Annual Report - 2015

EVALUATION OF POSTHARVEST TREATMENTS FOR MANAGEMENT OF GRAY MOLD, BLUE MOLD, AND OTHER DECAYS OF STORED PEARS IN CALIFORNIA

J. E. Adaskaveg, Department of Plant Pathology, University of California, Riverside, CA 92521
Collaborators: H. Förster, D. Thompson, and D. Cary (UC Riverside)

Special thanks to Naumes Packing, Marysville, CA, for their cooperation and for donation of fruit used in these trials.

ABSTRACT

Our pear postharvest research focused on new treatments for the management of decays to provide solutions for conventional and potentially also for organic fruit production. Treatments are developed for long-term usage because they are integrated with anti-resistance strategies. The final pre-mixture formulation of fludioxonil and difenoconazole (Academy), the bio-fungicide and fermentation product polyoxin-D, and the new experimental fermentation product EXP-13 that has a different mode of action from all other postharvest fungicides were evaluated. The latter two fungicides potentially could be registered with an exempt-from tolerance status in the US and may even be registered as organic fungicides. Laboratory and experimental packingline studies were conducted using inoculated Bosc and Comice pear fruit.

- The new pre-mixture Academy (fludioxonil + difenoconazole) has a broad spectrum of activity (blue mold, gray mold, Alternaria rot, bull's eye rot, Mucor decay, Rhizopus rot) and is very effective against these postharvest decays. Both components of the pre-mixture are very effective against blue mold and therefore, the pre-mixture provides a strategy for resistance management. Canada held up the joint registration with US-EPA and lower rates of Academy had to be evaluated. Registration in the US is now expected in Jan 2016.
- Polyoxin-D (Tavano, Oso) showed high efficacy against gray mold, Alternaria rot, and bull's eye rot, but, as previously stated, was not effective against blue mold. Although the marketing of the product has yet to be determined in the United States for postharvest use on fruit crops, this chemical deserves to be further explored for postharvest use on pears.
- In laboratory and experimental packingline studies the spectrum of activity of EXP-13 included gray mold, Alternaria rot, bull's eye rot, and Mucor decay. In contrast to apples and some pears, EXP-13 by itself was not effective against blue mold of Bosc or Comice pears, but was highly effective when mixed with a low rate of Scholar.

- We are currently investigating the reason for the lack of efficacy of EXP-13 against blue mold of selected pear varieties. Possibly, EXP-13 undergoes conformational changes in some environments, and these changes may cause a change in spectrum of activity.
- In baseline sensitivity studies with EXP-13, EC_{50} values for inhibition of mycelial growth ranged from 0.25 to 1.98 mg/L (mean 0.79 mg/L) for *B. cinerea* and 0.38 to 1.64 mg/L (mean 0.92 mg/L) for *A. alternata*. These values are in a similar range as those for *P. expansum* that we reported on previously. The lack of efficacy on fruit of selected pear varieties is being investigated.

INTRODUCTION

Gray mold, caused by *Botrytis cinerea,* and blue mold, caused by *Penicillium expansum* and several 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 caused by *Neofabraea* spp. Entry points for all pathogens are wounds caused by abiotic or biotic agents before or during harvest. Infections by *Neofabraea* spp. are mostly initiated in the orchard during fruit development. While some postharvest decay fungi like *Rhizopus* species are suppressed at storage temperatures of 0°C (32°F), *B. cinerea, P. expansum, Mucor, Alternaria,* and *Neofabraea* spp. 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, however, can reduce the incidence of postharvest gray mold when field bins of fruit are not washed and placed directly into cold storage, but they may increase the likely-hood of selecting for resistance of postharvest pathogens by over-usage (number of applications per season) and incomplete coverage. Fungicides are more effectively used as postharvest treatments. Currently registered postharvest fungicides including Penbotec (pyrimethanil - 2005), Scholar (fludioxonil - 2005) and Judge (fenhexamid – 2007) 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 fungicide resistance development in postharvest pear pathogens is high because most registered materials have a single-site mode of action and because fruit are often stored for extended periods of time. Furthermore, when fruit receive more than one postharvest treatment, repeated selection allows the survivors to become the dominant pathogen population. Although five fungicides (Captan, TBZ, Scholar, Penbotec, Judge) are now registered for postharvest use on pears, only two of them (Scholar, Penbotec) are highly effective against TBZ-resistant blue mold. Difenoconazole is not effective against gray mold, but highly effective against blue mold and also bull's eye rot (that is not controlled with fludioxonil). It is pending registration on pome fruit as a component in a pre-mixture with fludioxonil. The use of pre-mixtures is an effective anti-resistance strategy. Our laboratory selection studies indicated that pyrimethanil and fludioxonil have a similar high risk to develop resistance, but the risk for difenoconazole was determined to be lower, but still present. In collaboration with the registrant of Scholar, Syngenta Crop Protection, and IR- 4 Specialty Crop Program, we optimized usage rates and application methods for difenoconazole, and we evaluated different formulations of a pre-mixture. A final formulation called Academy (difenoconazole and fludioxonil) has been selected and most studies have been completed. Registration of the new pre-mixture is expected for Jan. 2016. The 2014 registration date was delayed after Canada requested additional rates to be included on the label.

As additional postharvest treatment alternatives, we are evaluating the bio-fungicide polyoxin-D that has obtained an exempt registration status in the US and the experimental EXP-13 that also has potential for an exempt status and an organic registration. Both compounds are natural fermentation products. Marketing companies for EXP-13 and polyoxin-D in the United States have currently not been determined by the registrants. Polyoxin-D is very effective against gray mold and Alternaria rot. Our results with EXP-13 on pome fruit have been very mixed. On apples, we found it to be consistently very effective against blue mold, gray mold, and bull's eye rot, as well as being moderately effective against Alternaria rot and Mucor decay. On pears, it was mostly effective against gray mold, Mucor decay, and Alternaria rot, but generally not against blue mold. After many years of usage of EXP-13 in other food applications resistance has never been reported in *Penicillium* species. Additionally, because the manufacturer had asked us to continue to define the spectrum of activity, we did additional studies in 2015 to evaluate the efficacy against different decays. From previous comparative studies we concluded that the activity of polyoxin-D and EXP-13 is also highly dependent on fruit maturity and the timing of fungicide application after wound inoculation. Both compounds could have a critical role in resistance management. The pre-mixture of fludioxonil with difenoconazole will reduce the risk for resistance development in *Penicillium* populations. A potential mixture of fludioxonil with polyoxin-D could reduce the risk for resistance development in *B. cinerea*; whereas a mixture with EXP-13 could be a broad-spectrum resistance management tool.

Thus, in 2015, our pear postharvest research focused on the management of major decays to provide solutions for conventionally treated and potentially also for organic fruit production. We continued evaluating polyoxin-D and EXP-13 and also the pre-mixture Academy.

OBJECTIVES

Objectives

1) Comparative evaluation of new postharvest fungicides
    a. Evaluate polyoxin-D against gray mold, Alternaria decay, and bull's eye rot and compare to pyrimethanil and fludioxonil.
    b. Evaluate formulations of EXP-13 against blue mold, gray mold, Alternaria decay, Mucor decay, and bull's eye rot.
2) Optimize efficacy of polyoxin-D and EXP-13.

a. Determine their post-infection activity at different times after inoculation and evaluate the effect of fruit maturity on fungicide performance.
b. Evaluate pH effects on the activity of EXP-13 in laboratory studies.
c. Evaluate buffered solutions of EXP-13 as treatments on Bosc and Comice pear.

3) Determine baseline sensitivities for polyoxin-D and EXP-13 for decay fungi of pear.
4) Efficacy of ozone treatments in commercial storage rooms.
    a. Evaluate the effectiveness for decay control of inoculated fruit and for sanitizing contaminated fruit.
    b. Evaluate combinations of potential exempt from tolerance bio-fungicides with ozone treatment during storage.

PROCEDURES

Comparative evaluation of new postharvest fungicides. The efficacy of Academy (the final A20682B formulation), polyoxin-D (Tavano, Oso), EXP-13 (a 50% powder formulation), and selected mixtures was evaluated in laboratory and experimental packingline studies. Comice or Bosc pears were wound-inoculated with decay pathogens of pears: *P. expansum*, *B. cinerea*, *Neofabraea perennans*, *Mucor piriformis*, or *Alternaria alternata* using spore concentrations as indicated in the figures of the results. Fruit were incubated for selected times at 20°C and then treated. In laboratory studies, treatments were done by dipping for 15 sec. In experimental packingline studies, treatments were done using high-volume, in-line drench applications that were followed by low-volume spray applications (CDA) with fruit coating (Decco 231, a carnauba-based coating) or by CDA applications in fruit coating. After treatment, fruit of all studies were stored at 20°C, 95% RH for 6 to 14 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 9.4.

Determination of baseline sensitivities. Baseline sensitivities for EXP-13 were determined for a limited number of isolates of *B. cinerea* and *A. alternata* from pome fruit. Concentrations to inhibit mycelial growth by 50% were determined on amended potato dextrose agar using the spiral gradient dilution method as described previously.

RESULTS AND DISCUSSION OF 2015 RESEARCH

Comparative evaluation of new postharvest fungicides. The new fludioxonil-difenoconaozle pre-mixture Academy was evaluated in one experimental packingline and in two laboratory studies. In the experimental packingline study, Academy at the 12-fl oz and 16-fl oz rates had a similar high efficacy as Scholar (176 ppm = 10 fl oz) in reducing the incidence of blue mold, gray mold, and Rhizopus rot of Bosc pear when applied as in-line drenches (Fig. 1). In comparison, Penbotec was only highly effective against blue mold and gray mold. In laboratory dip studies, Academy was also highly effective against blue mold and gray mold (Fig. 2), but also effective or highly effective against Alternaria rot (Figs. 2,3), and highly effective against bull's eye rot (Fig. 2) and Mucor decay (Fig. 3). Academy and Penbotec were effective when applied as in-line drench or CDA applications, but Scholar was significantly more effective when applied as an in-line drench (Fig. 1).

These results show that Academy has a broad spectrum of activity (blue mold, gray mold, Alternaria rot, bull's eye rot, Mucor decay, Rhizopus rot) and is very effective against these decays. Both components of the pre-mixture are very effective against blue mold and therefore, the pre-mixture provides a strategy for resistance management. Canada held up the joint registration of Academy with US-EPA because lower rates (e.g., 8 oz) had to be evaluated. The spectrum of activity and efficacy of currently registered and new (i.e., Academy) postharvest fungicides for pome fruits is summarized in Table 1.

In laboratory dip studies, polyoxin-D was highly effective against gray mold, Alternaria rot, and bull's eye rot, but, as previously stated, not effective against blue mold (Fig. 2). Although the marketing of the product has yet to be determined in the United States for postharvest use on fruit crops, this chemical deserves to be further explored for postharvest use on pears, especially since it is a natural fermentation product with potential for organic registration.

Our evaluations with EXP-13 continued in 2015. Laboratory and experimental packingline studies confirmed its spectrum of activity and efficacy with generally high efficacy against *B. cinerea*, *A. alternata, N. perennans*, and *M. piriformis*, but little or no efficacy against *P. expansum* (Figs. 1-4). Thus, EXP-13 has a fairly wide spectrum of activity against different fungal pathogens. In mixture with a low rate of Scholar, the treatment was also highly effective against *P. expansum* (Fig. 4).

In view of the high efficacy of EXP-13 against blue mold of apples, the reason for its lack of efficacy against blue mold of pears is still unknown. Previous exploratory studies ruled out several possible reasons (see our 2014 Annual Report). Because pH was considered a factor, this was evaluated in 2015. Applications with EXP-13 made in buffered citric acid or sodium citrate solutions at pH 3 or pH 4 were also not effective. In discussions with the manufacturer, our current hypothesis is that EXP-13 undergoes conformational changes in some environments, and these changes may cause a change in spectrum of efficacy. Conformational changes were also predicted for EXP-13 in the presence of chlorine, but in our studies on decay management of citrus, EXP-13 was similarly effective in the presence of chlorine than when without. Thus, characterization of EXP-13 toxicity and efficacy on some fruit crops when applied in chlorine solutions has been confirmed for managing postharvest decays.

Determination of baseline sensitivities. EC_{50} values for inhibition of mycelial growth by EXP-13 were determined for 26 isolates of *B. cinerea* and 40 isolates of *A. alternata* from pome fruit. EC_{50} values ranged from 0.25 to 1.98 mg/L (mean 0.79 mg/L) for *B. cinerea* and 0.38 to 1.64 mg/L (mean 0.92 mg/L) for *A. alternata*. These values are in a similar range as those for *P. expansum* that we reported on previously (range from 0.77 to 1.55 ppm (average 1.14 ppm). Therefore, in vitro sensitivity to selected postharvest pathogens does not explain the difference in efficacy that we observed for the different postharvest decays. Additional isolates of postharvest pathogens will be evaluated in the future.

Table 1. Efficacy of postharvest fungicides for management of postharvest decays of pear

Fungicide	Alternaria rot	Gray mold	Blue mold	Bull's eye rot	Rhizopus Rot	Mucor Decay
Captan	+	+	+	+	---	---
TBZ	---	++	++	++	---	---
Pyrimethanil	+	+++	+++	+++	---	---
Fenhexamid	+	+++	---	---	---	---
Fludioxonil	+++	+++	+++	++	+++	++
Difenoconazole	+++	+	+++	+++	+	---
Fludioxonil-Difeno.	+++	+++	+++	+++	+++	++

Pathogens are *Alternaria alternate*, *Botrytis cinerea*, *Penicillium expansum*, *Neofabraea perennans*, *Rhizopus stolonifer*, and *Mucor piriformis*.