

## Annual Report - 2013

Prepared for the California Apple commission

Project Title: Evaluation of new bactericides for control of fire blight of apples caused by *Erwinia amylovora* and evaluation of new postharvest fungicides for pome fruit

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### SUMMARY

#### Fire blight

1. All strains of *E. amylovora* were found to be sensitive against the antibiotics oxytetracycline and kasugamycin; whereas some strains were streptomycin-resistant and persisted in commercial orchards.
2. In toxicity studies with three biocontrol agents against chemicals used for fire blight control in our field studies, streptomycin, oxytetracycline, kasugamycin, captan, and mancozeb at 40 ppm were all inhibitory against *Streptomyces lydicus* (Actinovate) and *Bacillus amyloliquifaciens* (Double Nickel 55). In contrast, *Aureobasidium pullulans* (Blossom Protect) was not inhibited in growth by the three antibiotics at 40 ppm, but was inhibited by captan and mancozeb. These data indicate that in field applications only Blossom Protect could be safely used in combination with the three antibiotics.
3. In a field trial on the management of fire blight on Granny Smith and Fuji apple, kasugamycin continued to be highly effective. The product performed well by itself, but also in mixtures with copper, Firewall, or Actigard. Registration of Kasumin is expected for 2014.
4. The phosphonates Prophyt, K-phite, and Ko-phite did not show significant activity in a field trial.

#### Postharvest decay control

1. Postharvest experimental packingline studies focused on new treatments for the management of major decays to provide solutions for conventionally treated and potentially also for organic fruit production. Treatments are being developed based on anti-resistance strategies. The final pre-mixture formulation of fludioxonil and difenoconazole (e.g., Academy), the new multi-pack formulation of fludioxonil and TBZ (e.g., Scholar Max MP), the bio-fungicide polyoxin-D, and a new active ingredient for postharvest decay control (i.e., N-1) were evaluated. The latter two are exempt from tolerance by the US-EPA.
2. Experimental packingline studies again demonstrated that re-circulating high-volume drench applications are the most effective method to provide excellent coverage and decay control.
3. Applications with Academy, Scholar Max MP, Scholar, and Penbotec were all highly effective in reducing blue mold (caused by TBZ-sensitive and -resistant strains of *P. expansum*) and gray mold. Academy and Scholar Max MP also reduced the incidence of bull's eye rot to low levels.
4. Academy, Scholar Max, and Scholar were also highly effective against Alternaria rot and bitter rot (caused by *Colletotrichum acutatum*). This further broadens the spectrum of activity of the fludioxonil-difenoconazole pre-mixture Academy that includes now blue mold, gray mold (TBZ-sensitive and -resistant pathogen populations), bull's eye rot, Alternaria rot, and bitter rot.
5. Polyoxin-D was less effective against gray mold in this year's studies as compared to last year, but highly effective against Alternaria rot. In a timing study, however, polyoxin-D was very effective against gray mold when applied within 10 h of inoculation. It is suggested that fruit cultivar and maturity are critical for the optimum performance of this compound and these parameters need to be further evaluated.
6. N-1 showed moderate efficacy against blue mold and good efficacy against gray mold and Mucor rot. This compound was also more effective when treatments were applied after shorter post-inoculation incubation periods. As with polyoxin-D, further studies are needed to optimizing performance of this compound. This is important because both polyoxin-D and N-1 potentially could be used for organic production and they also could be used in mixtures to prevent resistance of gray mold to fludioxonil.

## INTRODUCTION

**Epidemiology and management of fire blight.** Fire blight, caused by the bacterium *Erwinia amylovora*, is a very destructive disease of pome fruit trees worldwide. It is one of the most difficult diseases to manage. The infection period is long, and moreover, very few effective treatments are available. Integrated programs that combine sanitation and orchard management with chemical and biological controls are the best approaches. If the disease is in its early stage and only a few twigs are blighted, it often can be eliminated by pruning. Thus, aggressive and regular scheduled pruning of diseased tissue is essential for keeping inoculum levels low in an orchard.

Current chemical control programs for fire blight control are based on protective schedules, because available compounds are contact treatments and are not systemic. Control with copper compounds is only satisfactory when disease severity is low to moderate. Copper treatments can be effective, especially under lower disease pressure, but are not commonly being used because they may be phytotoxicity on fruit and cause russetting. New formulations of copper, however, allow for reduced rates based on the metallic copper equivalent (MCE) and thus, extended usage past the bloom period may provide an effective rotational treatment or mix-partner without causing phytotoxicity. Additionally, copper products are approved for organic production and will have to be more extensively used because antibiotic use may become restricted in the future.

The antibiotic streptomycin has been used for many years; whereas the less effective antibiotic oxytetracycline (terramycin) has been used on apples for the last 7 years in California. Because of lack of alternative control materials, resistance developed against streptomycin at many locations in California. We identified a new streptomycin-resistance mechanism in California and this is currently being summarized in a manuscript. In our previous antibiotic resistance surveys over several years, we also detected strains of *E. amylovora* with reduced sensitivity to oxytetracycline at several locations. At one of these locations field treatments with oxytetracycline (e.g., Mycoshield, Fireline) were reported to be ineffective in controlling the disease. Thus, field resistance has occurred in some locations.

In the past years, in our evaluations of new materials for fire blight control, kasugamycin (Kasumin) was identified as the most effective alternative treatment with an efficacy equal or higher to streptomycin or oxytetracycline. This compound also showed very good efficacy in controlling fire blight in field trials in other pome fruit growing areas of the country. Although concerns have been expressed by regulatory agencies regarding the use of antibiotics in agriculture, kasugamycin is not used in human and animal medicine and has a different mode of action from streptomycin or oxytetracycline (no cross-resistance). Through our efforts, registration of Kasumin in California is pending in 2014. Kasugamycin was again effectively used in our field trials in 2013. It was applied by itself or in mixtures with selected other materials, including other antibiotics, copper, mancozeb, and Actigard that is enhancing host defense mechanisms in some plants. These evaluations were done to identify effective mixture treatments that would reduce the potential for resistance development. In the past, we also successfully evaluated rotation programs with Kasumin. In 2013, we also tested the phosphonates Prophyt, K-Phite, and Ko-phite, the biocontrols Blossom Protect and Actinovate, and several mixture treatments.

**Management of postharvest decays.** Apples, like other pome fruit, can be stored for some period of time using the correct storage environments. Still, postharvest decays caused by fungal organisms can cause serious crop losses. The major postharvest decays of apples include *Penicillium expansum*, *Botrytis cinerea*, *Alternaria alternata*, and *Mucor piriformis* causing blue mold, gray mold, black mold, and Mucor decay, respectively. Bull's eye rot caused by *Neofabraea* species can be a major problem in apple growing areas of the Pacific Northwest, but can also cause losses in California. Bitter rot caused by *Colletotrichum acutatum* mostly occurs in wet climates.

New 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 TBZ-resistance (Mertect 340F or Alumni) is widespread in populations of *B. cinerea* and *P. expansum*. These new treatments are just recently being utilized in California and the Pacific Northwest (PNW) because many countries had to establish maximum residue limits (MRLs) to allow the import of fruit.

Although five fungicides (Captan, TBZ, Scholar, Penbotec, Judge) are now registered for postharvest use on apple, only two of them (Scholar, Penbotec), are highly effective against TBZ-resistant blue mold. Resistance to Penbotec in the field and in the packinghouse, however, has already been reported in other pome fruit growing

areas of the US (e.g., PNW). Anti-resistance strategies include the use of fungicide rotations and mixtures. For this, we are identifying additional potential postharvest fungicides, and we continued our evaluation of the sterol biosynthesis inhibitor difenoconazole. We have been working in close collaboration with the registrant of Scholar and difenoconazole. One goal is to ultimately provide a pre-mixture of these fungicides that is both highly efficacious and cost-effective. For this, we have been optimizing usage rates, application methods, and we have been evaluating different fludioxonil-difenoconazole pre-mixture formulations for managing gray mold, blue mold, *Alternaria* rot, and bull's eye rot. Although this latter decay is only of sporadic importance in California (but very important in the Pacific Northwest), management strategies need to be known in the event of a disease outbreak.

As an additional alternative, we are evaluating the bio-fungicide polyoxin-D that has obtained an exempt registration status in the United States as a potential postharvest treatment for organic production. We obtained excellent gray mold reduction in previous studies using this compound, and in 2013 we continued its evaluation. Furthermore, another compound (N-1) was evaluated as a postharvest treatment on pome fruit and other crops. N-1 is known for its activity against *Penicillium* species and it has been used as a food additive for many years. The compound has the potential to obtain an exempt status and an organic registration because it is a natural fermentation product. Furthermore, over all the years in use, resistance in *Penicillium* species against N-1 has not occurred. N-1 was never evaluated on pome fruit and thus, we conducted studies on its use as a postharvest treatment.

These latter two alternative treatments could also be used as components of anti-resistance management for currently registered fungicides. Thus, for fludioxonil (Scholar), difenoconazole has been developed to prevent resistance in *Penicillium* populations. Polyoxin-D or N-1 could take this role for *B. cinerea*, because difenoconazole has little activity against gray mold. Thus, in 2013, our apple postharvest research focused on new treatments for the management of major decays to provide solutions for conventionally treated and potentially also for organic fruit production. Treatments are being developed for long-term usage because they are integrated with anti-resistance strategies.

## OBJECTIVES FOR 2013

### *Fire blight research*

1. Evaluate the efficacy of treatments for managing fire blight and characterize antibiotic resistance.
  - A. Laboratory in vitro tests to evaluate the bactericidal activity of antibiotics with and without biofilm inhibitors such as 2-aminoimidazole using spiral gradient dilution assays.
  - B. Small-scale hand-sprayer tests using different treatment-inoculation schedules to evaluate bio-film inhibitors in combination with antibiotics and/or low MCE copper products.
  - C. Field trials with protective air-blast spray treatments:
    - i. New formulations of copper (e.g., Kocide 3000, Badge X2) with and without antibiotics.
    - ii. Plant defense activators (e.g. ProAlexin, Actigard, PM-1) with and without antibiotics.
    - iii. Evaluate the efficacy of biological controls (e.g., Actinovate, Blossom Protect, Double Nickel 55), and natural products (e.g., Cerebrocide) in integrated programs using antibiotics and low MCE copper products.
  - D. Characterization of streptomycin- and oxytetracycline-resistant strains using molecular approaches: characterize plasmids that harbor the resistance genes and compare to *E. amylovora* populations from other parts of the country.

### *Postharvest research*

2. Comparative evaluation of new postharvest fungicides
  - A. Evaluate difenoconazole, fludioxonil, and difenoconazole-fludioxonil pre-mixtures at selected rates against gray mold, blue mold, *Alternaria* decay, and bull's eye rot and compare to pyrimethanil.
  - B. Evaluate polyoxin-D and Nm-1 against gray mold, *Alternaria* decay, and bull's eye rot and compare to pyrimethanil and fludioxonil.
  - C. Evaluate treatment effects on fungicide residues on apple fruit – determine the effect of temperature differences between treatment solution and fruit on uptake of fludioxonil and difenoconazole of different apple cultivars.
  - D. Determination of baseline sensitivities. Baseline sensitivities for fludioxonil and difenoconazole will be continued to be developed for additional isolates of *Alternaria* spp. that are collected.

## MATERIALS AND METHODS

**Isolation of *E. amylovora*, bacterial culturing, and verification of species identity.** Diseased apple blossoms with fire blight symptoms were obtained in the spring and early summer of 2013. Surface-disinfested, infected plant material (fruit, stems, and pedicels) was incubated in sterile water for 15 to 30 min to allow bacteria to ooze out. Suspensions were streaked onto YDC plates. Single colonies were transferred and the identity of strains as *E. amylovora* was verified by colony morphology and by PCR (using primers for the *E. amylovora* plasmid pEA29). Detection of a DNA fragment using gel electrophoresis confirmed a positive identification.

**Laboratory studies on the toxicity of bactericides against *E. amylovora*.** Kasugamycin (Kasumin 2L, Arysta Life Sciences, Cary NC), streptomycin (Sigma, St. Louis, MO), oxytetracycline (Sigma), and the biofilm inhibitor 2-aminoimidazole (along with an analog) were evaluated for their in vitro toxicity using the spiral gradient dilution method. For this, a radial bactericidal concentration gradient was established in nutrient agar media in Petri dishes by spirally plating out a stock concentration of each antimicrobial using a spiral plater (Autoplate 4000; Spiral Biotech, Inc., Norwood MA). After radially streaking out suspensions of the test bacteria ( $10 \mu\text{l}$  of  $10^8$  cfu/ml-based on optical density at 600 nm and use of a standard curve) along the concentration gradient, plates were incubated for 2 days at 25°C. Measurements were visually taken for two inhibitory concentrations: i) the lowest inhibitory concentration (LIC; the lowest concentration where inhibition of bacterial growth was observed, i.e., where the bacterial streak became less dense visually), and ii) the minimal concentration that inhibited growth by >95% (MIC). The actual antibiotic concentrations were obtained by entering the radial distances of inhibition (measured from the center of the plate) into the Spiral Gradient Endpoint program (Spiral Biotech, Inc.).

**Toxicity of chemicals used for fire blight control in our studies against three biocontrol agents.** The spiral gradient dilution method was used to evaluate the toxicity of streptomycin, oxytetracycline, kasugamycin, captan, and mancozeb against *Streptomyces lydicus* (Actinovate), *Aureobasidium pullulans* (Blossom Protect), and *Bacillus amyloliquifaciens* (Double Nickel 55). Stock concentrations of the chemical were used that resulted in maximum concentrations in the agar medium of approximately 40 ppm. The biocontrol agents were radially streaked along the concentration gradients, and plates were evaluated after two days. When growth of the biocontrol was inhibited, it was considered sensitive to the chemical.

**Field studies on fire blight using protective treatments during the growing season.** In a field study in an experimental orchard at KARE, treatments were applied at 25% bloom (3-18) and 85% bloom (3-25-13) to cv. Granny Smith and at 20% bloom (3-25) and 95% bloom (4-3-13) to cv. Fuji using an air blast sprayer at 100 gal/A. Trees were inoculated with *E. amylovora* using an air-blast sprayer on 4-1-13. Disease was evaluated on 4-22-2013, the number of diseased spurs per tree was counted, and potential phytotoxic effects of the treatments were recorded. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.1.

**Efficacy of postharvest treatments and application methods using single fungicides and mixtures.** The efficacy of Academy (the final formulation of a difenoconazole-fludioxonil pre-mixture), Scholar Max MP (a new multi-pack formulation of fludioxonil and TBZ) was evaluated in comparison with Scholar 230SC and Penbotec. Applications were done using high-volume in-line drench and low-volume CDA spray applications on an experimental packingline using the suggested commercial rates. Granny Smith apples were wound-inoculated with TBZ-resistant isolates of *P. expansum* ( $5 \times 10^5$  conidia/ml), or with *B. cinerea* ( $10^5$  conidia/ml), *Neofabraea perennans* or *M. malicortices* ( $10^6$  conidia/ml), *Alternaria alternata* ( $10^5$  conidia/ml), or *Colletotrichum acutatum* ( $10^5$  conidia/ml) incubated for 15-17 h at 20°C, and then treated. Fungicides were applied on an experimental packing line at KARE 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) or by low-volume CDA spray application at a rate of 25 gal/200,000 lb fruit. For N-1, two formulations (a 50% powder and a 5% liquid formulation) were used and applications were done using either method or a combination of the two.

A timing study was conducted with polyoxin-D and N-1 on apple fruit. Fruit were inoculated and incubated for selected times (4, 6, 9, or 12 h) at 20°C. Treatments with aqueous fungicide solutions were done using a hand-sprayer. 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 9.1.

## RESULTS AND DISCUSSION

**Antibiotic sensitivity among *E. amylovora* strains collected in California.** Strains of *E. amylovora* were confirmed for species identity by PCR amplification of a 1-kb DNA fragment using specific primers for plasmid pEa29 that is ubiquitously found in this bacterium. All strains were found to be sensitive against the antibiotics oxytetracycline and kasugamycin; whereas some strains were streptomycin-resistant. Thus, streptomycin-resistant strains are persistent in commercial orchards. The biofilm inhibitor 2-aminoimidazole along with an analog reported to be similar were shown to be not toxic to the pathogen.

**Toxicity of chemicals used for fire blight control in our studies against three biocontrol agents.** Results from our in vitro assays indicated that streptomycin, oxytetracycline, kasugamycin, captan, and mancozeb at 40 ppm were all active against *Streptomyces lydicus* (Actinovate) and *Bacillus amyloliquifaciens* (Double Nickel 55) (Table 1). In contrast, *Aureobasidium pullulans* (Blossom Protect) was not inhibited in growth by the three antibiotics at 40 ppm, but was inhibited by captan and mancozeb. These data indicate that in field applications only Blossom Protect could be safely used in combination with the three antibiotics.

Biocontrol product and agent	Streptomycin	Oxytetracycline	Kasugamycin	Captan	Mancozeb
Actinovate ( <i>Streptomyces lydicus</i> )	+*	+	+	+	+
Blossom Protect ( <i>Aureobasidium pullulans</i> )	-	-	-	+	+
Double Nickel 55 ( <i>Bacillus amyloliquifaciens</i> )	+	+	+	+	+

\* - Activity was determined using the spiral gradient dilution assay. + = chemical is active against the biocontrol agent, - = chemical is not effective at maximum concentration of 40 ppm tested.

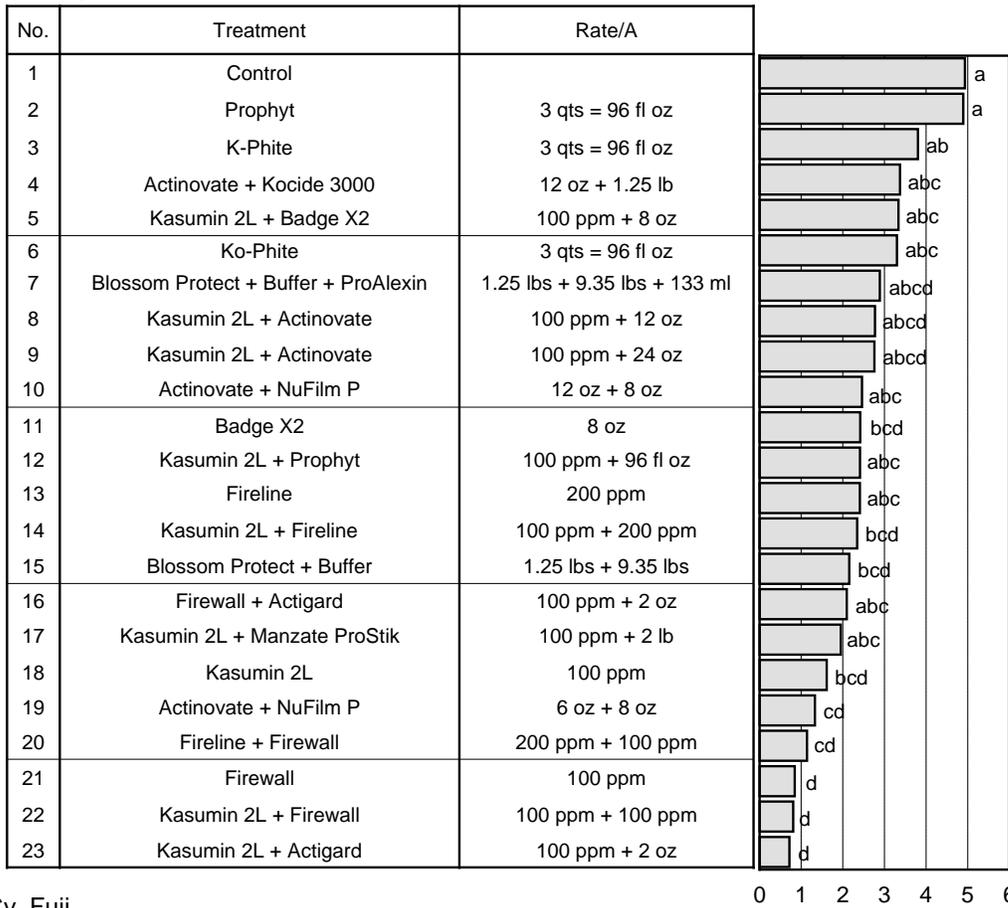
*Field studies on fire blight using protective treatments during the growing season.*

In a field trial on Granny Smith and Fuji apple, 23 and 11 treatments were evaluated, respectively. Kasumin continued to perform very well and was numerically the best treatment on cv. Fuji (Fig. 1). The product was also very effective in mixtures with copper, Firewall, or Actigard. Still, there was no additional disease reduction when Kasumin was used with copper or Actigard as compared to using Kasumin by itself. Mixtures of Kasumin with Firewall or copper can be considered anti-resistance strategies because each mixture component is active against the pathogen. Actigard is a systemic acquired resistance compound (SAR) and stimulates host defense systems in some plants. It has no direct effect on the pathogen and is generally not very active against fire blight when used by itself. Thus, there is no benefit of using this material in mixture treatments with Kasumin (Fig. 1A,B). Actigard also did not improve the performance of Firewall (Fig. 1A).

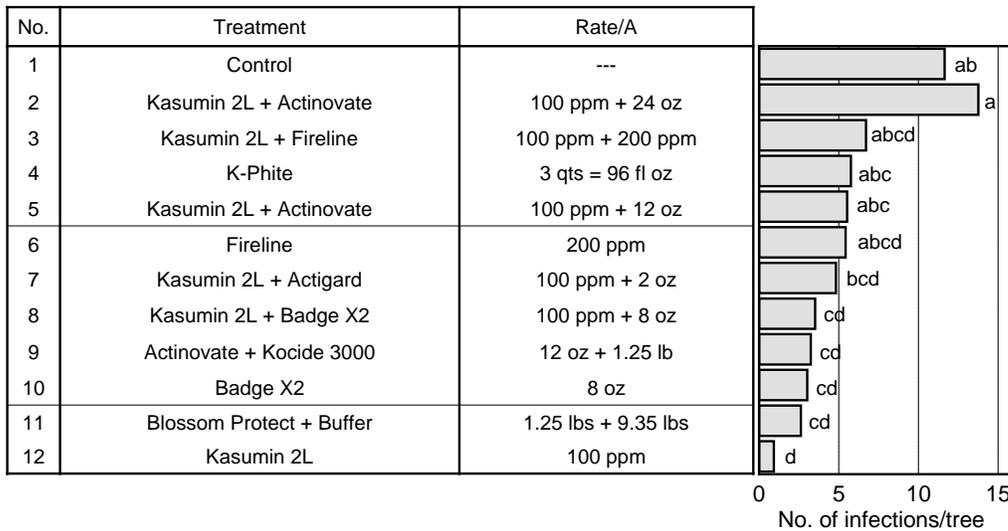
The biocontrol Blossom Protect was also among the best treatments (Fig. 1A,B). The biocontrol Actinovate was effective when used by itself at the 6-oz rate (Fig. 1A), but not in mixtures with Kasumin (Fig. 1A,B) or copper (Fig. 1A), reflecting the in vitro inhibition of *Streptomyces lydicus* by kasugamycin (see above; copper was not tested in this assay). Copper oxychloride/copper hydroxide (Badge) used by itself also significantly reduced the amount of disease from the control, but the phosphonates Prophyt, K-Phite, and Ko-phite did not show significant activity (Fig. 1AB). The biofilm inhibitor 2-aminoimidazole was not evaluated in the field due to high costs, low amount of material available, and non-inhibitory results in laboratory assays (see above).

Fig. 1. Efficacy of bactericides for fire blight management on Granny Smith and Fuji apple in a field trial at Kearney Ag Center 2013

A. Cv. Granny Smith



B. Cv. Fuji



Treatments were applied at 25% bloom (3-18) and 85% bloom (3-25-13) to cv. Granny Smith and at 20% bloom (3-25) and 95% bloom (4-3-13) to cv. Fuji using an air blast sprayer at 100 gal/A. Trees were inoculated with *E. amylovora* using an air-blast sprayer on 4-1-13. Disease was evaluated on 4-22-2013, and the number of diseased spurs per tree was counted.

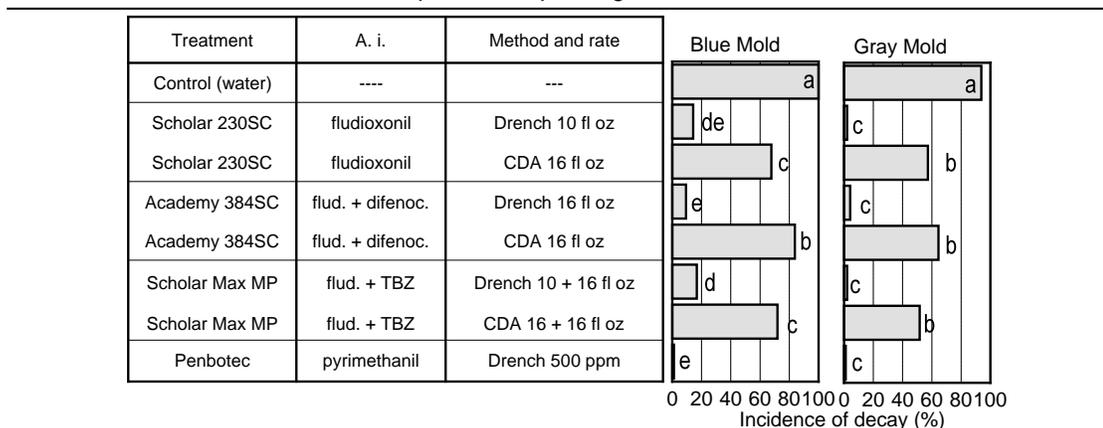
In summary, our project on the identification of integrated fire blight programs with copper, fungicides, antibiotics, and biocontrols has identified new treatments that can be adopted by the California pome fruit industries. Registration of Kasumin for use in California is pending in 2014.

**Evaluation of postharvest treatments using single-fungicides, mixtures, and pre-mixtures.** Experimental packing line studies using Granny Smith apples were conducted to evaluate new pre-mixture treatments in comparison with single-fungicides, as well as polyoxin-D and a new active ingredient for postharvest decay management, i.e., N-1. Decays studied included blue mold, gray mold, bull’s eye rot, and Alternaria rot. The latter decay can be quite serious on injured pome fruit, but was never before included in out postharvest studies. We also evaluated the efficacy against bitter rot. This disease occurs in California but is a major problem in wetter climates.

For the evaluation of the final pre-mixture formulation of fludioxonil-difenoconazole (i.e., Academy) and of a new fludioxonil-TBZ multi-pack formulation (i.e., Scholar Max MP) in comparison with fludioxonil (Scholar) alone, the efficacy was compared using re-circulating, in-line drench versus low-volume, CDA spray applications (Figs. 2-3). In all cases, the efficacy of each treatment was increased when applied as a drench application. During the application on a roller bed, the apple fruit did not rotate well on the roller bars, and thus, fungicide coverage of the inoculation site was poor using a low-volume spray application. Under commercial conditions, certain devices are used to improve fruit rotation during fungicide application, and thus, efficacy of low-volume applications is likely higher. Still, re-circulating high-volume drench applications are the most effective method to provide excellent coverage and decay control.

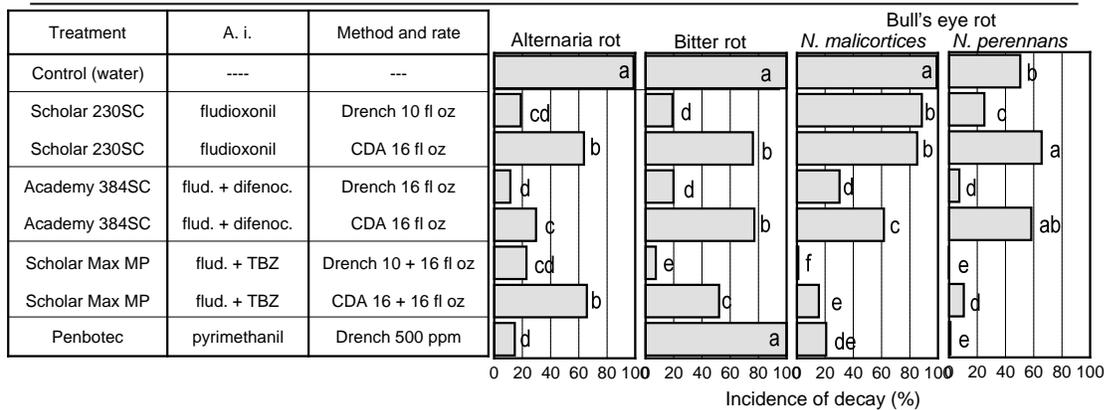
In-line drench applications with Academy, Scholar Max MP, Scholar, and Penbotec were all highly effective in reducing blue mold (caused by TBZ-resistant strains of *P. expansum*) and gray mold (Fig. 2). Decay incidence was reduced from almost 100% in the control to less than 20%. Penbotec was also highly effective. As indicated previously, Scholar is not effective against bull’s eye rot. However, the fludioxonil-difenoconazole mixture Academy and the fludioxonil-TBZ mixture Scholar Max reduced the incidence of decay to low levels on fruit inoculated with *N. perennans* or *N. malicorticis*, similar to Penbotec (Fig. 3). Resistance against pyrimethanil has developed in some populations of the three decay fungi at some locations and thus, Penbotec has to be rotated with different modes of action. Although difenoconazole is not effective against gray mold, and generally did not provide an additive effect in blue mold control when used in mixtures with Scholar as compared to using Scholar alone, registration of the pre-mixture will be an important tool to decrease the risk of fungicide resistance to develop in populations of *Penicillium* spp.

Fig. 2. Evaluation of postharvest applications with new fungicide pre-mixtures for management of blue and gray mold decay of Granny Smith apples in experimental packingline studies



Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* ( $5 \times 10^5$  conidia/ml) or with a TBZ-sensitive isolate of *B. cinerea* ( $10^5$  conidia/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnauba fruit coating (Decco 231). CDA applications were done using 25 gal/200,000 lb fruit and treatments were done in carnauba fruit coating. Rates for CDA applications are for 200,000 lb fruit. For Scholar Max MP rates are given separately for the two components, whereas for the pre-mixture Academy the rate is given as a total of the two components. 10 fl oz Scholar = 180 ppm, 16 fl oz = 480 ppm, 16 fl oz Academy = 480 ppm = 10 fl oz Scholar + 10.7 fl oz A8574D. Fruit were then incubated at 20 C for 6 days.

Fig. 3. Evaluation of postharvest applications with new fungicide pre-mixtures for management of *Alternaria* decay, bitter rot, and bull's eye rot of Granny Smith apples in experimental packingline studies

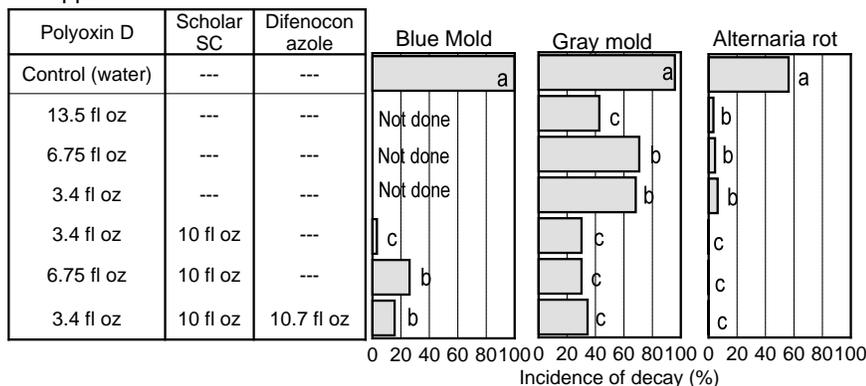


Fruit were inoculated with conidia of *Alternaria alternata*, *Colletotrichum acutatum*, *Neofabraea malicortices* (all at  $10^5$  conidia/ml), or *N. perennans* ( $10^6$  conidia/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnauba fruit coating (Decco 231). CDA applications were done using 25 gal/200,000 lb fruit and treatments were done in carnauba fruit coating. Rates for CDA applications are for 200,000 lb fruit. For Scholar Max MP rates are given separately for the two components, whereas for the pre-mixture Academy the rate is given as a total of the two components. 10 fl oz Scholar = 180 ppm, 16 fl oz = 480 ppm, 16 fl oz Academy = 480 ppm = 10 fl oz Scholar + 10.7 fl oz A8574D. Fruit were then incubated at 20 C for 6 days.

For control of *Alternaria* rot where non-fungicide-treated fruit developed 100% decay, Scholar, Academy, Scholar Max, and Penbotec were similarly effective (Fig. 3). Difenoconazole and fludioxonil were tested last year for their in vitro activity against *Alternaria* sp. and the low EC<sub>50</sub> values obtained (0.01 to 0.04 ppm for difenoconazole, 0.011 to 0.025 ppm for fludioxonil) support their high effectiveness against this decay. Bitter rot was also reduced to low levels using Scholar, Academy, or Scholar Max (Fig. 3). This further broadens the spectrum of activity of the fludioxonil-difenoconazole pre-mixture with blue mold, gray mold, bull's eye rot, *Alternaria* rot, and bitter rot. Studies on *Alternaria* rot will need to be repeated next year. Gray mold, blue mold, bull's eye rot, and *Alternaria* rot (but not *Mucor* decay or bitter rot) are also controlled by Penbotec. Resistance against pyrimethanil, however, has developed in populations of *Penicillium*, *Botrytis*, and *Neofabraea* spp. at some locations and thus, this fungicide has to be rotated with different modes of action.

Fig. 4. Evaluation of polyoxin-D (CX-10440) as a potential new postharvest treatment for management of blue mold, gray mold, and *Alternaria* rot of Granny Smith apples in experimental packingline studies

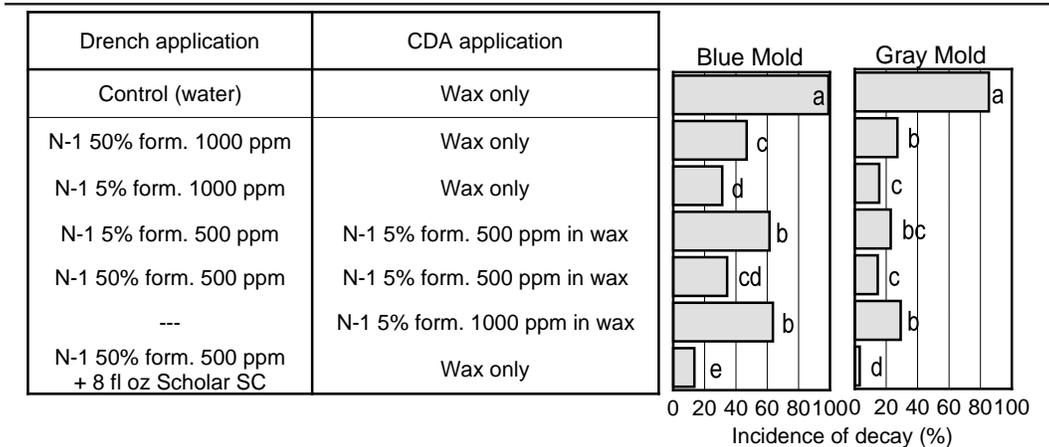
In-line drench applications



Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* ( $5 \times 10^5$  conidia/ml), *B. cinerea* ( $10^5$  conidia/ml) or *Alternaria alternata* (100,000 conidia/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnauba fruit coating (Decco 230). For difenoconazole, the A8574D formulation was used. 10 fl oz Scholar = 180 ppm, 10.7 fl oz A8574D = 300 ppm. Fruit were then incubated at 20 C for 6 days.

In a postharvest packing line study with CX-10440 (polyoxin-D), this treatment was moderately effective against gray mold when used by itself at the 13.5-oz rate or in combination with Scholar (Fig. 4). In last year's studies, it was shown to be highly effective. This indicates that fruit maturity may be critical for the effectiveness of this treatment (see below). Polyoxin was highly effective against Alternaria rot (Fig. 4).

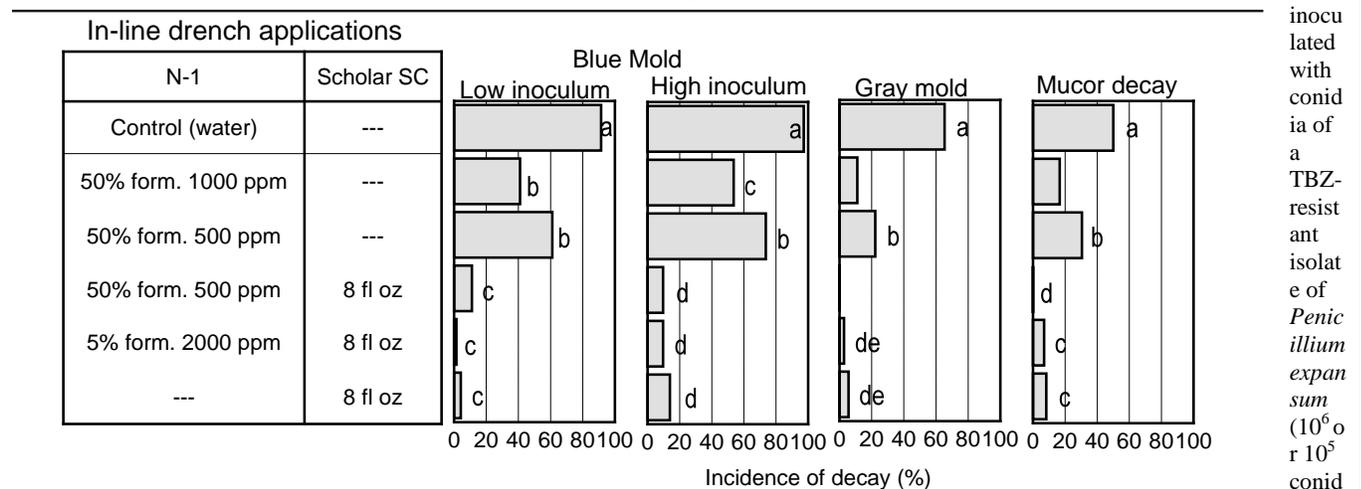
Fig. 5. Evaluation of two formulations of N-1 as a potential new postharvest treatment for management of blue and gray mold decay of Granny Smith apples in experimental packingline studies



Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* ( $5 \times 10^5$  conidia/ml) or with *B. cinerea* ( $10^5$  conidia/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnauba fruit coating (Decco 231). Fruit were then incubated at 20 C for 6 days.

N-1 is a new active ingredient for postharvest use that we evaluated for the first time in 2013. In-line drench treatments or drench-CDA combination treatments were very effective against gray mold and moderately effective against blue mold and Mucor decay (Figs. 5,6). In mixtures with low concentrations of Scholar (8 fl oz = approx. 150 ppm) decay was reduced to very low levels. As with polyoxin-D, the efficacy of this compound may be highly dependent on fruit maturity and the best application strategies still need to be defined.

Fig. 6. Evaluation of two formulations of N-1 as a potential new postharvest treatment for management of blue mold, gray mold, and Mucor decay of Granny Smith apples in experimental packingline studies



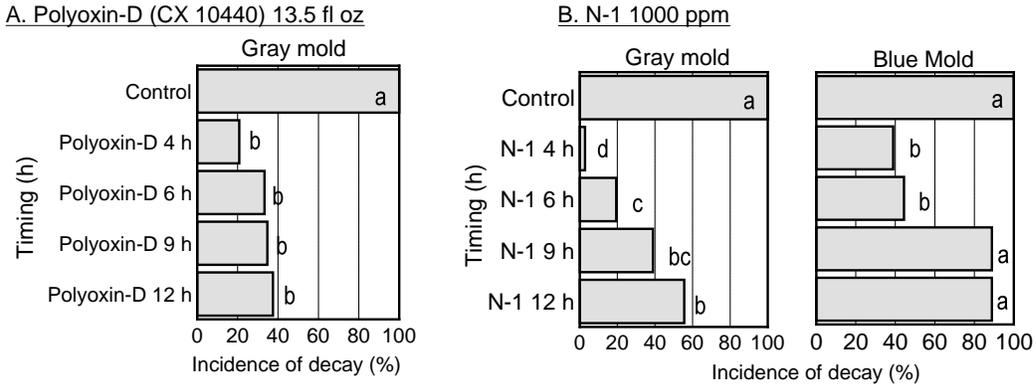
Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* ( $10^6$  or  $10^5$  conid

ia/ml), *B. cinerea* ( $5 \times 10^4$  conidia/ml) or *M. piriformis* ( $10^5$  spores/ml) and were incubated for 15-17 h at 20C. Treatments with aqueous fungicide solutions were done by in-line re-circulating drench applications that were followed by a CDA application with carnauba fruit coating (Decco 231). Fruit were then incubated at 20 C for 6 days.

In a timing study where treatments with polyoxin-D or N-1 were applied to apple fruit selected times after inoculation, efficacy was shown to be highly dependent on the timing. Thus, for N-1, treatments applied 4 or 6 h after inoculation were significantly more effective than when applied after 9 or 12 h (Fig. 7). A trend for better efficacy in the 4-h timing was also observed for polyoxin-D. Considering that highly susceptible, senescent fruit were used in this latter study, higher efficacy is expected when treating fruit immediately after harvest. Thus, both

compounds will have to be continued to be evaluated. This is important because both potentially could be used for organic fruit production. They also could be used in mixtures to prevent resistance of gray mold to fludioxonil in packinghouses using conventional treatments. Fludioxonil is currently the only highly effective gray mold material used commercially where no resistance has been found. Thus, its activity needs to be protected with registration of additional materials.

Fig. 7. Effect of application timing after inoculation on the efficacy of postharvest treatments with polyoxin-D and N-1 for management of blue mold and gray mold in laboratory studies



Fruit were inoculated with conidia of a TBZ-resistant isolate of *Penicillium expansum* or with *B. cinerea* ( $10^5$  conidia/ml each) and were incubated for selected times at 20C. Treatments with aqueous fungicide solutions were done using a hand-sprayer. Fruit were then incubated at 20 C for 6 days.