse, rinsed with water, and are currently being stored at 1 C under commercial conditions for 3-4 months.

Efficacy of new postharvest fungicides. Fungicides evaluated include Elevate 50WG, Scholar 50WP, Penbotec 400SC, Pristine 38WG, and fluoxystrobin. In laboratory studies, Bartlett or Bosc pears were wound-inoculated with TBZ-resistant isolates of *B. cinerea* or *P. expansum*, incubated for 10-12 h, and then spray-treated with fungicides. Fungicides were applied as aqueous solutions or in a carnauba-based fruit coating. In an experimental packing line trial at the Kearney Agricultural Center with wound-inoculated Bosc and Bartlett pear fruit, applications of Scholar, Penbotec, or mixtures of Scholar with TBZ were done as in-line drench applications. Fruit were stored at 20 C, 95% RH for 7 to 10 days and then evaluated for 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. Inoculated fruit were evaluated after 7 weeks and results for natural incidence are still pending. 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, fenhexamid, and fludioxonil. A total of 60 isolates of *Penicillium* spp. were collected in December of 2004 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 as described previously.

Cultural and molecular characterization of *Penicillium* spp. Isolates of *Penicillium* spp. were grown on selected media at different temperatures (Pitt, 1991) and growth characteristics were evaluated and compared to descriptions provided in the literature and to reference cultures. For molecular characterization, fungal DNA was extracted from fungal mycelium and amplified in PCR reactions. For identification of *P. expansum,* specific primers from the polygalacturonase gene (Marek et al., Int. J. Food Microbiol. 89, 139-144, 2003) were used. To differentiate all species occurring on pears, a fragment ca. 450 bp in size of the beta-tubulin gene was amplified using published primers (Glass and Donaldson, Appl. Envir. Microbiol. 61, 1323-1330, 1995). The fragment was then digested with four restriction enzymes and the resulting DNA bands were separated in agarose gels and visualized under UV light after ethidium bromide staining. Banding patters were compared with those of reference cultures and isolates were grouped according to their banding patterns. Additional molecular comparisons of isolates were done using four random primers in RAPD analyses and amplified fragments were again separated and banding patterns were compared.

RESULTS AND DISCUSSION OF 2005 RESEARCH

Evaluation of preharvest fungicide applications for postharvest decay control. Preharvest treatments for postharvest decay control of pears were conducted on Bartlett, Bosc, and YaLi Asian pears in 2005. After wound-inoculation, 1-day preharvest treatments of Asian pears with either Elevate or Vangard did not reduce the incidence of gray mold or blue mold (Fig. 1). Currently, fruit being evaluated for postharvest decay from our preharvest trials is still being incubated and thus, data are pending. Our previous studies over several years indicate that preharvest fungicide treatments of pears can sometimes have additional benefits to postharvest treatments with TBZ when TBZ-resistant pathogen populations have to be controlled and thus, can be beneficial in an integrated management program. Still, the efficacy was always inconsistent and generally much lower than when fruit is postharvest treated with the new ‘reduced-risk’ fungicides that we are developing in our studies.

Efficacy of new postharvest fungicides for management of decays of Bartlett and Bosc pears. In laboratory postharvest studies, the new fungicide fluoxystrobin was evaluated on wound-inoculated Bosc pears, however, it was found to be ineffective against gray mold and blue mold at the low rates (3.5 to 7 fl oz/100 gal or 130-260 ppm) evaluated (Fig. 2). Elevate was very effective against gray mold in this study, and Pristine (at 1000 ppm – 336 ppm pyraclostrobin + 664 ppm boscalid) reduced gray mold and blue mold to zero levels. Thus, higher rates of fluoxystrobin have to be tested similar to those of pyraclostrobin in the premix Pristine. Previously we reported on phytotoxic effects on Pristine-treated Bartlett fruit when the

fungicide was applied in a fruit coating. In 2005 we again evaluated potential negative effects of this fungicide on Bartlett pear fruit and also included fluoxystrobin in these studies. Pristine was very effective against gray mold when applied as an aqueous treatment (Fig. 3). Treatments in two different carnauba-based fruit coatings, however, were not effective. Phytotoxicity was observed on the fruit as brown blotches on the surface after aqueous, as well as treatments of Pristine in fruit coatings. Thus, as observed with Pristine-treated nectarines and peaches, efficacy and phytotoxic effects of this fungicide depend on the application method, the adjuvants in the fruit coating used, and the duration of wetness after treatment. Treatments with fluoxystrobin in both carnauba fruit coatings resulted in high levels of phytotoxicity. This, together with the lack of efficacy indicates that fluoxystrobin may not be suitable for postharvest treatments of pears, especially considering that much higher rates will have to be used.

Additional laboratory studies were conducted on the efficacy of the new fungicides Pristine, Scala (Penbotec), and Scholar against three species of *Penicillium* that cause blue mold of pears. In this study, decay caused by *P. expansum, P. solitum,* or *P. commune* was reduced to very low or zero levels (Fig. 4). This indicates that the three species are all highly sensitive to the fungicides tested.

In an experimental packingline study Bosc and Bartlett pears were treated with in-line drenches of Scholar, Penbotec, or mixtures of Scholar and TBZ. All treatments, including Scholar at rates between 150 and 300 ppm, were highly effective against both gray mold and blue mold (Fig. 5). In previous years we have demonstrated that in-line drench applications are generally more effective than low-volume CDA applications. In this year's trial we additionally showed that very low rates of Scholar are still very effective in these drench applications. Fungicide residues as shown in Fig. 5 were very low ($\leq$0.1 ppm fludioxonil) indicating that a good treatment efficacy is not always related to high fungicide residues on the fruit and the application method needs to be considered when evaluating residue data. In theory, high-volume, dilute aqueous applications deposit fungicide residues in fruit wounds and leave less residues on the intact fruit surface, whereas low-volume, concentrated applications of fungicides in fruit coatings deposit more residues on the hydrophobic fruit surface.

In a commercial packingline study the efficacy of CDA treatments with Pristine, Scholar, Scala (Penbotec), and mixtures of Scholar and Scala or Elevate and fluoxystrobin were compared. As in 2004, the efficacy of some fungicide treatments (e.g., Pristine, Scholar) was higher on Bartlett than on Bosc pear (Fig. 6). In 2004 we tried to explain the reduced efficacy of Pristine by the long storage of the fruit where decay development may resume after applications of fungicides with only fungistatic action. Still, other factors do contribute to this, because on Bartlett pear, Pristine was very effective. Fluoxystrobin was more effective in this study as compared to the laboratory evaluation and blue mold decay was reduced by 60% in the Elevate-fluoxystrobin mixture. As in 2004, the Penbotec-Scholar mixture was a highly effective treatment. In 2005, the Scholar-TBZ treatments were also very effective. This indicates that mixtures may be the most effective strategy not only for decay management, but also for resistance management.

Cultural and molecular characterization of *Penicillium* spp. Different colony morphologies were found after culturing a collection of *Penicillium* isolates from pears on specific growth media. *P. roquefortii* could be most easily differentiated from the other species of *Penicillium* by its faster growth rate and spreading colony. Reference cultures of *P. solitum* and *P. commune* looked very similar.

In a molecular analysis using *P. expansum*-specific PCR amplification primers a DNA fragment was amplified from the majority of isolates. When an amplified beta-tubulin fragment was digested with four restriction enzymes, different banding patterns were observed among different *Penicillium* isolates. Isolates could be placed into one of four banding groups that correlated with reference cultures of *P. expansum, P. solitum, P. commune,* and *P. roquefortii*, respectively (Fig. 7). Isolates that successfully amplified a fragment using the *P. expansum*-specific primers all grouped with *P. expansum*. Using four random primers in a RAPD analysis, banding patterns within these RFLP groups were identical or very similar (Fig. 8). Thus, we were able to differentiate species of *Penicillium* from pear that have a similar cultural appearance and are not

easily distinguished by traditional taxonomic methods using relatively simple molecular methods that do not involve DNA sequence analysis.

The frequency of each of the four species of *Penicillium* was summarized for our 2003 and 2004 collections. In both years, *P. expansum* with 68.3 to 88% of the total isolates was the most common species involved in blue mold decay of pears (Table 1). *P. solitum* and *P. commune* were more frequently isolated in 2003 than in 2004. In pathogenicity tests, *P. expansum* was found to be the most virulent species, resulting in the largest decay lesions. Inoculations with *P. solitum* and *P. commune* also resulted in a high incidence of decay. Lesion sizes, however, were much smaller than for *P. expansum*. Decay development after inoculation with *P. roquefortii* was much more erratic and lesion sizes were very variable and thus, this species is the least virulent of the four species of *Penicillium*. Based on these results, the etiology of blue mold of pears in California is more complex than previously thought, but still similar to that described in the Pacific Northwest growing region.

Pathogen sensitivities to TBZ, fenhexamid, and fludioxonil. Collections of isolates of *Penicillium* spp. obtained in late 2004 from fruit stored in one packinghouse were evaluated for their in vitro sensitivity to TBZ, fludioxonil, and pyrimethanil. Only few isolates of *B. cinerea* were obtained during the sampling. Among the 60 isolates of *Penicilllium* evaluated, 22 isolates were highly resistant to TBZ ($EC_{50} \geq 18$ ppm) (Fig. 9). Thus, pathogen populations resistant to TBZ are consistently identified in California packinghouses over the years, emphasizing the need for new postharvest treatments. Sensitivities against fludioxonil ranged between 0.008 and 0.021 ppm. This range is similar to the one established in 2003 (0.010 to 0.032 ppm). A wider range of sensitivities was found for pyrimethanil with EC_{50} values between 0.007 and 1.21 ppm as compared to a range of between 0.105 and 0.669 ppm in 2003. Implications from these baseline studies on fungicide sensitivities suggest that new materials with a broad range of toxicity to target pathogen populations need to be mixed with other fungicides to prevent the development of resistance.

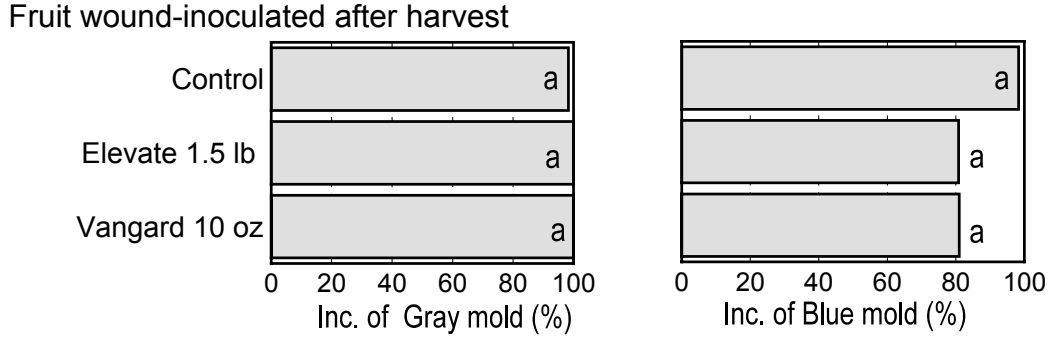
Evaluate the new biocontrol Arabesque as a biofumigant for management of postharvest decays. Our studies on other fruit crops have indicated that treatments with Arabesque can effectively reduce the incidence of postharvest gray mold. To date, we have not tested this material against any postharvest Penicillium decay. Studies on postharvest decay control of pears were planned but because of administrative delays in signing secrecy and material transfer agreements with the manufacturer (i.e., AgraQuest) of the biofumigant product, no material was available and no studies could be done. In the meantime, however, we were able to sign agreements and Arabesque will be available to us for evaluation in the coming season.

Table 1. Species of *Penicillium* causing blue mold decay of stored pears in California

Species	Number of isolates	%	Number of isolates	%
P. expansum	41	68.3	66	88
P. solitum	9	15	2	2.7
P. commune	6	10	2	2.7
P. roquefortii	4	6.7	4	5.3
Total	60	100	75	100

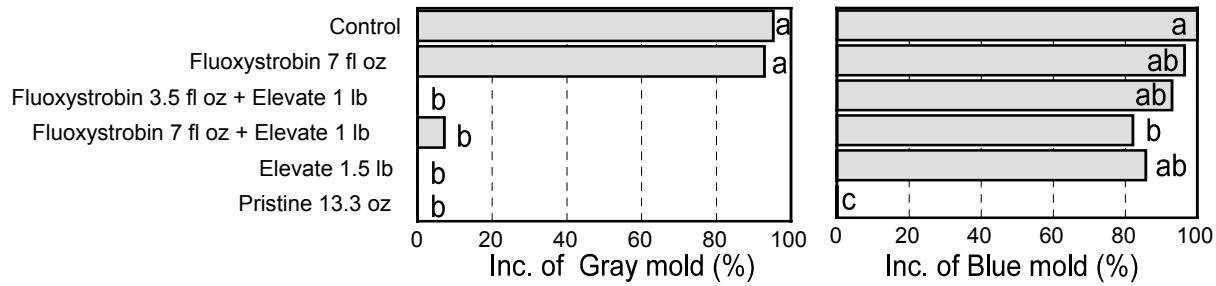
Isolates of Penicilium were obtained from decayed Bartlett and Bosc pears that were stored at a packinghouse for 3-5 months. Species identification was done by cultural morphology and by a DNA identification method that is based on restriction analysis of a beta-tubulin gene fragment.

Fig. 1. Evaluation of 1-day preharvest treatments with new fungicides for management of gray mold and blue mold decay of YaLi Asian pears 2005



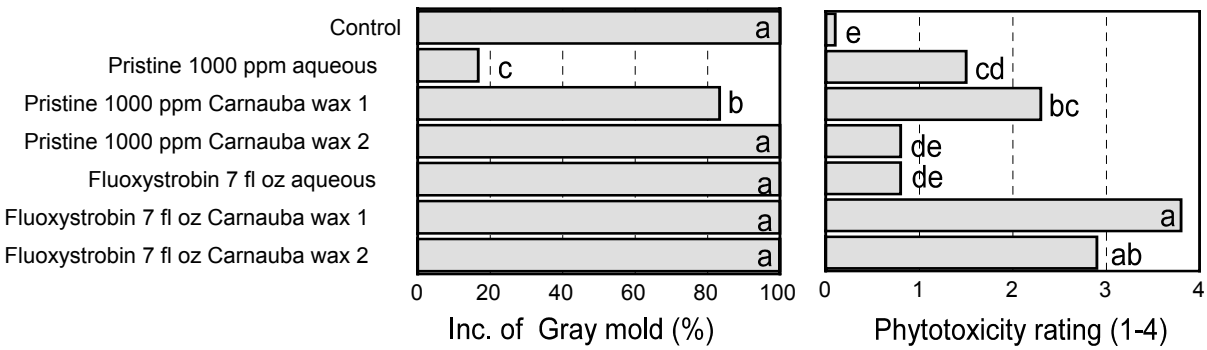
applications were done in the field 1 day before harvest using an air-blast sprayer at a rate of 100 gal/A. Fruit were harvested, wound-inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, and incubated for 7 days at 20C.

Fig. 2. Evaluation of postharvest treatments with new fungicides for management of gray mold and blue mold decay of Bosc pears in a laboratory study 2005



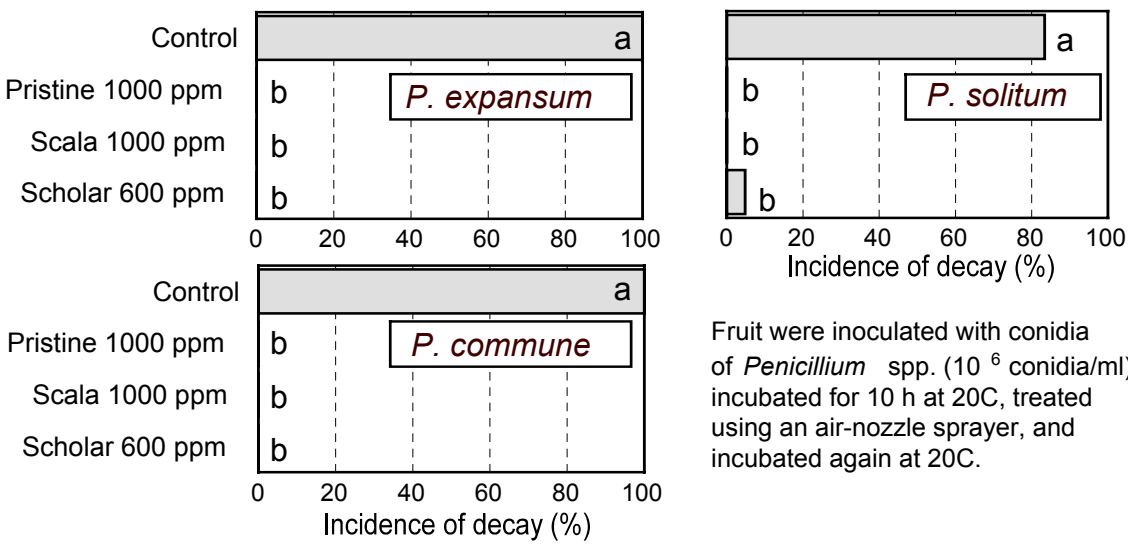
Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum* , incubated for 10 h at 20C, treated using an air-nozzle sprayer (high volume - 100gal/200,000 lb), and incubated again at 20C.

Fig. 3. Evaluation of postharvest treatments with new fungicides for management of gray mold of Bartlett pears in a laboratory study 2005



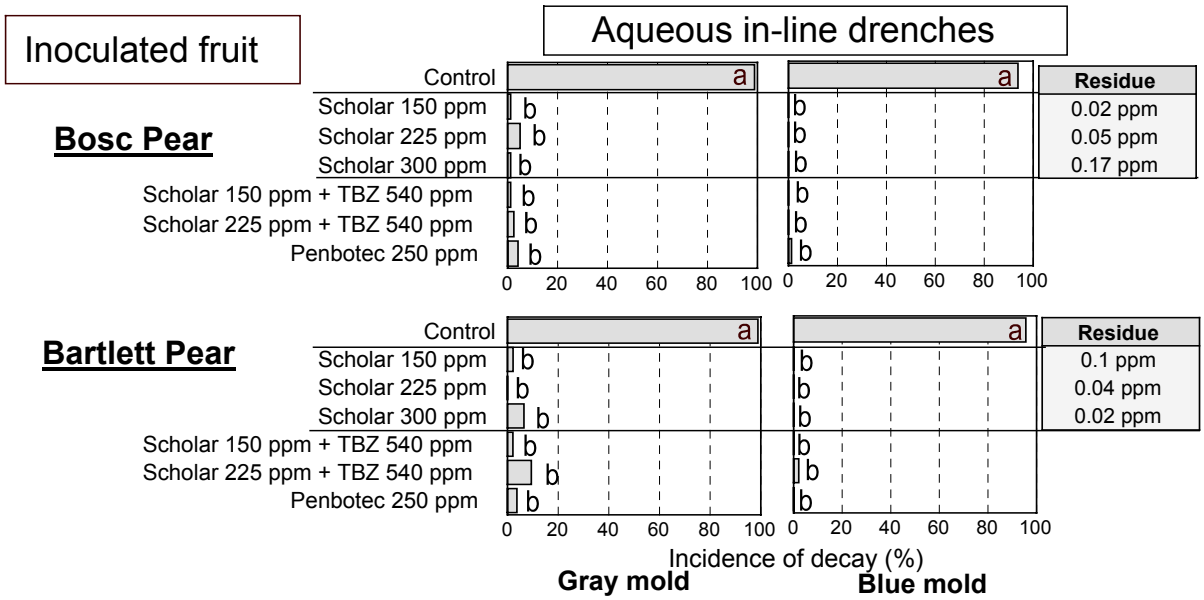
Fruit were inoculated with conidia of a TBZ-resistant isolate of *Botrytis cinerea* , incubated for 10 h at 20C, treated using an air-nozzle sprayer, and incubated again at 20C. Treatments were applied either as aqueous applications or in a carnauba-based fruit coating.

Fig. 4. Efficacy of postharvest fungicides against decay of Bartlett pears caused by three different blue mold pathogens



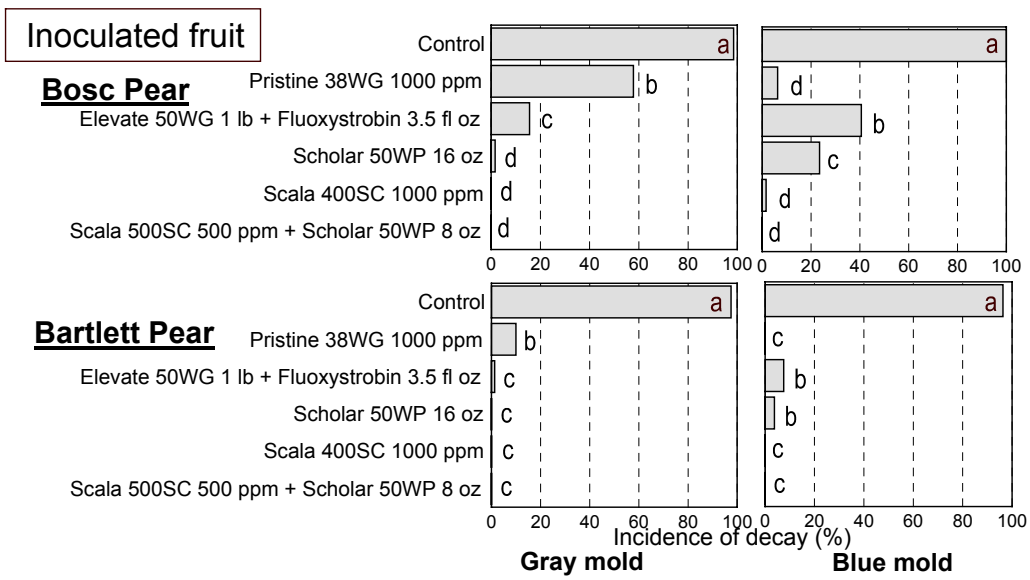
Fruit were inoculated with conidia of *Penicillium* spp. (10^6 conidia/ml), incubated for 10 h at 20C, treated using an air-nozzle sprayer, and incubated again at 20C.

Fig. 5. Evaluation of treatments with Scholar 230SC for management of gray mold and blue mold decay of pears
Experimental packingline study 2005



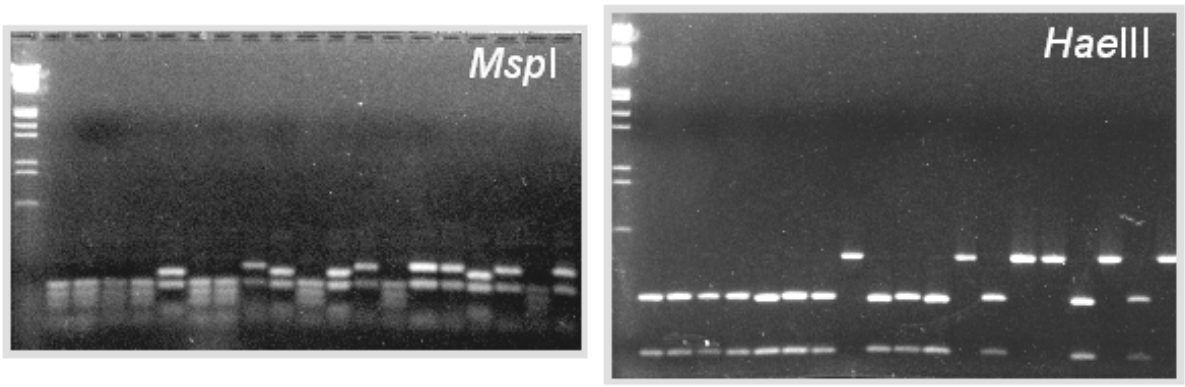
Fruit were wound-inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* ($5x10^4$ conidia/ml) or *Penicillium expansum* (10^5 conidia/ml). Treatments were applied 14-16 h after inoculation. Drenches were applied as aqueous solutions.

Fig. 6. Evaluation of postharvest treatments for management of gray mold and blue mold decay of pears
Commercial packingline study 2005



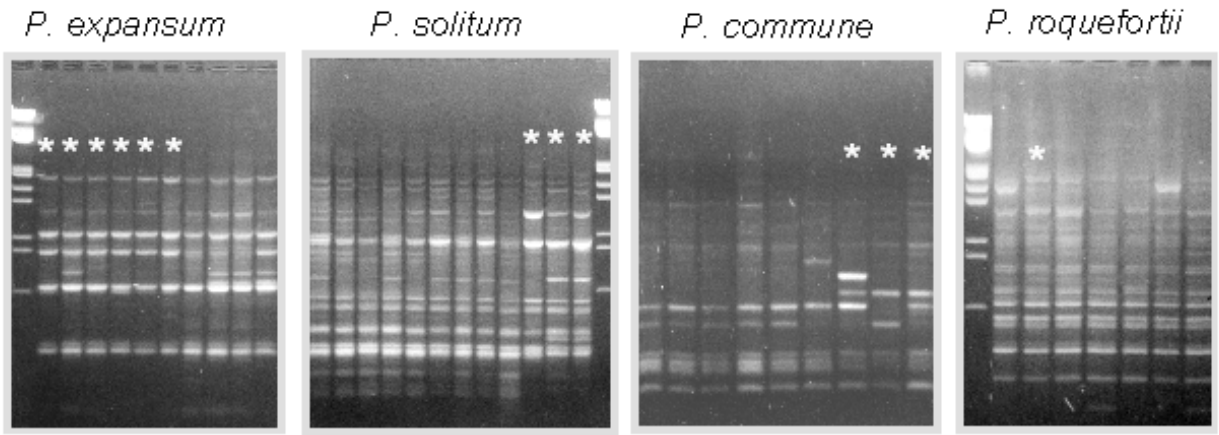
Fruit were wound-inoculated with conidia of TBZ-resistant isolates of *B. cinerea* ($5x10^4$ conidia/ml) or *P. expansum* (10^5 conidia/ml). Treatments were applied as aqueous CDA applications at a rate of 33 gal/200,00 lb. Fruit were stored at 0 C for 7 weeks.

Fig. 7. Identification of *Penicillium* species from pear by RFLP analysis of an amplified beta-tubulin DNA fragment



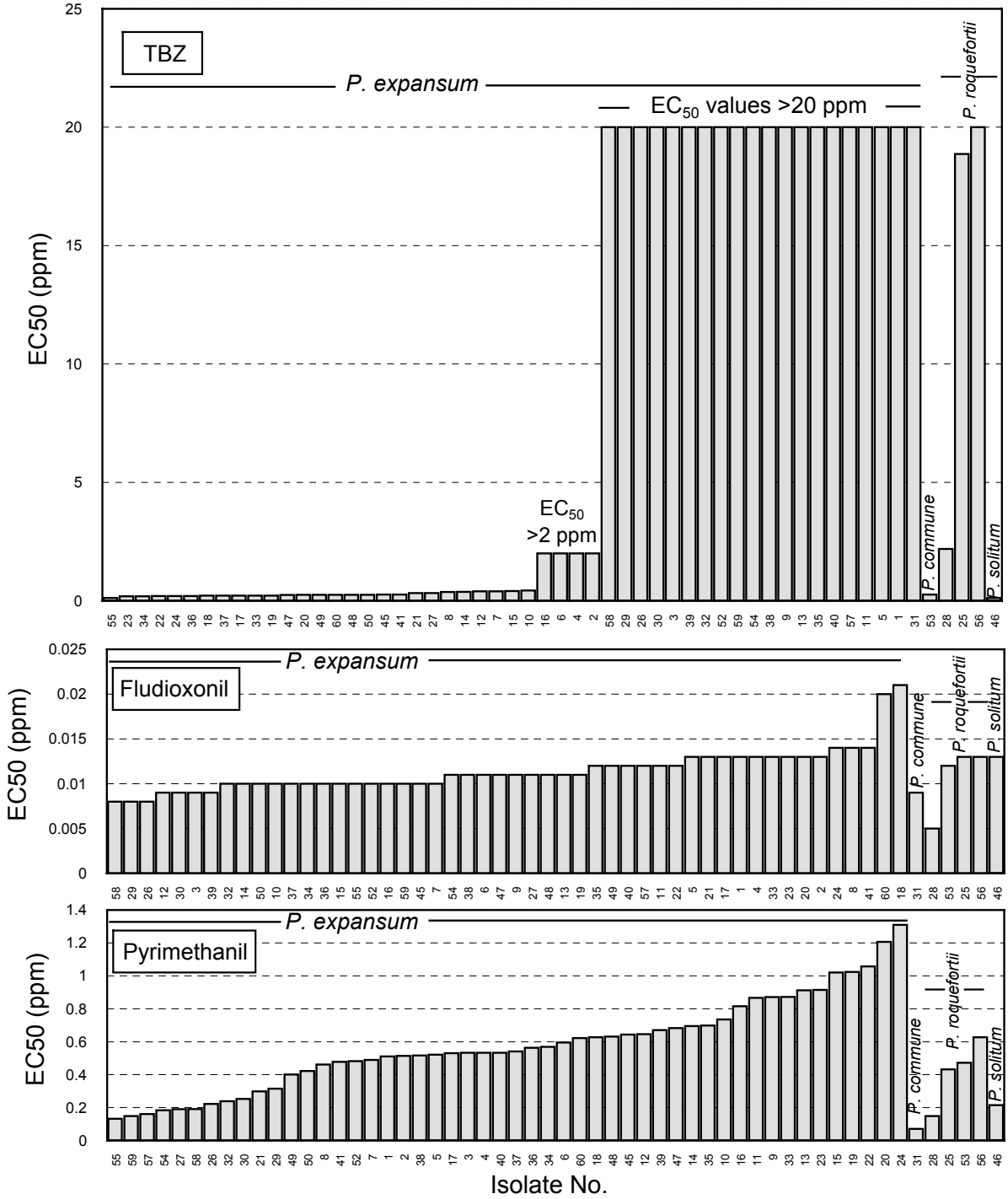
Using published primers, a ca. 450 bp fragment of the beta-tubulin gene was amplified from isolates from *Penicillium*. The amplified fragment was then digested with restriction enzymes *Msp*I (left) or *Hae*III (right) and DNA bands were separated in agarose gels.

Fig. 8. Molecular diversity within RFLP groups of *Penicillium* species from pear based on RAPD analysis



DNA from from isolates from *Penicillium* from each of the RFLP groups was amplified using random primers. After agarose gel electrophoresis, banding patterns of unkown isolates were compared with those of reference cultures (indicated by *).

Fig. 9. Fungicide sensitivities for TBZ and baseline sensitivities for fludioxonil and pyrimethanil for isolates of *Penicillium* spp. from pear in California - 2005



Isolates of *Penicillium* spp. were collected from decayed Bartlett and Bosc pear fruit in a packinghouse in December of 2004. 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.

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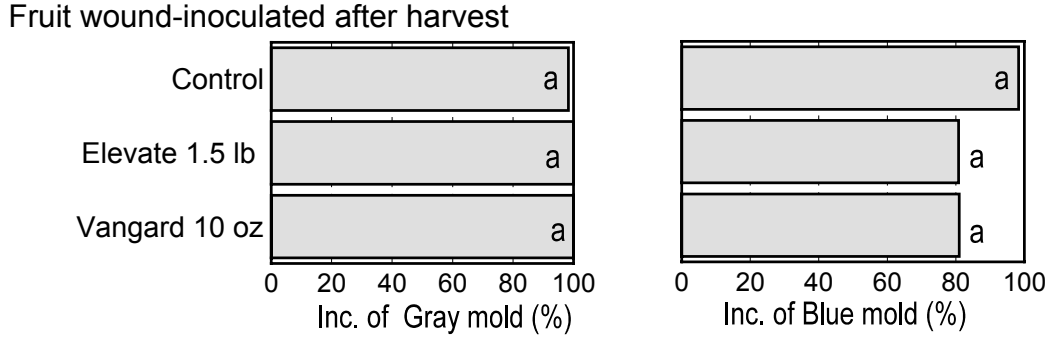
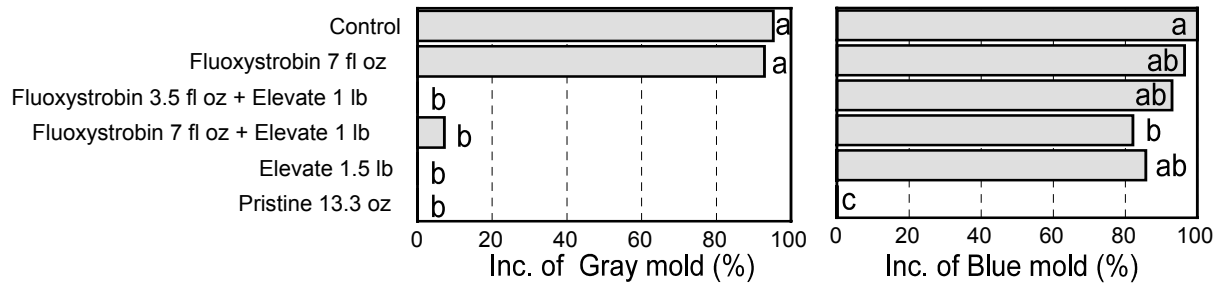


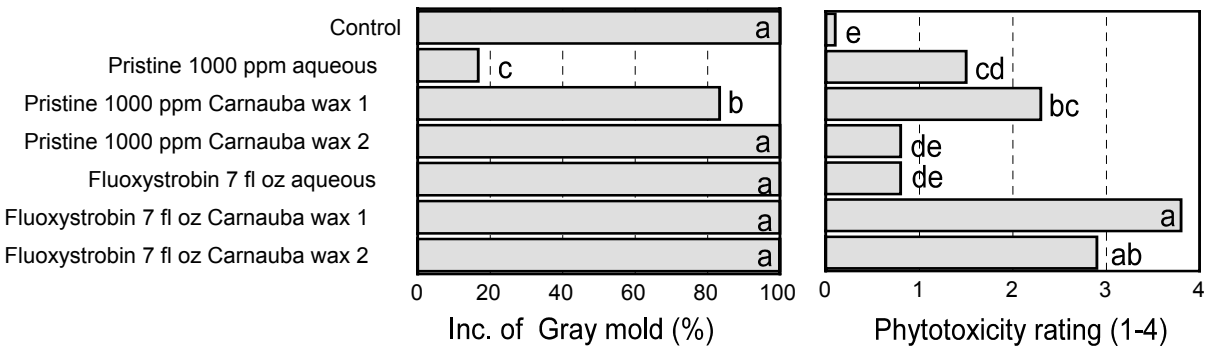
applications were done in the field 1 day before harvest using an air-blast sprayer at a rate of 100 gal/A. Fruit were harvested, wound-inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum*, and incubated for 7 days at 20C.

Fig. 2. Evaluation of postharvest treatments with new fungicides for management of gray mold and blue mold decay of Bosc pears in a laboratory study 2005



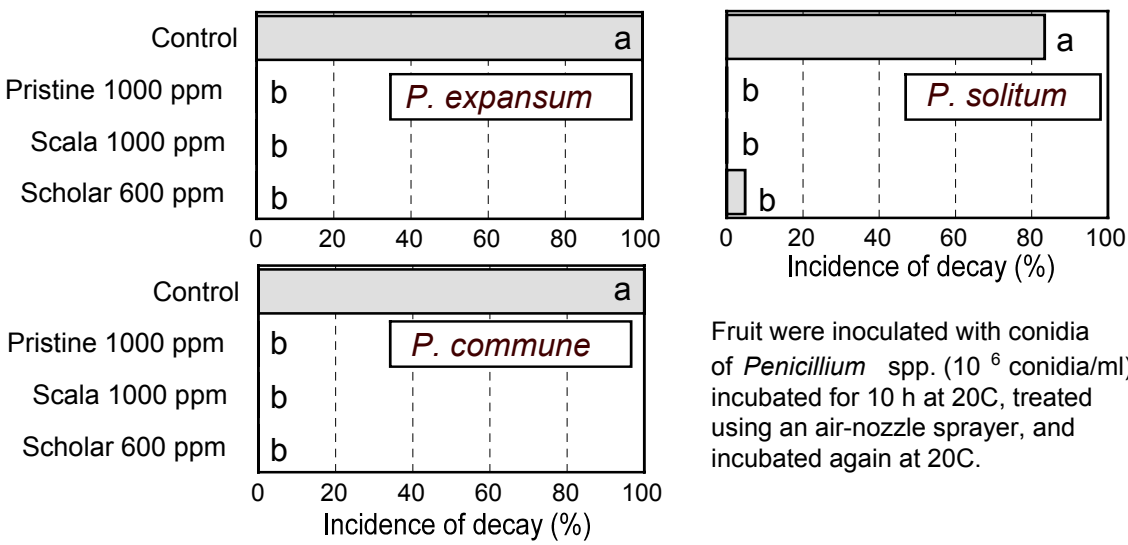
Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* or *Penicillium expansum* , incubated for 10 h at 20C, treated using an air-nozzle sprayer (high volume - 100gal/200,000 lb), and incubated again at 20C.

Fig. 3. Evaluation of postharvest treatments with new fungicides for management of gray mold of Bartlett pears in a laboratory study 2005



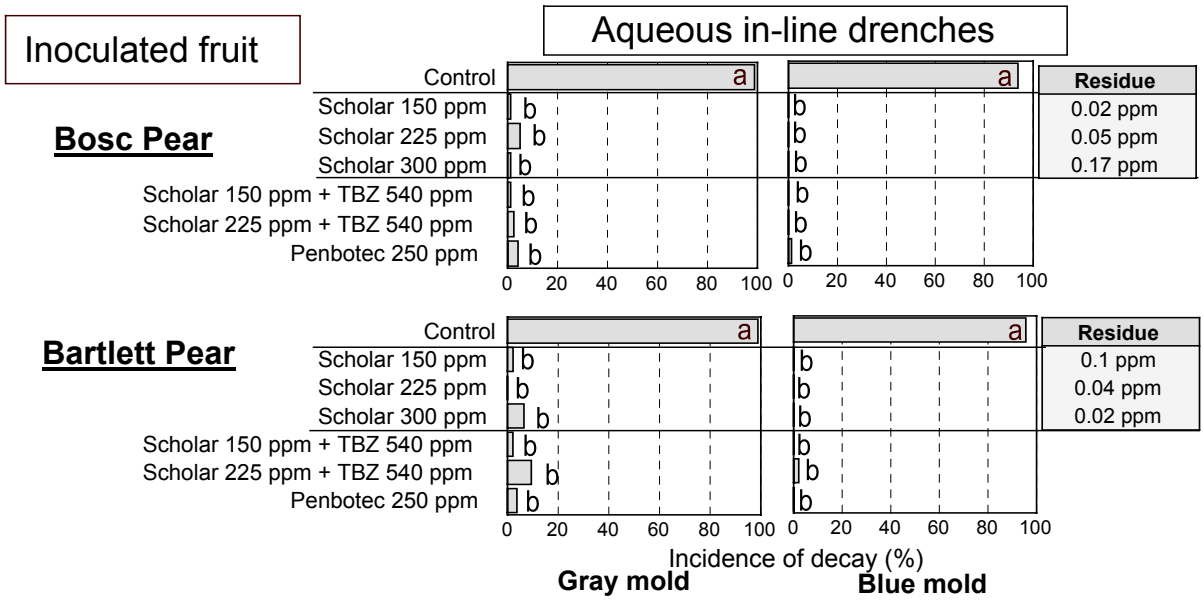
Fruit were inoculated with conidia of a TBZ-resistant isolate of *Botrytis cinerea* , incubated for 10 h at 20C, treated using an air-nozzle sprayer, and incubated again at 20C. Treatments were applied either as aqueous applications or in a carnauba-based fruit coating.

Fig. 4. Efficacy of postharvest fungicides against decay of Bartlett pears caused by three different blue mold pathogens



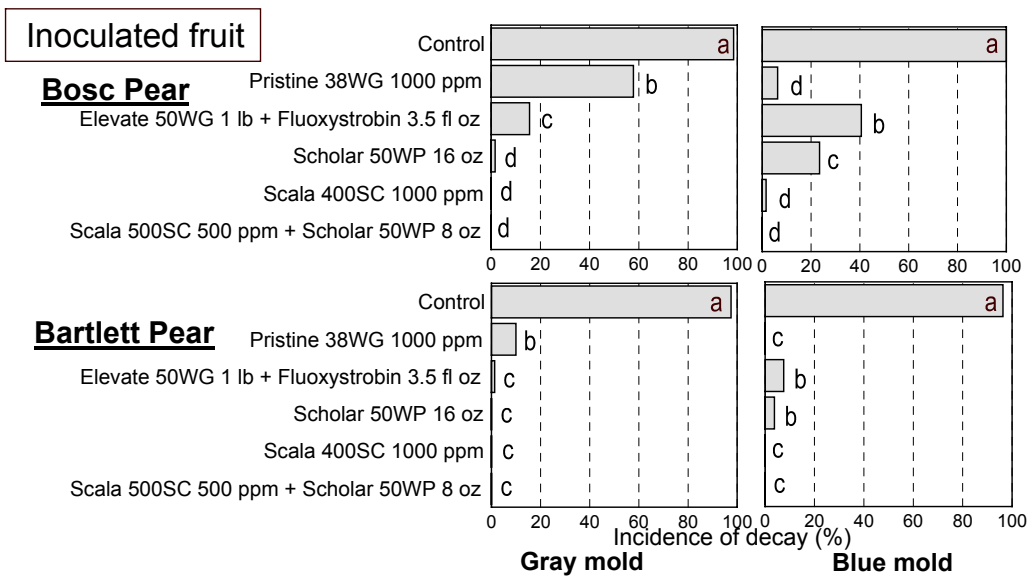
Fruit were inoculated with conidia of *Penicillium* spp. (10^6 conidia/ml), incubated for 10 h at 20C, treated using an air-nozzle sprayer, and incubated again at 20C.

Fig. 5. Evaluation of treatments with Scholar 230SC for management of gray mold and blue mold decay of pears
Experimental packingline study 2005



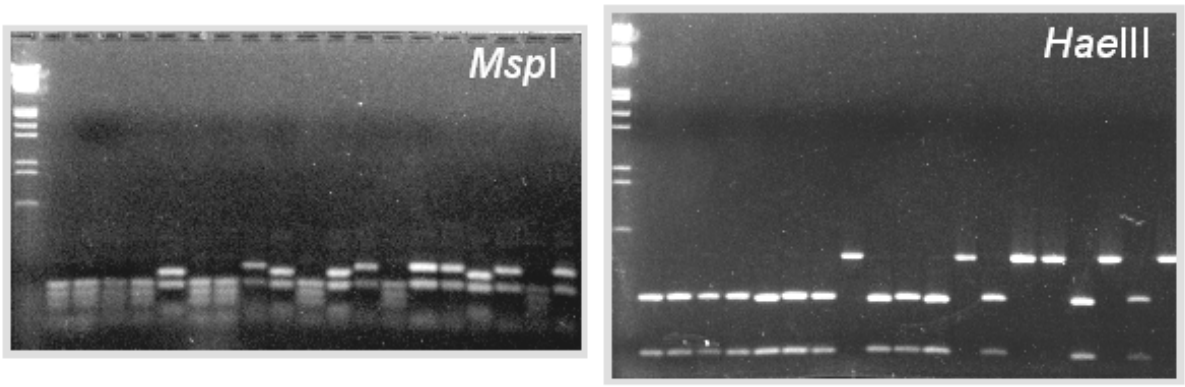
Fruit were wound-inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* ($5x10^4$ conidia/ml) or *Penicillium expansum* (10^5 conidia/ml). Treatments were applied 14-16 h after inoculation. Drenches were applied as aqueous solutions.

Fig. 6. Evaluation of postharvest treatments for management of gray mold and blue mold decay of pears
Commercial packingline study 2005



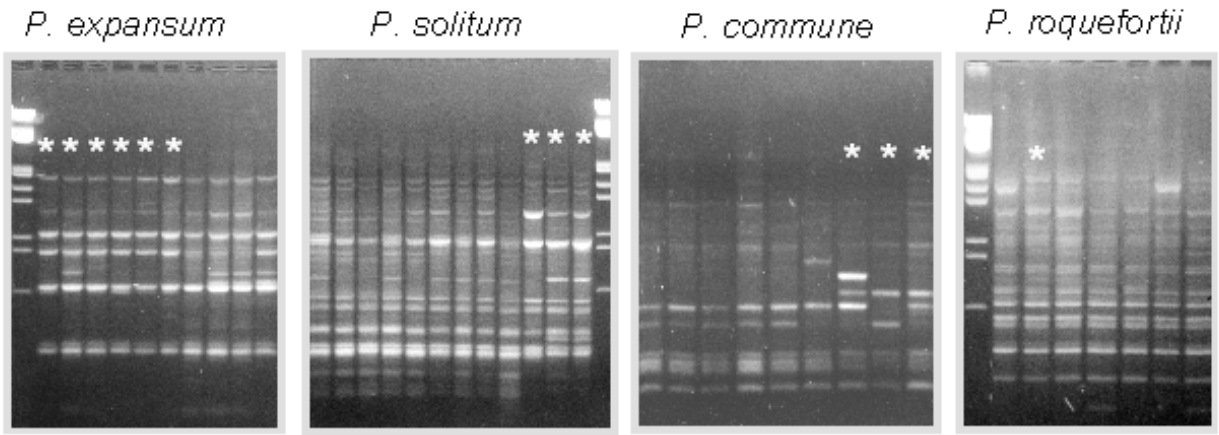
Fruit were wound-inoculated with conidia of TBZ-resistant isolates of *B. cinerea* ($5x10^4$ conidia/ml) or *P. expansum* (10^5 conidia/ml). Treatments were applied as aqueous CDA applications at a rate of 33 gal/200,00 lb. Fruit were stored at 0 C for 7 weeks.

Fig. 7. Identification of *Penicillium* species from pear by RFLP analysis of an amplified beta-tubulin DNA fragment



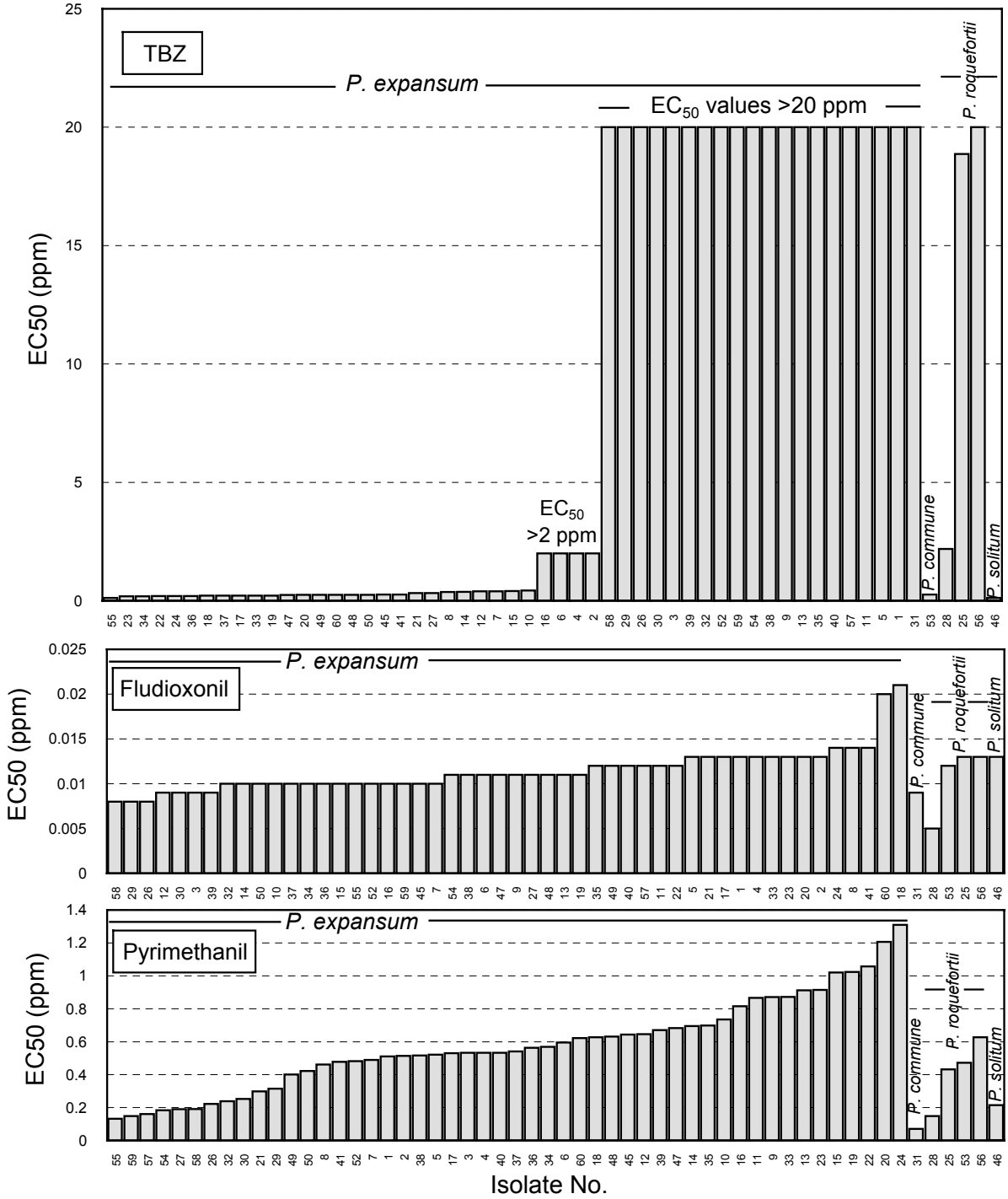
Using published primers, a ca. 450 bp fragment of the beta-tubulin gene was amplified from isolates from *Penicillium*. The amplified fragment was then digested with restriction enzymes *Msp*I (left) or *Hae*III (right) and DNA bands were separated in agarose gels.

Fig. 8. Molecular diversity within RFLP groups of *Penicillium* species from pear based on RAPD analysis



DNA from from isolates from *Penicillium* from each of the RFLP groups was amplified using random primers. After agarose gel electrophoresis, banding patterns of unkown isolates were compared with those of reference cultures (indicated by *).

Fig. 9. Fungicide sensitivities for TBZ and baseline sensitivities for fludioxonil and pyrimethanil for isolates of *Penicillium* spp. from pear in California - 2005



Isolates of *Penicillium* spp. were collected from decayed Bartlett and Bosc pear fruit in a packinghouse in December of 2004. 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.