Annual Report - 2009

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 2009 RESEARCH

1. Experimental packingline studies were conducted on the management of postharvest decays of pears to identify new fungicides that could be used in mixtures with registered fungicides to increase treatment efficacy and to help in the prevention of resistance development in pathogen populations.
2. The SBI fungicide difenoconazole was not effective against gray mold, but highly effective against blue mold of Bosc pear, similar to Scholar or Penbotec. On Bartlett pear, difenoconazole was more effective when fruit inoculations were done at lower spore concentrations.
   - In mixture treatments of Scholar with difenoconazole, no negative interaction in activity was observed as compared to single treatments and thus, these two fungicides will be compatible in a pre-mixture.
   - Rate effects with Scholar (150 to 300 ppm) or Scholar-difenoconazole (150 – 300 ppm/300-700 ppm) were generally not observed.
   - The addition of the nonionic surfactant Triton X-100 to difenoconazole or Scholar-difenoconazole reduced the efficacy of the treatments.
   - Difenoconazole was also highly effective against bull's eye rot.
   - IR-4 residue studies with difenoconazole were approved and will be conducted with the new SC formulation in 2010.
3. A new single-bin fungicide drench treatment method that potentially can be used in the field or packinghouse immediately after harvest, significantly reduced the incidence of decay of inoculated fruit that were placed into the bottom or on the top of fruit-filled bins. When keeping the total amount of fungicide applied per bin the same, a higher application volume (8 gal/bin) was more effective than a lower (2 gal/bin) volume. Subsequent studies demonstrated that fungicide-treated fruit have to be air-dried before being immersed into the float tank to maintain high fungicide performance.
4. Sanitation treatments (chlorine, Perasan, captan, quaternary ammonia compounds) effectively reduced gray mold decay, but not blue mold decay, of non-wound inoculated Bosc fruit. Treatments containing captan also reduced gray mold decay after wound inoculation.

INTRODUCTION

Gray mold, caused by *Botrytis cinerea*, and blue mold, caused mainly by *Penicillium expansum* in addition to some less common species of *Penicillium*, are the most important storage diseases of pears in California. Other decays that may cause significant losses include Alternaria, Phomopsis, Rhizopus and Mucor rots, 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 (i.e., ziram, captan, Pristine, Elevate etc.) 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). Until the recent registration of Penbotec and Scholar, only thiabendazole and captan (Captan 50WP) were available for postharvest use on pears. New fungicides were developed by us and others because resistance against TBZ (Mertect 340F) is wide-spread among the pome fruit pathogens *B. cinerea* and *P. expansum* and captan at the registered postharvest rate of 2 lb/200,000 lb is ineffective against blue mold. 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). We continued our evaluation of new materials against blue and gray mold, as well as bull's Eye rot.

The sterol biosynthesis inhibiting fungicide difenoconazole was suggested to the registrant by our lab as a mix partner or alternative to be evaluated on pome fruits specifically to prevent resistance in populations of *Penicillium* spp. No funding was provided by the California Pear Board for this project in 2008. Still, we continued our evaluations on the efficacy of new postharvest decay control treatments of pears using different application methods. 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 with fungicide mixtures and rotations. This is important due to the known risk of resistance development in the postharvest pear pathogens to fungicides such as TBZ 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. To date, resistance has not been reported for the new fungicides in commercial packinghouses. In our studies on resistance potential in 2008 and 2009, however, we demonstrated a high risk for resistance development against pyrimethanil and fludioxonil in populations of *Penicillium expansum* and other species of *Penicillium* infecting pear fruit. 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 evaluation of difenoconazole in a mixture with fludioxonil and to have the two fungicides eventually being marketed as a pre-mixture. This way, with every application, there is a reduced pressure for resistant individuals to be selected as compared to single-fungicide treatments.

Objectives

1) Evaluation of postharvest fungicides (fludioxonil - Scholar, pyrimethanil – Penbotec, and difenoconazole) for postharvest management of gray mold, blue mold, and bull's eye rot. 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 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 captan, chlorine, acidified hydrogen peroxide (Perasan), and quaternary ammonia materials (e.g. Deccosan) 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 (two SC formulations – N and O), Scholar 230SC, and mixtures of these two fungicides was evaluated using different rates and were compared to treatments with Penbotec. For selected treatments Triton X-100 was used at a final concentration of 0.02%. Bartlett or Bosc pears were wound-inoculated with TBZ-resistant isolates of *B. cinerea* or *P. expansum*, incubated for 10-14 h, and then treated with fungicides. Fungicides were applied on an experimental packingline at the Kearney AgCenter as aqueous solutions using in-line drench or T-Jet applications that were followed by low-volume spray applications with fruit coating (Decco 231, a Carnaubabased coating). 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 a study to evaluate the performance of difenoconazole against *Neofabraea* spp. that cause bull's eye rot of pear, isolates of four species were obtained from Dr. Robert Spotts who identified the species using molecular techniques as: *N. perennans, N. malicortis, N. alba,* and *N.* sp. *nova*. Fruit were wounded and inoculated using a conidial suspension of each species at a concentration of 10^6 conidia/ml, incubated for 14-16 hrs at 20 C, not treated or treated with difenoconazole (500 ppm), difenoconazole-Scholar 230SC (fludioxonil) (500-150 ppm), or Scholar 300 ppm alone (300 ppm) using an experimental in-line drench system, and incubated at 12 days at 20 C. Data were analyzed using analysis of variance and least significant difference mean separation procedures of SAS 9.1.

Evaluation of a new postharvest single-bin fungicide treatment system. The treatment system consisted of a hand-held spray wand that was connected to a fungicide nurse tank. Spray volumes could be adjusted by a control system. Fruit were wound inoculated with isolates of *B. cinerea* (10^5 spores/ml) or *P. expansum* (10^6 spores/ml), stored for 2 or 18 h, and then placed either on the top or in the bottom of filled field picking bins (4 x 4 x 2 ft). Bins were treated with an aqueous solution of Scholar where 1 g active ingredient of fludioxonil was applied per bin at application volumes of 2, 4, or 8 gal per bin. Control fruit were treated with water at 4 gal/bin. Fruit were then taken to the laboratory and incubated at 20 C. Data were analyzed using analysis of variance and least significant difference mean separation procedures of SAS 9.1.

In an additional study, inoculated and treated fruit were either air-dried or non-air-dried and then were dipped in pear float or water for 1 min. Fruit were then taken to the laboratory and incubated at 20 C. The incidence of decay was assessed and data were analyzed as indicated above.

RESULTS AND DISCUSSION OF 2009 RESEARCH

Efficacy of postharvest treatments and application methods using single fungicides and mixtures. Experimental packingline studies were conduced to identify mixture and rotation materials for registered fungicides and to optimize treatment efficacy. The SBI fungicide difenoconazole was used as two SC formulations (N and O that were designed for postharvest use with approved inert ingredients), and efficacy was compared to Scholar 230SC and Penbotec 600SC (i.e., Scala 600SC).

In a trial using a T-Jet application system, the performance of the two fungicides fludioxonil (Scholar 230SC) and difenoconazole (SC formulation) when used alone or in mixtures was evaluated for managing blue mold and gray mold of Bartlett pear fruit that were wound-inoculated using high and low levels of inoculum of each pathogen (Fig. 1). Scholar at 150 (4 fl oz/100 gal) and 225 (6 fl oz/100 gal) ppm was highly effective in reducing blue and gray mold decay at low inoculum levels, whereas at high inoculum levels the 225-ppm rate performed significantly better that than the low rate for blue mold and there was a similar trend for gray mold. The fungicide was originally registered at 300 ppm (8 oz of the 50WP formulation) using a CDA applicator. With high-volume application systems such as T-Jet (or drenches), lower rates of the fungicide can be used. Difenoconazole was ineffective at the low and medium rates (300 and 500 ppm) against gray mold at both inoculum concentrations and only significantly reduced gray mold at the 700-ppm rate using low inoculum levels (Fig. 1). The fungicide was highly effective against blue mold at all rates using the low inoculum, but at the high inoculum level, it was most effective using the high rate (700 ppm). The combination fungicide treatment with 150 ppm of fludioxonil and either 500 or 700 ppm of difenoconazole was highly effective against both pathogens and at both inoculum levels (Fig. 1). There was no negative interaction in fungicide mixtures or with the carnauba fruit coating that was applied using a CDA system staged immediately after the T-Jet fungicide application treatment.

In another study using in-line drench applications of inoculated Bartlett pears, two rates of a soluble concentrate of difenoconazole (250 and 500 ppm) were used in combination with Scholar 230 SC at either150, 225, or 300 ppm. Blue and gray mold decays were significantly reduced from that of the control with >90% reduction in decay (Fig. 2). Inoculum levels for both fungi were considered at high levels and thus, this was considered a severe test. As in the T-Jet trial, there were no negative interactions when using fungicide combinations or carnauba fruit coating that was applied using a CDA system staged immediately after the drench treatment. Thus, a soluble concentrate (SC) pre-mixture product at a rate of $\geq$150 ppm fludioxonil and $\geq$300 ppm difenoconazole would be a highly effective treatment against blue and gray mold and would be effective in reducing the potential for resistance development in populations of *Penicillium* spp.

In two other studies on Bartlett and Bosc pear using T-Jet applications, the nonionic surfactant Triton X-100 was added to solutions of difenoconazole or Scholar-difenoconazole mixture treatments to possibly improve treatment efficacy. In one of the studies, two SC formulations of difenoconazole (N and O) were compared. As indicated in Figs. 3 and 4, the addition of 0.02% Triton X-100 in most cases significantly reduced the efficacy of the treatments. Additionally, both formulations of difenoconazole performed similarly when used alone or in mixtures with Scholar (Fig. 4). Formulation N is the one that has been selected by the registrant to be used for IR-4 residue trials and this formulation was used in all 2009 studies where the specific formulation is not indicated in the Figures of the Results. The Scholar (150 ppm) - difenoconazole (500-700 ppm) mixture treatment again was highly effective, similar to treatments with Penbotec.

In a study to evaluate the efficacy of difenoconazole and Scholar against bull's eye rot, these fungicides were used alone or in combination in treating wound-inoculated fruit using four species of *Neofabraea* (Fig. 5). Scholar was ineffective, whereas difenoconazole was similarly highly effective against all four species in these wound inoculation studies. The mixture of the two fungicides was also effective and negative interactions were not observed.

In summary, in these postharvest studies we found that mixtures of Scholar with difenoconazole were highly effective in managing gray and blue molds of pear. The SC formulation of difenoconazole showed to be compatible with the 230SC formulation of Scholar (fludioxonil). Because Syngenta Crop Protection is the registrant for both active ingredients, the marketing of a 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 a new postharvest single-bin fungicide treatment system. A new postharvest single-bin drench system was evaluated to potentially treat fruit with a postharvest fungicide immediately after picking in the field or in the packinghouse. This system would dramatically shorten the time period from inoculation (i.e., occurring during fruit picking) to treatment and thus, maximize the efficacy of the fungicide treatment. A fungicide rate was chosen for most of our trials (i.e., 1 g active ingredient of Scholar per bin or 800-1000 lb) that is equivalent to a postharvest high-volume drench treatment (i.e., approximately 4 oz of Scholar 50WP or 8 fl oz of Scholar 230 SC/50,000 lb). In the three trials conducted on Bosc pear, although not as effective as a comparative dip treatment (with less than 10% decay) significant reductions in the incidence of gray mold (one trial only – Fig. 8) and blue mold were observed when inoculated fruit were placed either in the top or in the bottom of a full bin (Figs. 6-8). The incidence of decay was generally lower for fruit placed in the top of the bin. For both, fruit on top and in the bottom of the bin, increasing application volumes (while maintaining the same amount of total fungicide applied) often resulted in an increased performance. Thus, the 8-gal application rate was more effective than the 2-gal rate, but fungicide run-off was minimal with the 4 gal rate. The addition of a surfactant (Kinetic) to the fungicide solution resulted in a slight improvement in efficacy of the fungicide treatment in two of the trials where this comparison was done (Figs. 6 and 7).

In subsequent studies we demonstrated that fungicide-treated fruit have to be air-dried before being immersed into the float tank to maintain high fungicide performance. When fruit were not air-dried after fungicide treatment, the incidence of decay was 60 and 2.8% for inoculations done 24 h before treatment and was 22.8% and 0% for inoculations done 1 h before treatment for non-air-dried and air-dried fruit, respectively (Fig. 9).

These results indicate that these single-bin drench treatments can potentially be effectively used in the field or in the packinghouse, especially during the peak of the picking season when postharvest in-line treatments cannot always be done in a timely way or when fruit are transported from distant orchards to packinghouses. Because of the simplicity of the spray system, it can be easily set up and operated. Thus, it has great potential to be used commercially.

Evaluation of new fruit sanitation treatments. In a experimental packingline trial was conducted on the comparative evaluation of sanitation treatments. In treatments of fruit that were wound-inoculated 4 h before T-Jet wash treatments, treatments that contained captan (either alone or in mixture with chlorine or Perasan) significantly reduced the incidence of gray mold (Fig. 10). A significant reduction of blue mold decay was only observed for the captan only treatment. When fruit were non-wound inoculated, treated with sanitizers, and then wounded and incubated, none of the treatments were effective against blue mold, whereas all treatments significantly reduced the incidence of gray mold. Among the sanitizers, chlorine was the least effective treatment (Fig. 10). Efficacy of the sanitizers was higher using non-wounded, inoculated fruit as compared to wound-inoculated fruit, indicating that the sanitizers killed inoculum on fruit surfaces but did not penetrate or were inactivated inside fruit wounds. This emphasizes the need for residual fungicides as postharvest treatments for preventing decays of fruit is storage.

Deccosan 315 was evaluated as a potential quaternary ammonia-based material for treating fruit because the IR-4 program was interested in developing alternative sanitation treatments to chlorine. One product from South Africa (Exp JBL-08A or Sporekill) was evaluated in 2008 on other crops where it was highly effective against some decays (e.g., brown rot), and was less effective against other decays (e.g., Rhizopus rot, gray mold). Quaternary ammonia compounds such as Deccosan are currently registered for sanitizing postharvest equipment provided that a water rinse is done before fruit is processed. Due to the limited activity of Deccosan against Penicillium decay in non-wound inoculated fruit and limited activity against both gray mold and *Penicillium* decays in wound-inoculated fruit, the material was not considered high priority for development. Perhaps if other materials such as JBL-08A with better activity are available these materials can be considered for registration as alternatives to chlorine sanitizers. JBL-08A is registered on fruit crops in some other parts of the world, but no registrations are currently available for these materials in the United States.

Perasan is commercially available and was used at 80 ppm because at this concentration no subsequent water rinse is required. It proved effective against gray mold in non-wound inoculations and in combination with captan also in wound-inoculations. In summarizing these fruit sanitation experiments, Perasan at 80 ppm hydrogen peroxide overall showed equivalent or higher efficacy than chlorine at 100 ppm for the control of gray mold.