Annual Report - 2010

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 2010 RESEARCH

- Experimental packingline studies were conducted on the management of postharvest decays of pears. The DMI fungicide difenoconazole was not effective against gray mold, but highly effective against blue mold of Bosc pear, similar to Scholar or Penbotec.
- Rates of fludioxonil-difenoconazole mixtures were evaluated. A newly developed pre-mixture of the two fungicides was similarly effective as the tank mixture and thus, will be pursued for registration on pome fruit. Using in-line drench applications, a very high efficacy was obtained with low fungicide rates (180 ppm fludioxonil + 540 ppm difenoconazole).
- Treatment additives (such as Tween 80 or Perasan) provided no additional benefit to fungicide (e.g., difenoconazole or difenoconazole – fludioxonil mixtures) treatments that were the highly efficacious at rates tested.
- Lower (55F) fruit incubation temperatures between inoculation and treatment enhanced fungicide efficacy in some cases as compared to higher temperatures (68F). This could be correlated to fungal biology (spore germination rates at the two temperatures). This temperature effect will provide some more flexibility for postharvest fungicide treatment timing in locations/at times with low temperatures during harvest and packing, but less likely for California conditions where temperatures can still be high in late summer and fall. The effects of fruit and fungicide solution temperature on fungicide uptake by the fruit is currently under investigation.
- A formulation of polyoxin-D (Ph-D) that potentially could be used for organic fruit production was effective against gray mold decay, but not as effective as Scholar or Penbotec. Blue mold was not controlled by Ph-D.
- Most of the fludioxonil- and pyrimethanil-resistant isolates that were obtained in laboratory selection assays were stable in culture and were pathogenic in fruit inoculations.
- In fruit co-inoculations with a sensitive wild-type isolate of *P. expansum*, isolates highly resistant to fludioxonil were not recovered, whereas isolates highly resistant to pyrimethanil or with an intermediate level of resistance to both fungicides competed against the wild-type isolate. These data indicate that with repeated fungicide applications some isolates may become predominant, and that proper anti-resistance strategies have to be followed with the use of these new fungicides.
- The in vitro sensitivity of mycelial growth of *Penicillium expansum* and other *Penicillium* spp. to difenoconazole was evaluated. The baseline sensitivity range was 0.004 to 0.015 ppm. Sensitivity of isolates was normally distributed with most isolates sensitive between 0.006 and 0.01 ppm in a frequency histogram.
- Results of sanitation studies are pending.

INTRODUCTION

Gray mold, caused by *Botrytis cinerea,* and blue mold, caused by *Penicillium expansum* and 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, as well as occasionally bull's eye rot. 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. Preharvest treatments with fungicides (e.g., Ziram, Captan, Pristine, Elevate) to manage postharvest decays have been inconsistent and generally unsatisfactory in their efficacy when fruit are sanitized and washed immediately after harvest. These treatments significantly reduced the incidence of postharvest gray mold decay when field bins of fruit were not washed and only placed into cold storage. Still, these treatments were not as effective as when used as postharvest treatments (i.e., Elevate, Judge). Until the recent registration of Penbotec (pyrimethanil) and Scholar (fludioxonil), only thiabendazole and captan (Captan 50WP) were available for postharvest use on pears. New fungicides were developed by us and others because Captan at the registered postharvest rate of 2 lb/200,000 lb is ineffective against blue mold and resistance against TBZ (Mertect 340F) is widespread in populations of *B. cinerea* and *P. expansum.* The risk of resistance development in the postharvest pear pathogens to fungicides is high because fruit 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. Additionally, the pathogens produce abundant spores, favoring the selection of resistant individuals. Scholar and Penbotec were registered in California in 2005, whereas Elevate/Judge was federally registered in 2007. Although five fungicides (Captan, TBZ, Scholar, Penbotec, Judge) are now registered for postharvest use on pears, only two of them are highly effective against TBZ-resistant blue mold (Scholar, Penbotec). The latter two fungicides were evaluated for their risk to develop resistance. We were able to select isolates of *P. expansum* resistant to fludioxonil and/or pyrimethanil from sensitive wild type populations in laboratory studies. The average resistance frequencies for pyrimethanil and fludioxonil were estimated to be 3.5×10^{-7} and 2.2×10^{-7}, respectively. Thus, the risk of resistance to develop against these two fungicides is considered high. In 2010 we continued to characterize fludioxonil- and pyrimethanil-resistant isolates of *P. expansum* to obtain additional information for estimating resistance risk.

To prevent field resistance from developing in packinghouses, anti-resistance strategies need to be followed. This includes the use of fungicide rotations and mixtures. To identify additional potential postharvest fungicides, we continued our evaluation of new materials against blue and gray mold. The sterol biosynthesis inhibiting fungicide difenoconazole was suggested by us to the registrant as a mix partner to prevent resistance in populations of *Penicillium* spp. In 2010 we continued our evaluations on the efficacy of this compound used by itself and in mixtures with Scholar to optimize usage rates and application methods. We have been working in close collaboration with the registrant of Scholar, Syngenta Crop Protection that is very supportive of these studies. One goal of this collaboration is the ultimate design of a fludioxonil-difenoconazole pre-mixture. Using a pre-mixture, with every postharvest application, there is a reduced pressure for resistant individuals to be selected as compared to single-fungicide treatments. Additionally, we evaluated polyoxin-D (Ph-D) as a postharvest treatment. We used a formulation that potentially could be registered for organically grown fruit to provide new treatment options for this market.

The development of additional postharvest fungicides is critical and timely, because the new treatments pyrimethanil (Penbotec), fludioxonil (Scholar), and fenhexamid (Judge) are just recently being utilized in California because many countries also had to establish maximum residue limits (MRLs) to allow marketing of fruit with our trade partners. Our goal is to have several highly effective new fungicides with different modes of action registered for postharvest use on pear in order to be able to design resistance management strategies.

Objectives

1) Comparative evaluation of postharvest fungicides (fludioxonil - Scholar, pyrimethanil – Penbotec, and difenoconazole) 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. Evaluation of application technologies for postharvest fungicides (e.g., dips, drenches, and low-volume single-bin drench treatments in the field immediately after harvest and in the packinghouse).
    ii. Experimental packingline treatments with postharvest fungicides (especially difenoconazole) either alone or in mixtures with other registered fungicides such as Scholar and Penbotec.
2) Evaluation of the pathogenicity, virulence, and fitness of naturally occurring isolates of *P. expansum* that are resistant to fludioxonil or pyrimethanil.

i. Evaluation of pathogenicity and virulence: Inoculation of pear fruit and comparison of decay development using sensitive and resistant isolates.
ii. Evaluation of fitness: Co-inoculation of pear fruit with sensitive and resistant isolates and determination of the proportion of sensitive and resistant spore progeny produced on the decaying fruit.

3) Evaluation of captan, chlorine, acidified hydrogen peroxide (Perasan), and JBL-08A as sanitizers of fungicide drench solutions or other water tank systems (e.g., float tanks).
i. Experimental packing line treatments with sanitizers used alone or in mixtures with fungicides.
ii. Evaluation of application technologies for sanitizers without using pear float (e.g., elevators in aqueous dump tanks, dry dumps on impact-absorbing foam rollers, etc.).

MATERIALS AND METHODS

Efficacy of postharvest treatments and application methods using single fungicides and mixtures. The efficacy of difenoconazole (formulation A8574D), Scholar 230SC, as well as a mixture and a pre-mixture (i.e., A18720A) of these two fungicides were evaluated using different rates and were compared to treatments with Penbotec. In addition, treatments with polyoxin-D (Ph-D) were also done. For selected treatments, Tween 80 was added tot a final concentration of 0.05% to potentially increase efficacy. In another test, Perasan at 80 ppm was added to a drench treatment with difenoconazole. Bosc pears were wound-inoculated with TBZ-resistant isolates of *B. cinerea* or *P. expansum*, incubated for ca. 16 h, and then treated. Fruit were first sprayed with chlorine at 100 ppm and then rinsed with water. Fungicides were applied on an experimental packingline at the Kearney AgCenter as aqueous solutions using in-line drench applications that were followed by low-volume spray applications with fruit coating (Decco 231, a carnauba-based coating). Alternatively, fungicides were applied directly in fruit coating by low-volume spray (CDA) application at a rate of 25 gal/200,000 lb fruit. After treatment, fruit were stored at 20 C, 95% RH for 6 to 8 days and then evaluated for the incidence of decay. Data were analyzed using analysis of variance and least significant difference mean separation procedures of SAS 9.1.

In some of the tests, we compared incubation temperatures of 55F (12.5C) and 68F (20C) from the time of inoculation to treatment. This was done to simulate environmental conditions during harvest and packing in late summer/fall when temperatures can vary widely under California conditions, but are generally low under Pacific Northwest conditions.

Baseline sensitivities for difenoconazole against mycelial growth of *P. expansum* and other species of *Penicillium*. A total of 69 isolates of *Penicillium* spp. (mainly *P. expansum* in addition to several isolates of *P. commune* and *P. solitum*) from our packinghouse collections from 2005-2008 were included in the evaluation. 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.

Evaluation of the pathogenicity, virulence, and competitiveness of naturally occurring isolates of *P. expansum* that are resistant to fludioxonil or pyrimethanil. For this study, representative isolates resistant to fludioxonil (moderately or highly resistant), pyrimethanil (all highly resistant), or moderately resistant to both fungicides that were generated in laboratory selection studies were used. For evaluation of pathogenicity and virulence, fruit were inoculated with conidial suspensions (5×10^5 conidia/ml) and lesion diameters were determined after 6 days of incubation at 20C. For evaluation of competitiveness, fruit were co-inoculated with equal amounts of conidia from a sensitive wild-type isolate of *P. expansum* and one of the resistant isolates. Fruit were incubated for 6-7 days at 20C and conidia from the decay lesion were collected, single-spored, and 10-12 single-spore cultures from each mixture inoculation were evaluated for their fungicide sensitivity. Competiveness was based on the proportion of resistant to sensitive progeny. As controls, fruit were also inoculated with each of the individual isolates and conidia were collected and characterized as described above. Experiments were done twice for each isolate combination. One pyrimethanil-resistant isolate, 2 isolates moderately resistant to fludioxonil and pyrimethanil, and 2 isolates highly resistant to fludioxonil were evaluated.

RESULTS AND DISCUSSION OF 2010 RESEARCH

Efficacy of postharvest treatments and application methods using single-fungicides, mixtures, and pre-mixtures. Experimental packingline studies were conduced to evaluate single-fungicide, mixture, and pre-mixture materials to optimize treatment efficacy. Aspects evaluated included: comparison of efficacy of fludioxonil, difenoconazole, and pyrimethanil; rate optimization of fludioxonil-difenoconazole mixtures; efficacy of a pre-

mixture; efficacy of Ph-D; evaluation of treatment additives and comparison of low-volume spray and in-line drench applications; comparison of fruit incubation temperatures after inoculation.

Comparison of efficacy fludioxonil, difenoconazole, and pyrimethanil. As previously established, fludioxonil (Scholar) was highly effective against gray mold and blue mold decays even when applied at a low rate of 8 fl using the SC formulation (equivalent to 138 ppm when applied at 100 gal/200,000 lb) (Figs. 1,2). Penbotec applied at 12.8 fl oz (383 ppm) was also very effective, whereas difenoconazole at 16 or 20 fl oz (430 or 540 ppm) was only highly effective against blue mold decay (Figs. 1,2). These fungicides provided a very high efficacy although treatments were applied 16 h after inoculation and incubation at 20C.

Rate optimization of fludioxonil-difenoconazole mixtures and efficacy of a pre-mixture. Both rates of the fludioxonil-difenoconazole mixture evaluated were similarly highly effective in reducing gray mold and blue mold decays when applied as in-line drench applications (Figs. 2,4). The pre-mixture of the two fungicides (when applied at the same rates as the mixture: 540 ppm difenoconazole, 172/180 ppm fludioxonil) was similar (Fig. 2) or slightly less effective (Fig. 1) as compared to the mixture. Thus, these results will facilitate the development of this new pre-mixture.

Efficacy of Ph-D. Polyoxin-D was evaluated in one study. Incidence of gray mold was reduced by ca. 50% as compared to the control, but there was no effect against blue mold (Fig. 3). Thus, the efficacy was not as high as for the conventional fungicides evaluated. Still, because Ph-D was evaluated using an organic formulation, this compound could be of benefit for organic production under conditions, where gray mold is the major pathogen, e.g., on Asian pears.

Evaluation of treatment additives and comparison of low-volume spray and in-line drench applications. When the sanitizing agent Perasan was added to difenoconazole, the efficacy in reducing decay was not increased as compared to difenoconazole alone (Fig. 3). When Tween 80 was added to the fludioxonil–difenoconazole pre-mixture, the efficacy was similarly high as when the pre-mixture was used by itself (Fig. 4). This additive should be evaluated again under conditions where a higher incidence of decay is obtained with the pre-mixture treatment, for example, by extending the time between inoculation and treatment or by using lower fungicide rates. In 2009 we had evaluated Triton X-100 (0.02% v/v) as an additive and we found that this compound in most cases significantly reduced fungicide efficacy.

When CDA and in-line drench applications were compared in their efficacy, in-line drenches were generally more effective than the spray applications (Fig. 1: Scholar and difenoconazole for blue mold; Fig. 3: difenoconazole). An equally high efficacy of treatments using both methods was demonstrated in other studies (Fig. 1: Scholar gray mold; Fig. 4: pre-mixture) due to the inherent high activity of these treatments. In-line drench applications are recommended as a postharvest treatment method for pears because over the years they have provided consistent high decay control in our studies.

Comparison of fruit incubation temperatures after inoculation. When fruit were incubated at 12.5 C (55F) for 16 before treatment, the efficacy of difenoconazole against blue mold was much higher than fruit were incubated at 20C (68F) for the same timer period (Fig. 3). Gray mold was not reduced or only slightly reduced by the fungicide using both temperatures. These efficacy data correlated well with microscopic observations of fungal spores that were incubated on agar plates for the same time periods at the same temperatures. Spores of *P. expansum* had germinated >95% when incubated at 20C and germ tubes were rather long, whereas at 12.5C spores were still in the pre-germination phase (i.e., swelling). This explains why treatments with difenoconazole were more effective using the 12.5C- incubation. In contrast, spores of *B. cinerea* had germinated >95% at both temperatures (although germ tubes were longer at 20C). This explains that there was no major difference in efficacy against gray mold using the two incubation temperatures. These studies can explain observations of the high treatment efficacy under cool-temperature conditions. Still, differential fungicide uptake using solutions at different temperatures and of fruit at different temperatures also needs to be considered. This is currently under investigation.

Summary of postharvest fungicide treatments. In these postharvest studies we found that mixtures of Scholar with difenoconazole were highly effective in managing gray and blue molds of pear. The new pre-mixture also showed to be very effective and was compatible with the carnauba fruit coating used. Because Syngenta Crop Protection is the registrant for both active ingredients, the marketing of this pre-mixture will be feasible. This is the strategy that we are developing with other crops (e.g., stone fruit – Scholar and Mentor; citrus – Graduate, Mentor, and Diploma). Although difenoconazole is not effective against gray mold, and generally did not provide an additive effect in blue mold control when they were used in mixtures with Scholar as compared to using Scholar alone, registration of a pre-mixture will be an important tool to decrease the risk of fungicide resistance to develop in populations of

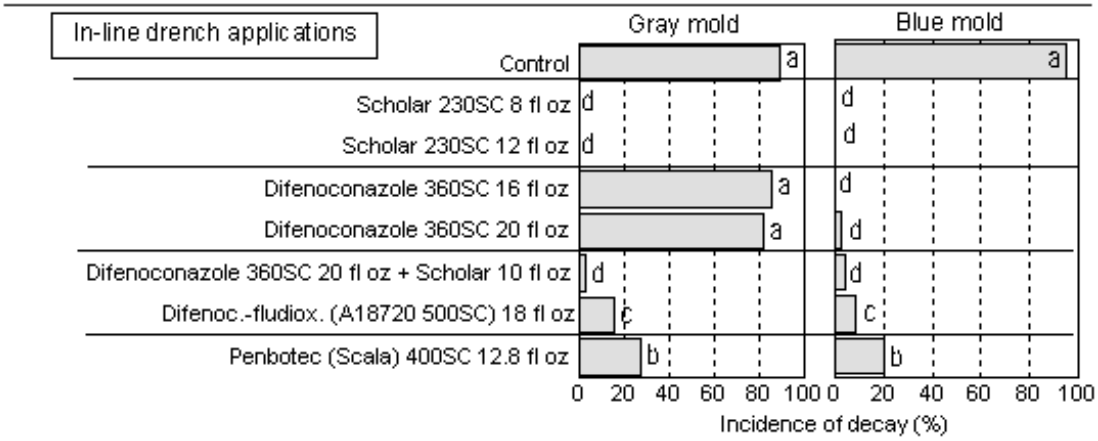
Penicillium spp. Additionally, because difenoconazole is also very effective against bull's eye rot, this pre-mixture will increase the spectrum of activity for postharvest decay control. These results support our plans to support a difenoconazole registration for postharvest use on pears through the IR-4 program.

Evaluation of the pathogenicity, virulence, and fitness of naturally occurring isolates of *P. expansum* that are resistant to fludioxonil or pyrimethanil. Most of the fludioxonil- and pyrimethanil-resistant isolates that were obtained in laboratory selection assays were stable in culture and were pathogenic in fruit inoculations. Isolates resistant to pyrimethanil were all highly virulent. Fludioxonil-resistant isolates were variable in their virulence but there was no correlation to the degree of resistance or colony type. The competitiveness of these isolates was evaluated in co-inoculations of fruit with a sensitive wild-type isolate of *P. expansum*. Competiveness was based on the proportion of resistant to sensitive progeny that were grown from conidia collected from decaying fruit. Either of two isolates highly resistant to fludioxonil (EC_{50} >40 mg/L as compared to <0.02 mg/L for sensitive isolates) were not recovered after co-inoculation with the sensitive isolate, whereas when using an isolate highly resistant to pyrimethanil (EC_{50} >75mg/L as compared to <0.70 mg/L for sensitive isolates) 27.1 to 33.3% of conidia from decaying fruit displayed the resistant phenotype. In co-inoculations with either of two isolates of *P. expansum* with an intermediate level of resistance to both fungicides (EC_{50} 0.12 or 2.42 mg/l for fludioxonil, 1.74 or 2.08 mg/L for pyrimethanil), 22.9 to 35.4% of the collected conidia displayed the double-resistant phenotype. These data indicate that differences in competitiveness exist among resistant isolates, that with repeated fungicide applications some isolates may become predominant, and that proper anti-resistance strategies have to be followed in the use of these new fungicides.

Baseline sensitivity studies for difenoconazole. The in vitro sensitivity of mycelial growth for 69 isolates of *Penicillium* spp. (mainly *P. expansum*) to difenoconazole was determined. The range of EC_{50} values was from 0.004 to 0.015 ppm (Fig. 5). Sensitivity of isolates was normally distributed with most isolates sensitive between 0.006 and 0.01 ppm in a frequency histogram (Fig. 6). Baseline sensitivities for fludioxonil and pyrimethanil were determined by us previously. These sensitivity ranges will serve as reference points in fungicide resistance monitoring. The baseline sensitivity range was 0.004 to 0.015 ppm.

Evaluation of captan, chlorine, acidified hydrogen peroxide (Perasan), and JBL-08A as sanitizers of fungicide drench solutions or other water tank systems (e.g., float tanks). Results are pending after incubation of treated fruit in storage and statistical analysis of summarized data. In general, we are demonstrating that captan as a water treatment in float tanks can inhibit gray mold infections caused by *B. cinerea* but has only a limited effect on fruit decays caused by *Penicillium* spp. Sanitation washes are still an important component of handling pears after fruit are floated out of the harvest bins. As an alternative or as a supplemental wash treatment, acidified hydrogen peroxide (e.g., Perasan) treatments can be effective especially when used in combination with neutral detergent washes. As an added advantage, no rinsing is required when acidified hydrogen peroxide is used at 80 ppm or less**.** The longer dwell time or contact time on the fruit improves the toxicity of the oxidizing treatment to fungal spores. The experimental JBL-08LA, a quaternary ammonium compound, was effective against some pathogens but not others. Unfortunately, after discussions with IR-4 and EPA representatives, the registration potential of this compound is low in the United States.

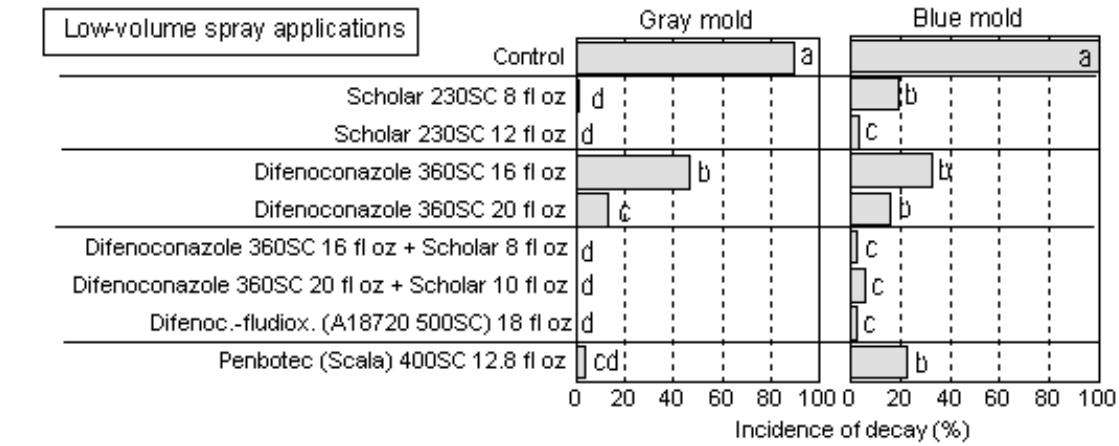
Fig. 1. Evaluation of new postharvest fungicides for management of decay of Bosc pears in experimental packingline studies



[1] - Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* ($7x10^4$ conidia/ml) or *Penicillium expansum* ($5x10^5$ conidia/ml), incubated for 16 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 (Decco 231). Fruit were then incubated at 20 C for 6 days.

[2]- Scholar 230SC 8 fl oz = 138 ppm, Scholar 230SC 12 fl oz = 208 ppm, difenoconazole 16 fl oz = 430 ppm, difenoconazole 20 fl oz = 540 ppm, A18720A 18 fl oz = 540 ppm difenoconazole + 180 ppm fludioxonil, Penbotec 12.8 fl oz = 383 ppm. For difenoconazole, the A8574D formulation was used.

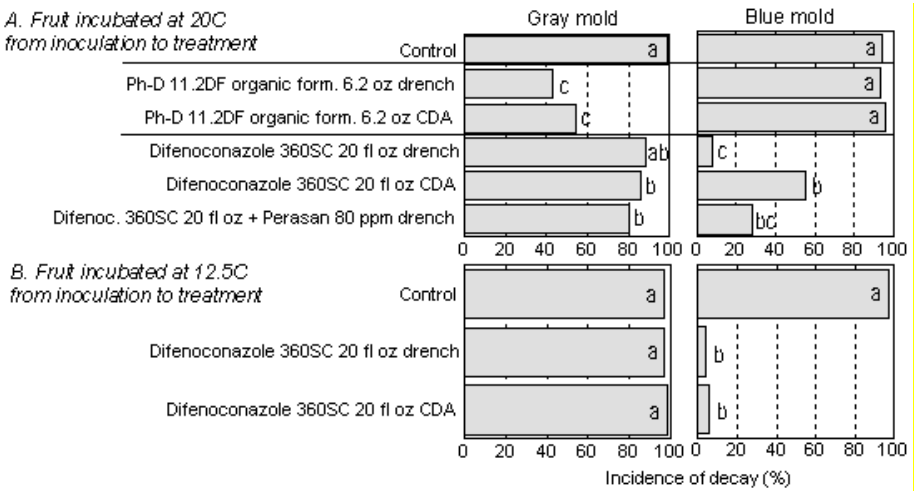
Fig. 2. Evaluation of new postharvest fungicides for management of decay of Bosc pears in experimental packingline studies



[1] - Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* ($7x10^4$ conidia/ml) or *Penicillium expansum* ($5x10^5$ conidia/ml), incubated for 16 h at 20C and treated by low-volume spray (CDA) applications at 25 gal/200,000 lb fruit. Fungicides were applied in a carnauba fruit coating (Decco 231). Fruit were then incubated at 20 C for 6 days.

[2] - Scholar 230SC 8 fl oz = 138 ppm, Scholar 230SC 12 fl oz = 208 ppm, Scholar 230SC 10 fl oz = 172 ppm, difenoconazole 16 fl oz = 430 ppm, difenoconazole 20 fl oz = 540 ppm, A18720A 18 fl oz = 540 ppm difenoconazole + 180 ppm fludioxonil, Penbotec 12.8 fl oz = 383 ppm. Rates in fl oz are for an application to 200,000 lb fruit. Concentrations in ppm are calculated for an application volume of 100 gal/200,000 lb (thus, they are four-times more concentrated using an application volume of 25 gal/200,000 lb). For difenoconazole, the A8574D formulation was used.

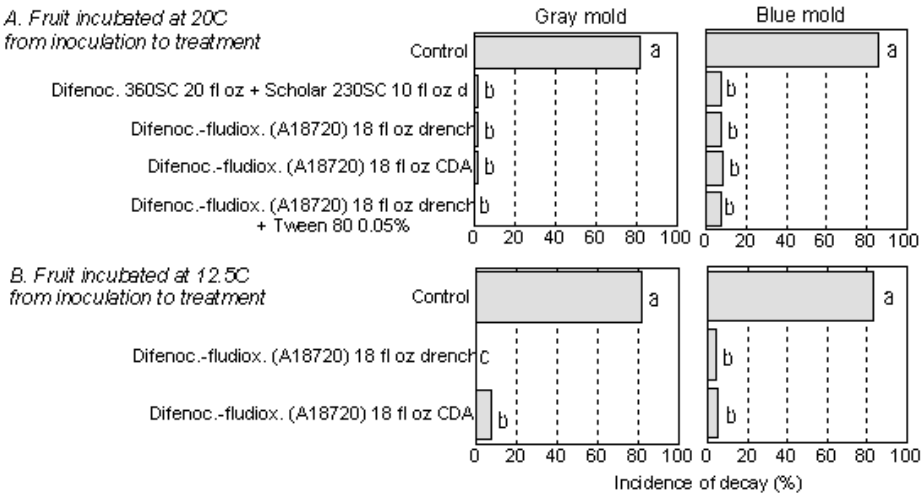
Fig. 3. Evaluation of new postharvest fungicides for management of decay of Bosc pears in experimental packingline studies
- In-line drench and low-volume spray applications -



[1] - Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* ($7x10^4$ conidia/ml) or *Penicillium expansum* ($5x10^5$ conidia/ml), incubated for 16 h at 20C and treated by low-volume spray (CDA) applications or in-line drench applications. CDA applications were done at 25 gal/200,000 lb fruit and fungicides were applied in a carnauba fruit coating (Decco 231). In-line drench applications were applied aqueous and were followed by a CDA application with fruit coating. Fruit were then incubated at 20 C for 6 days.

[2] - Difenoconazole 20 fl oz = 540 ppm. Rates in fl oz are for an application to 200,000 lb fruit. Concentrations in ppm are calculated for an application volume of 100 gal/200,000 lb (thus, they are four-times more concentrated using a CDA application volume of 25 gal/200,000 lb). For difenoconazole, the A8574D formulation was used.

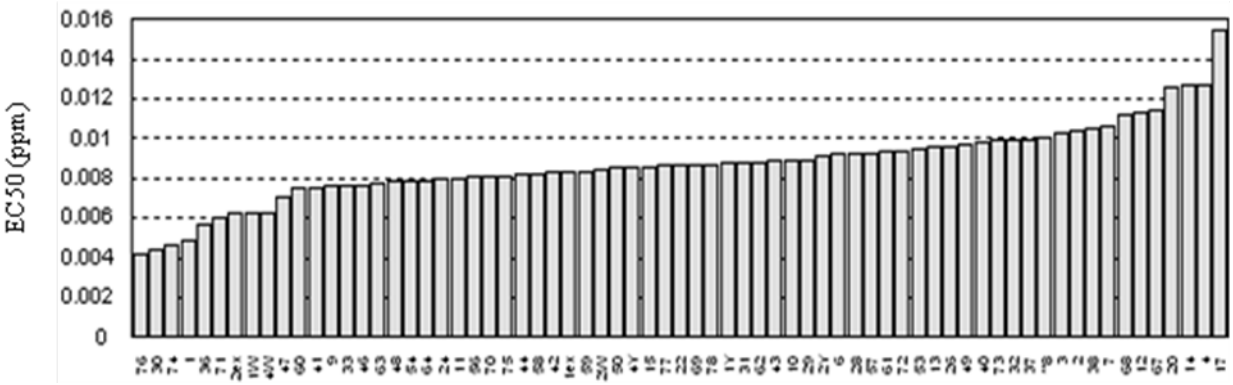
Fig. 4. Evaluation of new postharvest fungicides for management of decay of Bosc pears in experimental packingline studies
- In-line drench and low-volume spray applications -



[1] - Fruit were inoculated with conidia of TBZ-resistant isolates of *Botrytis cinerea* ($7x10^4$ conidia/ml) or *Penicillium expansum* ($5x10^5$ conidia/ml), incubated for 16 h at 20C and treated by low-volume spray (CDA) applications or in-line drench applications. CDA applications were done at 25 gal/200,000 lb fruit and fungicides were applied in a carnauba fruit coating (Decco 231). In-line drench applications were applied aqueous and were followed by a CDA application with fruit coating. Fruit were then incubated at 20 C for 6 days.

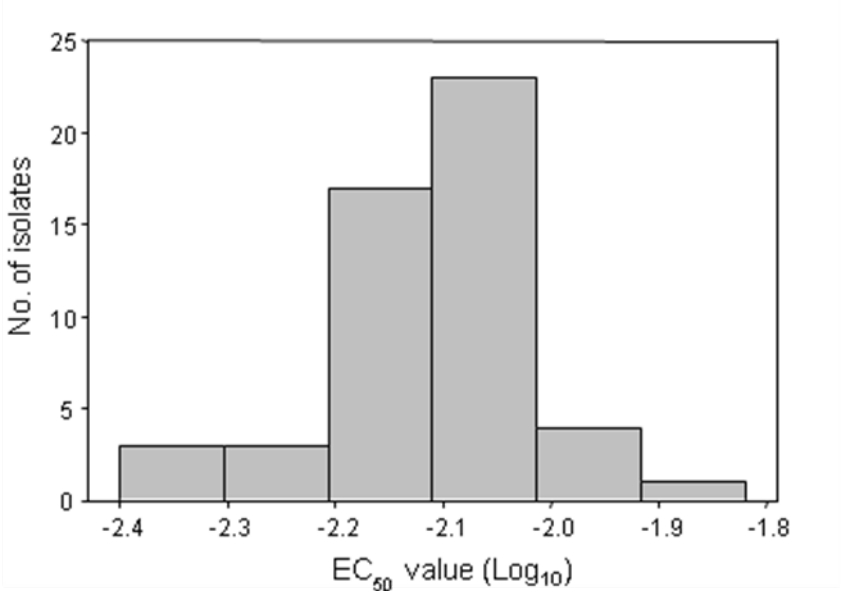
[2] - Scholar 230SC 10 fl oz = 172 ppm, difenoconazole 20 fl oz = 540 ppm, A18720A 18 fl oz = 540 ppm difenoconazole + 180 ppm fludioxonil. Rates in fl oz are for an application to 200,000 lb fruit. Concentrations in ppm are calculated for an application volume of 100 gal/200,000 lb (thus, they are four-times more concentrated using an application volume of 25 gal/200,000 lb). For difenoconazole, the A8574D formulation was used.

Fig. 5. Baseline sensitivities for difenoconazole for isolates of *Penicillium* spp. from pear in California collected 2005-2008



[1] - Isolates of *Penicillium* spp. were collected from decayed Bartlett and Bosc pear fruit in selected packinghouses. The majority of the 69 isolates evaluated (75%) were identified as *P. expansum*. Other species included *P. solitum* and *P. commune*. Fungicide sensitivities were determined using the spiral gradient dilution method.

Fig. 6. Frequency distribution of sensitivities for difenoconazole for isolates of *Penicillium expansum* from pear in California collected 2005-2008



[1] - Fifty-two isolates of *Penicillium expansum* were collected from decayed Bartlett and Bosc pear fruit in selected packinghouses. Fungicide sensitivities were determined using the spiral gradient dilution method. The frequency histogram is based on Scott's Method.