Annual Report - 2018

Prepared for the California Apple commission

Project Title:	Evaluation of new biological controls for management of fire blight of apples caused by *Erwinia amylovora* and evaluation of new natural products as organic postharvest fungicides for pome fruits
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SUMMARY

Fire blight management

1. Resistance in *E. amylovora* to streptomycin was found in 2017 in three of the seven orchards sampled. Results over the years support our recommendation that streptomycin can be used once a year effectively for most growers. In years with high- to moderate disease levels, pathogen populations exposed to multiple applications of streptomycin will be under selection pressure of the antibiotic, and this will allow re-emergence of resistant sub-populations. Data for 2018 collections are pending.
2. *E. amylovora* populations were found to be moderately copper-resistant. Additionally, we again frequently observed the occurrence of spontaneous mutant colonies emerging at higher copper concentrations, especially when using nutrient agar.
3. Field trials on the management of fire blight were conducted under high disease pressure on cvs. Granny Smith and Fuji, as well as on Bartlett pear.
   a. Among biological treatments, the rotation of Badge – Badge+lime sulfur – Blossom Protect/buffer showed the highest efficacy with a 41% reduction of disease compared with the control on 'Granny Smith'. Cueva, Blossom Protect, Serenade ASO+Badge, and Serenade ASO were less effective but disease was still significantly lower than the control. On pear, the preservatives Nisin and polylysine also resulted in reduction of disease.
   b. On 'Fuji' apple, the mixture of FireWall and Mastercop reduced the disease to the lowest level with a 72% reduction from the control. This treatment, however, resulted in an unacceptable high severity of fruit russeting.
   c. Mixture-rotation and rotation treatment programs with antibiotics (i.e., Kasumin, FireLine, FireWall) were very effective. Kasumin is currently considered a conventional treatment, however, efforts are underway to obtain an organic registration. The compound is a natural substance that is commercially produced by fermentation. In contrast to streptomycin and oxytetracycline, it has very minimal or no usage in human medicine.

Postharvest decay control

1. In laboratory studies, formulations of the bio-fungicide natamycin were compared, and two liquid formulations were found to be superior to the WP formulations in reducing decay. Natamycin was registered in 2016 under the trade name BioSpectra on citrus and stone fruits. Because it is a fermentation product, it is being proposed to the National Organic Standards Board (NOSB) as an organic treatment (PI submitted a letter of support to the NOSB).
2. In an experimental packingline study using in-line drench applications, BioSpectra was not very effective against blue mold, but was similarly effective against gray mold and Mucor rot when compared with Scholar or the newly registered Academy.
3. Academy (fludioxonil + difenoconazole) was highly effective against the three decays, similar to Scholar, and the addition of another 150 ppm of fludioxonil to the pre-mixture improved efficacy. Academy was previously also shown to be effective against bull's eye rot, Rhizopus rot, and Alternaria rot, and thus, has a wide spectrum of activity. Mixtures of BioSpectra with Scholar or Academy were also very effective against the three decays. This is important, because this presents an excellent resistance management strategy.

INTRODUCTION

Epidemiology and management of fire blight. Fire blight, caused by the bacterium *Erwinia amylovora,* is one of the most destructive diseases of pome fruit trees including apples. The disease causes a blackening of twigs, flowers, and foliage and is indigenous to North America but has since spread worldwide. In addition to cankers, the pathogen overwinters in flower buds, diseased fruit, small twigs, and branches. In the spring, blossoms are infected through natural openings in nectaries and pistils. After destroying the blossom, the bacteria spread into the peduncle, spur, and twig. Warm wet environments favor disease development. Inoculum may ooze as droplets from cankers or infected flowers, peduncles, and other infected tissues. Inoculum is spread by wind, rain, insects, birds, or by man, e.g., by means of contaminated pruning tools. Secondary infections may occur throughout the growing season.

Current chemical control programs for fire blight are based on protective schedules, because available compounds are contact treatments and are not systemic except for the antibiotic streptomycin. Control with conventional copper compounds is only satisfactory when disease severity is low to moderate. Historically, these treatments are only used during dormant and bloom periods because phytotoxicity commonly occurs on fruit as russeting. Subsequently, labeled rates of copper are at low amounts of metallic copper equivalent (MCE) that are at the limit of effectiveness. Additionally, in 2016-17, low to moderate levels of copper insensitivity in pathogen populations was again detected.

The antibiotics streptomycin and oxytetracycline have been used for many years for the management of fire blight, but they were removed from the approved list of organic treatments of apples and other pome fruits by the National Organic Standards Board (NOSB). Resistance to streptomycin was present at high incidence in populations of the fire blight pathogen in California between 2006 and 2011, but since then has declined to low levels in most orchards. Reduced sensitivity to oxytetracycline only has been found sporadically, and resistant populations did not persist. After a long delay, kasugamycin (Kasumin) is now registered in California. The antibiotic is currently not registered as an organic treatment and thus, organic growers have very limited choices for disease control.

New re-formulated copper products that can be used at reduced MCE rates and that have less contamination in their formulations that may cause phytotoxicity are available. Some of the coppers are OMRI-approved and these include Badge X2 (Gowan), CS-2005 (Magna Bon, Inc.), and Cueva (Certis). They have been reported to be effective against fire blight without causing phytotoxicity. Thus, research on OMRI-approved coppers needs to be continued especially if antibiotics are no longer approved, and these treatments were included in our 2018 field studies.

The biocontrol treatments Blight Ban A506 (*Pseudomonas fluorescens* strain A506) and Bloomtime Biological (*Pantoea agglomerans* strain E325), and the fermentation product of *Bacillus subtilis* Serenade (strain QST 713) have been very inconsistent over the years in their performance in our trials and were most effective under low inoculum levels and less favorable micro-environments. Serenade has become available as a new liquid formulation (ASO) that needed to be evaluated. The biocontrol Blossom Protect (*Aureobasidium pullulans*) was evaluated for the last several years and shown to be very effective under less to moderately favorable disease conditions and it is one of the most consistent biologicals that we have evaluated. In general, biocontrols are most effective when they are actively growing on the plant. Additives that can be used under field conditions have been evaluated, but their effect has also been inconsistent. Thus, we are evaluating other alternatives such as the natural fermentation compounds lactic acid, ε-poly-L-lysine, and Nisin that have known anti-bacterial activity and are used as natural preservatives in food. They potentially could qualify for organic production. In our 2018 studies, we prepared alginate formulations of the latter two products that have the potential to provide a slow release and higher persistence of the active compounds.

A novel way to inhibit bacterial pathogens could be the interference with vital processes such as the secretion of pathogenesis-related proteins. For this, the type III secretion system is used by many bacterial plant pathogens. Molecular work has led to the identification of inhibitors of this secretion system, and a laboratory has provided us with substantial amounts that could be tested in field studies. These potentially could qualify as organic treatments and therefore, were also evaluated in our 2018 studies. Our goal is to develop effective rotational programs for either organic farming practices with the use of copper and biologicals or conventional programs with the use of antibiotics alone or in mixtures with fungicides, copper, biologicals, or possibly SAR compounds during bloom or as cover sprays during early fruit development.

Management of postharvest decays. Apples like other pome fruits can be stored for some period of time using optimum fruit storage environments. Still, postharvest decays caused by fungal organisms can result in economic crop losses during storing and marketing of fruit. The major postharvest pathogens of apples include *Penicillium expansum, Botrytis cinerea, Alternaria alternata, Mucor piriformis,* and *Neofabraea* spp. causing blue mold, gray mold, Alternaria rot (black mold), Mucor decay, and bull's eye rot, respectively. There is a deficiency in postharvest biocontrols and natural products for preventing decays in storage. BioSave 100 is one of the few materials currently available in the United States, but its efficacy is limited. The product Aspire has been discontinued. Still, other biological products are registered in other countries and these potentially could be evaluated for California conditions if registrants decide to market their products in the U.S.

In previously found that the bio-fungicide polyoxin-D (Ph-D, Oso, Tavano) was very effective in reducing the incidence of gray mold and Alternaria rot, but not of blue mold. We also demonstrated the efficacy of another bio-fungicide, natamycin (formerly pimaricin or EXP-13). This compound was registered in late 2016 as BioSpectra as a postharvest treatment for citrus and stone fruits. Natamycin showed very good to good efficacy against decays caused by *Penicillium*, *Botrytis*, and *Mucor* spp. For many years, it has been a federally-approved food additive to prevent mold growth, including *Penicillium* species, on dairy and meat products in the United States and other countries. Over this time, resistance in *Penicillium* species against natamycin has not occurred. Natamycin has an exempt registration status and it has been submitted to the NOSB for organic registration. In our studies over the past years, we noted a somewhat inconsistent efficacy of natamycin. Therefore, a goal was to improve its performance. In 2017/18, we compared several formulations of the bio-fungicide and we continued to evaluate its efficacy in an experimental packingline study together with the newly registered Academy (pre-mixture of fludioxonil and difenoconazole) with the goal of having additional postharvest fungicides for the apple industry of California.

OBJECTIVES

Fire blight research

1. Evaluate the efficacy of treatments for managing fire blight.
   - A. Laboratory in vitro tests to identify and evaluate growth enhancers of biological control agents.
   - B. Laboratory in vitro tests on copper and zinc products (registered copper products and new nano-particles as they become available) with newly identified additives (lactic acid, poly-L-lysine, and experimentals called SBH derivatives) that enhance the activity of these bactericides.
   - C. Small-scale hand-sprayer tests using different treatment-inoculation schedules to evaluate coppers (e.g., Badge X2, CS-2005, Cueva, Champ), and biological treatments (e.g., Blossom Protect, Actinovate, Serenade, Taegro, Double Nickel 55) by themselves or in selected combinations (e.g., copper and Blossom Protect).
   - D. Field trials with protective air-blast spray treatments:
     - i. New formulations of copper (e.g., Badge X2, CS-2005, Cueva) possibly supplemented with nano-copper oxide (if laboratory assays show activity) with and without newly identified additives (lactic acid, poly-L-lysine, and an experimental called SDH).
     - ii. Biological treatments (Blossom Protect, Serenade, Double Nickel 55) with and without the addition of growth enhancers.
     - iii. Plant defense activators or SARs alone of in mixtures with other biological control treatments.

Postharvest research

2. Comparative evaluation of new postharvest fungicides
   - A. Evaluate natamycin (BioSpectra) and other new postharvest fungicides such as Academy at selected rates against gray mold, blue mold, Alternaria decay, and bull's eye rot and compare to pyrimethanil and fludioxonil.
   - B. Evaluate mixtures of these compounds.
   - C. Determine baseline sensitivities. Baseline sensitivities for natamycin will be continued to be developed for additional fungal pathogens that are collected.

PLANS AND PROCEDURES

Isolation and culturing of* E. amylovora *and sensitivity testing against antibiotics and copper. Fire blight samples were obtained from pome fruit trees in the spring of 2017 and 2018 from commercial orchards. Infected plant material was surface-disinfested for 1 min using 400 mg/L sodium hypochlorite, rinsed with sterile water, cut into small sections, and incubated in 1 ml of sterile water for 15 to 30 min to allow bacteria to stream out of the tissue. Suspensions were streaked onto yeast extract-dextrose-$CaCO_3$ agar (YDC). Single colonies were transferred and the identity of the isolates as *E. amylovora* was verified by colony morphology and by PCR using primers specific for *E. amylovora* (Appl. Environ. Microbiol. 58:3522-2536). Strains were tested for their sensitivity to streptomycin and oxytetracycline using the spiral gradient dilution (SGD) method. Copper sensitivity of strains was determined by streaking bacterial suspensions (70% transmission at 600 nm) on CYE (casitone, yeast extract, glycerol) or nutrient agar amended with 0, 10, 20, or 30 ppm MCE. Growth was recorded after 2 days of incubation at 25C and was rated as +++ (growth not inhibited, similar to the control), ++ (growth inhibited as compared to the control), or + (growth sparse).

Field studies on the management of fire blight using protective treatments. Air-blast field studies on the relative efficacy of protective treatments were conducted in experimental cvs. Granny Smith and Fuji apple orchards at the Kearney Agricultural Research and Extension Center (KARE). All trees received a copper treatment at bud break to help reduce the high amount of inoculum present in these orchards that made evaluation of bactericide treatments difficult in the last couple of years. Four applications were done starting at 5-10% bloom and followed by phenology-based treatments until petal fall. Several rotation or mixture rotation programs were evaluated. Incidence of blight was assessed in early to mid-June based on the number of infected flower clusters of 200 clusters evaluated for each of the four single-tree replications. Additionally, potential phytotoxic effects of the treatments (e.g., fruit russeting caused by copper) were evaluated. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.4.

For comparison, field studies on fire blight were also conducted on Bartlett pear. In these trials some novel treatments were included such as type III secretion inhibitors, Nisin and polylysine mixed with alginate, as well as zinc nitrate. Three applications were done, and disease was evaluated on 90 spurs of each of the four single-tree replications seven days after the last application.

Efficacy of new postharvest fungicides for managing apple decays in storage. A comparison of three natamycin formulations (50WP, 10SC, 5EC) was conducted on 'Shinko' apple pears in the laboratory. Fruit were inoculated with *P. expansum* or *B. cinerea*, and treated using an air-nozzle sprayer after 11 h. Fruit were then incubated for 7 days at 20C.

'Granny Smith' fruit that were treated similar to commercial practices concerning harvest, handling, packing, and temperature-management of fruit were used in an experimental packingline study at KARE. Fruit were wound-inoculated with conidial suspensions of several decay fungi (*B. cinerea, P. expansum,* and *Mucor piriformis*) and treated after 16 to 18 h with test fungicides by an in-line drench that was followed by a CDA application with a carnauba-based fruit coating (i.e., Decco 230). Treatments included natamycin (BioSpectra), Scholar, and Academy (fludioxonil – difenoconazole pre-mixture). For each of four replications, 24 fruit were used. Data were analyzed using analysis of variance and averages were separated using least significant difference mean separation procedures of SAS 9.4.

RESULTS AND DISCUSSION

Antibiotic and copper sensitivity of* E. amylovora *strains collected in California. All 26 strains from seven locations collected in 2017 in Sacramento and Lake Co. were determined to be sensitive to oxytetracycline (Table 1); whereas, 8 of 25 strains from Sacramento Co. were resistant to streptomycin. Five of these 8 strains were highly resistant (MIC values >2000 mg/L) and three were moderately resistant (MIC values <30 ppm) (Table 1). Resistance was found in three of the seven orchards sampled, and all six strains from one orchard were either moderately or highly resistant. Results over the years support our recommendation that streptomycin can be used once a year effectively for most growers. In years with high- to moderate disease levels, pathogen populations exposed to multiple applications of streptomycin will be under selection pressure of the antibiotic, and this will allow re-emergence of resistant sub-populations. Data for 2018 collections are pending.

All 26 strains from Sacramento and Lake Co. did not grow on CYE (a growth medium with a low copper-binding capacity) amended with 20 ppm MCE (Table 1). They all grew similar to the non-amended control on the nutrient-rich nutrient agar at 20 ppm MCE. One strain still grew well at 30 ppm MCE on nutrient agar, whereas growth of the other strains was reduced at this concentration. Thus, as in 2015 and 2016, we conclude that current *E. amylovora* populations are moderately copper-resistant. Additionally, we again frequently observed the occurrence of spontaneous mutant colonies emerging at higher copper concentrations, especially when using nutrient agar. These mutants were not stable when sub-cultured on copper-free media and reverted back to sensitivity. If these mutants also occur in the field, however, under continued presence of selection pressure (i.e., copper sprays) they may successfully compete and cause disease.

We consider several factors that likely contributed to the failure of copper applications to control fire blight in the past: 1) Highly conducive disease conditions may low allow for the pathogen to overcome the suppressive action of copper; 2) Only low rates of copper are registered for fire blight management (approx. 170 MCE for the 0.5 lb rate of Kocide 3000) and this may allow growth of moderately Cu-resistant strains; 3) There is moderate copper resistance in *E. amylovora;* and 4), Selection of populations (spontaneous mutants) with higher copper resistance after repeated applications may lead to disease in the presence of copper. Furthermore, copper is bacteriostatic and does not kill the pathogen. Thus, use as a pre-bloom/early bloom treatment may have some benefits in suppressing bacterial oozing from cankers. Applying a contact bactericide with low to moderate toxicity will only provide marginal benefits because the pathogen causes a deep internal infection (i.e., cankers) and has a high reproductive capacity. This means that the pathogen will ooze from cankers (unaffected by copper) and disseminate to unprotected tissues if copper is not routinely applied. If several copper applications are done, however, russetting will occur on pome fruit varieties.

Field studies on fire blight using protective treatments. Fire blight incidence in our research plots in the spring of 2017 was high, i. e., over 50% based on infected flower clusters of untreated control trees. On cv. Granny Smith apple, among organic treatments, the rotation of Badge – Badge+lime sulfur – Blossom Protect/buffer showed the highest efficacy with a 41% reduction of disease compared with the control (Fig. 1). Cueva, Blossom Protect, Serenade ASO+Badge, and Serenade ASO resulted in 38%, 34%, 32%, and 17% reductions, respectively, and these were all significantly lower than the control. Phytotoxicity on fruit after Cueva treatments had a rating of 1.2 on a scale from 0 to 4 (with 4 being the highest phytotoxicity). Treatments containing Kasumin (by itself or mixed with polylysine and zinc oxide or with Firewall) performed the best in this study with reductions in disease between 52% and 56%.

In the study on 'Fuji' apple, the mixture of FireWall and Mastercop reduced the disease to the lowest level with a 72% reduction from the control (Fig. 2). This treatment, however, resulted in an unacceptable high severity of fruit russeting (a rating of 3 of a maximum of 4). The mixture-rotation and rotation treatment programs with antibiotics (i.e., Kasumin, FireLine, FireWall) were also very effective with 62% and 57% reductions in disease, respectively. Phytotoxicity ratings were <0.3 for the latter treatments. Blossom Protect rotated or mixed with Serenade ASO was somewhat less effective, but still significantly the incidence of fire blight from the control, and there was no phytotoxicity.

Studies were also done on Bartlett pear where fire blight is generally more severe than on apple. New and experimental conventional and biological treatments were evaluated in two studies in three-spray programs, some of which could not be included in the apple studies (due to a limited number of trees available). In the first study, among biological treatments, the preservative Nisin was the most effective, reducing the incidence of blight from the untreated control by approximately 50% (Fig. 3). Nisin was less effective when prepared as an alginate formulation to provide a slower release of the material over time. One of the three type III secretion inhibitors (TS153) evaluated had a slight numeric, but not statistical, increase in disease as compared with Nisin; and Blossom Protect and Serenade ASO followed in efficacy with 34% to 35% reduction in disease. In this study, FireLine that was included as a standard conventional treatment, had the lowest incidence of disease in this plot with a 64% reduction as compared with the control (Fig. 3). The addition of the adjuvant Tactic did not increase efficacy of FireLine. Two type III secretion inhibitors and Serifel (*Bacillus amyloliquefaciens*) did not show efficacy in this study. Zinc nitrate and Serenade ASO by itself or mixed with Cueva showed some reduction of fire blight.

In the second study on pear, Kasumin mixed with FireWall showed the highest efficacy (as in many of our previous years' studies) with an 81% reduction in disease as compared with the control (Fig. 4). Other treatments containing Kasumin also performed well, except in the rotation with the natural product 1552 (treatments 1 and 3 in the rotation were done with 1552, treatment 2 was done with Kasumin). Cueva, polylysine (with or without alginate – zinc oxide), and Cueva mixed with the copper enhancer DAS-1 showed intermediate efficacy.

In conclusion, none of the new organic treatments or those of a natural origin (except Kasumin) showed high efficacy in the management of fire blight. In comparison with conventional treatments, those containing antibiotics were always the most effective. Blossom Protect was less effective than in many of our previous studies, but still significantly reduced the disease from the control. Other biological treatments to be considered are the liquid copper formulation Cueva and the preservatives Nisin and polylysine. We tried to improve the efficacy of the two preservatives with the addition of alginate. This was not very successful, but possibly, other additives could be tested. Kasumin is currently considered a conventional treatment, however, efforts are underway to obtain an organic registration. The compound is a natural substance that is commercially produced by fermentation of *Streptomyces* species. In contrast to streptomycin and oxytetracycline, it has very minimal or no usage in human and veterinary medicine. Thus, an organic registration seems plausible. A summary on the use of biological treatments for the management of fire blight has recently been prepared for the California Apple Commission.

High levels of overwintering cankers and disease on new growth in the spring were present at all of our field test sites because orchards were either experimental (apple) or were not commercially managed (pear). This made it difficult to obtain low disease levels for any of the treatments evaluated. Still, comparative efficacy data could be obtained, and absolute efficacy in a well-managed commercial orchard is expected to be higher.

Evaluation of postharvest treatments using single-fungicides, mixtures, and pre-mixtures. Postharvest studies focused on the efficacy of the new natural compound natamycin that is currently exempt-from-tolerance and registered as BioSpectra on citrus and stone fruits. The compound was submitted to the NOSB, and a letter was written by Dr. Adaskaveg in support of an OMRI listing. In laboratory studies, we compared the efficacy of several formulations in the control of blue mold and gray mold. Significant differences were observed, with the WP formulation the least effective (Fig. 5). The 5EC formulation was more effective than the 10SC formulation in the control of blue mold when used at 1000 ppm, but at 2000 ppm, these two performed the same. Still, Scholar used at 300 ppm was significantly more effective. For gray mold, both liquid formulations were highly effective, and the 10SC formulation resulted in similar low levels of decay than Scholar.

In an experimental packingline study using in-line drench applications, BioSpectra was not very effective against blue mold, but was similarly effective against gray mold and Mucor rot when compared with Scholar or the newly registered Academy (Fig. 6). Academy was highly effective against the three decays, similar to Scholar, and the addition of another 150 ppm of fludioxonil to the pre-mixture improved efficacy. Academy was previously also shown to be effective against bull's eye rot, Rhizopus rot, and Alternaria rot, and thus, has a wide spectrum of activity. Mixtures of BioSpectra with Scholar or Academy were also very effective against the three decays. This is important, because this represents an excellent resistance management strategy. Resistance to natamycin has not been reported previously to any *Penicillium* species, although the compound has been registered for food uses for over 20 years.

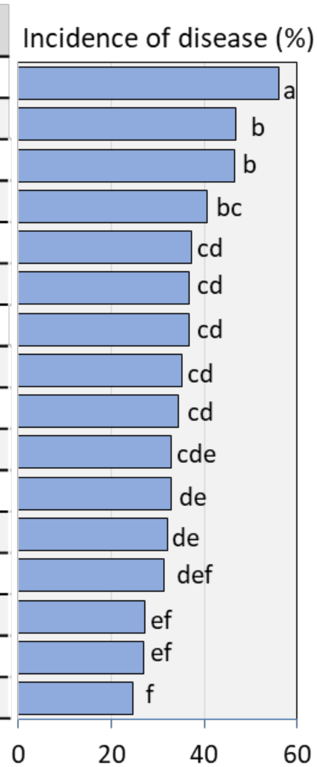
Table 1. Sensitivity of *E. amylovora* strains from California pome fruit orchards to streptomycin, oxytetracycline, and copper in 2017.

						Copper sensitivity - growth at:		
No.	Location No.	Isolate code	County	Strepto-mycin	Oxytetra-cycline	20 ppm CYE agar	20 ppm Nutrient agar	30 ppm Nutrient agar
1	1	1-1	Sacramento	S	S	-	++	+
2		1-2		S	S	-	++	+
3		1-3		S	S	-	++	+
4		1-4		S	S	-	++	+
5		1-5		S	S	-	++	+
6	2	2-1	Sacramento	HR	S	-	++	+
7		2-2		HR	S	-	++	+
8		2-3		MR	S	-	++	+
9		2-4		HR	S	-	++	+
10		2-5		HR	S	-	++	+
11		2-6		MR	S	-	++	+
12	3	3-1	Sacramento	HR	S	-	++	+
13	4	4-1	Sacramento	S	S	-	++	+
14	5	5-1	Sacramento	MR	S	-	++	+
15		5-3		S	S	-	++	++
16		5-5		S	S	-	++	+
17		5-6		S	S	-	++	+
18		5-7		S	S	-	++	+
19		5-9		S	S	-	++	+
20	6	6-1	Sacramento	S	S	-	++	+
21		6-2		S	S	-	++	+
22		6-3		S	S	-	++	+
23		6-4		S	S	-	++	+
24		6-5		S	S	-	++	+
25		6-6		S	S	-	++	+
26	7	7-1	Lake	S	S	-	++	+

Sensitivity to streptomycin and oxytetracycline was determined using the spiral gradient endpoint method. S = sensitive, MR = moderately resistant (MIC = <30 ppm), HR = highly resistant (MIC = >2000 ppm). Sensitivity to copper was determined by growth on amended CYE or nutrient agar. ++ = growth similar as in the non-amended control, + = reduction in growth.

Fig. 1. Efficacy of new mostly organic bactericides for management of fire blight of Granny Smith apples, Fresno Co. 2018

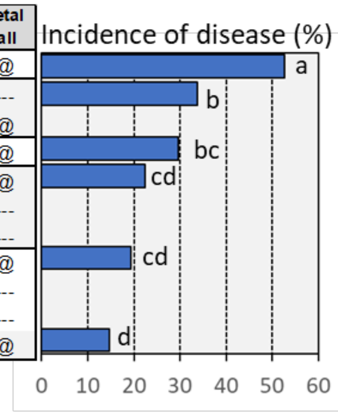
Treatment	Rate
Control	---
TS108 20% SL + Regulaid*	62.5 fl oz + 16 fl oz
Serenade ASO	64 fl oz
TS153 10% SL + Regulaid*	101 fl oz + 16 fl oz
Serenade ASO + Badge X2	64 fl oz + 16 oz
Blossom Protect + Buffer	20 oz + 8 lbs
TS28 10% SL + Regulaid*	107 fl oz + 16 fl oz
Cueva	3 qts
Kasumin 2L + Cueva	64 fl oz + 3 qts
Badge X2;X2+LS;BP+B;BP+B	16 oz
Kasumin 2L + Fireline	64 fl oz + 12 oz
ε Poly-L-Lysine + Zinc Oxide	13.2 oz + 32 oz
Kasumin 2L + Citric Acid	64 fl oz + 27 oz
Kasumin 2L	64 fl oz
Kasumin 2L + Firewall	64 fl oz + 8 oz
Kasumin 2L + ε Poly-L-lysine + Zinc Oxide	64 fl oz + 13.2 oz + 32 oz



Treatments were applied on 3-27 (5-10% bloom), 4-1 (20-40% bloom), and 4-9-18 (full bloom), and 4-14 (petal fall) using an air-blast sprayer at 100 gal/A. Disease was evaluated for 100 flower clusters (spurs) of each tree on 6-4-18. Abbreviations: X2 = Badge X2; LS= lime sulfur (6% rate); BP+B = Blossom Protect and buffer. All treatments had four, paired-tree replications (total of 8 trees). Limited material available for treatments using Regulaid thus only four, single-tree replications used.

Fig. 2. Efficacy of organic and conventional bactericides for management of fire blight of Fuji apples, Fresno Co. 2018

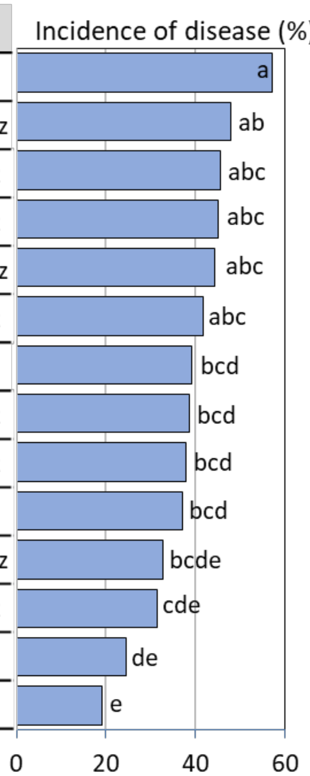
Treatment*	Rate/A	5-10% bloom	20-40% bloom	Full bloom	Petal fall
Control	—	@	@	@	@
Blossom Protect/Buffer	20 oz/128 oz	@	---	@	---
Serenade ASO	64 fl oz	---	@	---	@
Blossom Protect/Buffer + Serenade ASO	20 oz/128 oz + 64 fl oz	@	@	@	@
Kasumin + FireWall	64 fl oz + 8 oz	@	---	---	@
FireWall + FireLine	8 + 16 oz	---	@	---	---
Kasumin + FireLine	64 fl oz +16 oz	---	---	@	---
Kasumin	64 fl oz	@	---	---	@
FireWall	8 oz	---	@	---	---
FireLine	16 oz	---	---	@	---
FireWall + Mastercop	8 oz + 4 pt	@	@	@	@



Treatments were applied on 4-6 (5-10% bloom), 4-9 (20-40% bloom), and 4-13-18 (full bloom), and 4-19 (petal fall) using an air-blast sprayer at 100 gal/A. Disease was evaluated for 100 flower clusters (spurs) of each tree on 6-4-18. All treatments had four, paired-tree replications (total of 8 trees).

Fig. 3. Efficacy of new mostly organic bactericides for management of fire blight of Bartlett pear, Sutter Co. 2018

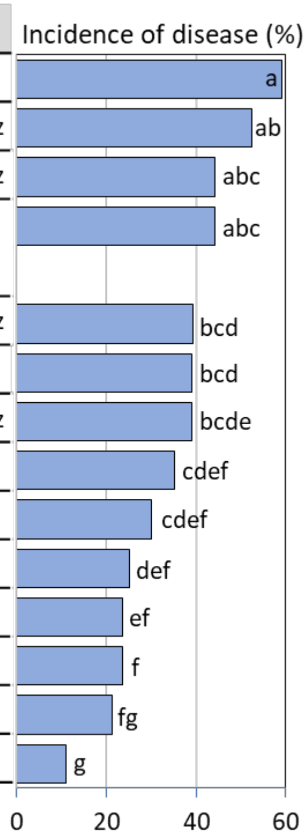
Treatment	Rate	Adjuvant
Control	---	---
TS28 10% SL	107 fl oz	Regulaid 16 fl oz
Nisin + Alginate	13.5 oz	NuFilmP 5 fl oz
Serifel	4 oz	NuFilmP 5 fl oz
TS108 20% SL	62.5 fl o	Regulaid 16 fl oz
Nisin + Alginate + Brandt ZnO	13.5 oz + 32 oz	NuFilmP 5 fl oz
Serenade ASO + Cueva	64 + 96 fl oz	---
Brandt ZnNO3	64 fl oz	NuFilmP 5 fl oz
Serenade ASO	64 fl oz	NuFilmP 5 fl oz
Blossom Protect + buffer	20 + 143 oz	---
TS153 10% SL	101 fl oz	Regulaid 16 fl oz
Nisin	13.5 oz	NuFilmP 5 fl oz
Fireline	16 oz	Tactic 8 fl oz
FireLine	16 oz	---



Treatments were applied on 3-28 (5% bloom), 4-3 (full bloom), and 4-11-18 (petal fall) using an air-blast sprayer at 100 gal/A. Early infections were observed on 4-11-18. Disease was evaluated for 90 spurs of each tree on 4-18-18. All treatments had four, single-tree replications used.

Fig. 4. Efficacy of new bactericides for management of fire blight of Bartlett pear, Sutter Co. 2018

Treatment	Rate	Adjuvant
Control	---	---
PolyLysine + Alginate	13.5 oz	NuFilmP 5 fl oz
Brandt ZnNO3	64 fl oz	NuFilmP 5 fl oz
Rotation: 1552	28 oz	---
Rotation: Kasumin	64 fl oz	
PolyLysine + Alginate + ZnO	13.5 + 32 oz	NuFilmP 5 fl oz
Cueva	96 fl oz	---
Polylysine	13.5 oz	NuFilmP 5 fl oz
DAS1 + Cueva	27 + 96 fl oz	---
Kasumin + Polylysine	64 fl oz + 13.5 oz	---
Kasumin	64 fl oz	---
Kasumin + Citric Acid	64 fl oz + 26.5 oz	---
Mycoshield	16 oz	Tactic 8 fl oz
Firewall	8 oz	---
Kasumin + Firewall	64 fl oz + 8 oz	---

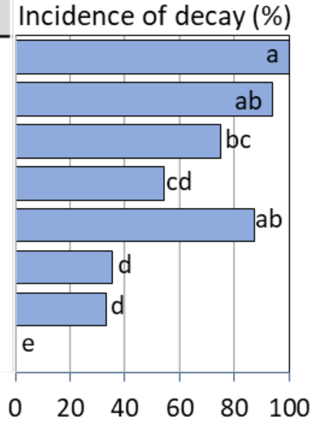


Treatments were applied on 3-28 (5% bloom), 4-3 (full bloom), and 4-11-18 (petal fall) using an air-blast sprayer at 100 gal/A. Early infections were observed on 4-11-18. Disease was evaluated for 90 spurs of each tree on 4-18-18. All treatments had four, single-tree replications used.

Fig. 5. Comparison of natamycin formulations for control of blue mold and gray mold in laboratory studies

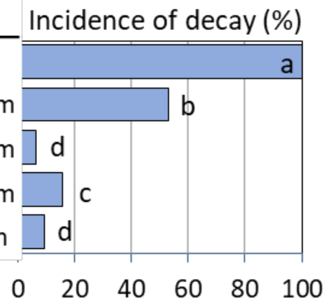
A. Blue mold

Treatment	Form.	Rate
Control	---	---
Natamycin	50WP	1000 ppm
Natamycin	10SC	1000 ppm
Natamycin	5EC	1000 ppm
Natamycin	50WP	2000 ppm
Natamycin	10SC	2000 ppm
Natamycin	5EC	2000 ppm
Scholar	230SC	300 ppm



B. Gray mold

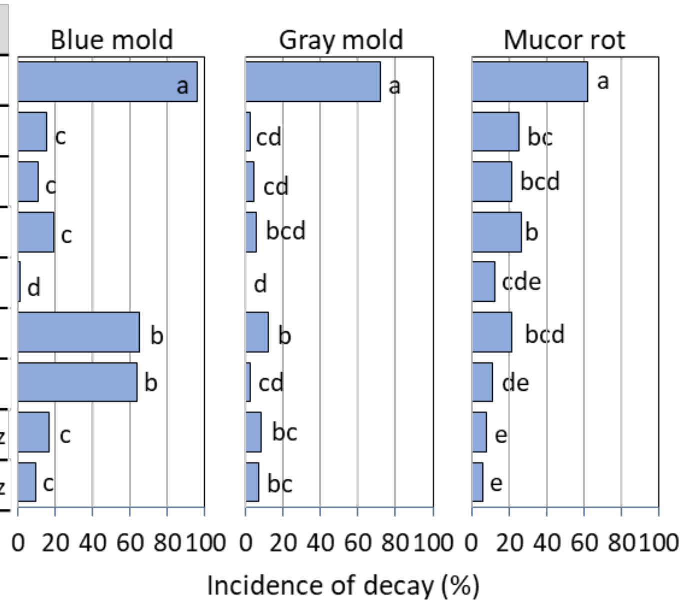
Treatment	Form.	Rate
Control	---	---
Natamycin	50WP	1000 ppm
Natamycin	10SC	1000 ppm
Natamycin	5EC	1000 ppm
Scholar	230SC	300 ppm



Apple pear fruit were wound-inoculated with *P. expansum* or *B. cinerea* and treated after 11 h of incubation at 20C using an air-nozzle sprayer. Fruit were then incubated for 7 days at 20C

Fig. 6. Evaluation of postharvest fungicides for managing postharvest decays of Granny Smith apple in an experimental packingline study

Treatment	Rate
Control	‘---
Scholar SC	16 fl oz
Scholar SC	32 fl oz
Academy SC	16 fl oz
Academy SC + Scholar	16 fl oz + 8 fl oz
BioSpectra	500 ppm
BioSpectra	1000 ppm
BioSpectra + Scholar	500 ppm + 16 fl oz
BioSpectra + Academy	500 ppm + 16 fl oz



Fruit were wound-inoculated with *P. expansum, B. cinerea,* or *M. piriformis* and treated after 16-18 h as in-line drenches that were followed by a CDA application with carnauba fruit coating (Decco 330). Fruit were then incubated at 20C.