Annual Report – 2019-20

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

I. Fire blight management

A. *Antibiotic and copper resistance surveys for populations of Erwinia amylovora in California pear growing areas were continued in 2019.*

1) Kasugamycin: All 117 strains from 14 orchard locations in Sacramento and 18 locations in Lake Co. were sensitive.
2) Streptomycin: Plasmid-based moderate- and chromosomal-based high-resistance was detected at eight locations in Sacramento Co. The incidence of resistance including that of high-resistant strains was sometimes very common in an orchard. Strains with moderate resistance to streptomycin was also found at three locations in Lake Co. Thus, populations of *E. amylovora* re-adjust rapidly to selection pressure (i.e., bactericide applications). Streptomycin should be used strategically, and these findings stress the importance of resistance management with mixtures or rotations and the development of new alternatives.
3) Oxytetracycline: Strains with high resistance levels (>40 ppm) were detected at three locations in Sacramento Co. At two of the locations, resistance was already found in 2018. These resistant strains were also highly resistant to streptomycin. In the location with the highest incidence of oxytetracycline resistance, nine applications of the antibiotic were applied between 2017 and 2019. Oxytetracycline resistance in *E. amylovora* has never been reported previously at this level, and this finding is a serious concern.

B. *Field trials on the management of fire blight*

1) On Granny Smith and Fuji apple, all treatments significantly reduced the disease from the control, and all treatments performed statistically similar. Numerically, Kasumin-FireWall and Blossom Protect-Buffer had the lowest incidence of disease on Granny Smith. Intermediate treatments on Granny Smith apple included copper products (MasterCop, CS2005 - MagnaBon), selected mixtures containing nisin and ε-poly-L-lysine, the natural product BacStop, as well as Kasumin and Mycoshield. On Fuji apple, ε-poly-L-lysine + Dart was numerically the most effective, and treatments with intermediate efficacy included nisin-mixtures, BacStop, and Kasumin.
2) In field studies on Bartlett pears in 2020, the natural incidence of fire blight was most effectively reduced using oxytetracycline (formulated as FireWall or Mycoshield) or Kasumin. Some of the new biological compounds under evaluation, however, performed statistically similarly, and among these were nisin + zinc nitrate, ε-poly-L-lysine + Dart, and TDA-NC-1.
3) 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. This is an ongoing process with UPL and the California Apple Commission.

II. Postharvest decay control

A. In an experimental packingline study, in-line drench applications of inoculated Granny Smith apple fruit with BioSpectra by itself or mixed with Scholar or Inspire as well as Academy provided excellent control of blue mold. The new experimental EXP-ADA was only moderately effective. When fruit were wounded and inoculated after treatment with *B. cinerea*, only Academy and BioSpectra mixed with Scholar were highly effective.

B. The efficacy of natamycin against postharvest decays of pome fruits is variable depending on the cultivar, source, and maturity of fruit. Optimization by mixing with other fungicides such as Scholar or Academy is the main strategy that we are pursuing for resistance management. Although the efficacy of Scholar and

Academy is not improved, these mixtures provide an anti-resistance strategy because resistance to natamycin has not been reported previously to filamentous fungi including decay pathogens of pome fruit. Natamycin has been registered for food uses for over 20 years.

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. Current control programs are based on protective schedules because available compounds are mostly contact treatments and are not systemic. Registered treatments include copper products, antibiotics, as well as natural products and biocontrol agents. Conventional copper compounds are only effective when disease severity is low to moderate. They may cause fruit russeting and therefore, labeled rates are at low amounts of metallic copper equivalent (MCE) that are at the limit of effectiveness. Newly formulated copper products are available that can be used at reduced MCE rates and cause less phytotoxicity. Some are OMRI-approved including Badge X2, CS-2005, and Cueva. Because only few treatments are permitted for organic apple production, research on OMRI-approved coppers needs to be continued. In our surveys, however, we detected low to moderate levels of copper insensitivity in pathogen populations.

Other organically approved treatments are the biocontrols 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). These showed inconsistent efficacy over the years in our trials and were most effective under low inoculum levels and less favorable micro-environments. The biocontrol Blossom Protect (*Aureobasidium pullulans*) has been very effective under less to moderately favorable disease conditions, and it is one of the most consistent biologicals that we have evaluated. Biocontrols are most effective when they are actively growing on the plant and already have colonized susceptible host tissues before infection events, whereas natural products can also have some direct toxicity to the pathogen during infection events. New natural products evaluated in 2020 included BacStop, EF400, Gargoil, ET91, Dart, TDA-NC-1, and the biocontrol Double Nickel was used in a rotation program.

We are also evaluating other bactericide 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 food preservatives. They potentially could qualify for organic production. Our initial evaluations with these compounds showed high toxicity in lab studies, but only moderate activity in the field. Therefore, we continued trying to improve their efficacy by using selected additives. New bacteriophage preparations were planned for evaluation against fire blight; however, the registrant only made a preparation available for walnut blight control. Bacteriophages are very host- (bacterial pathogen-) specific even to strains of the same species. New strategies include blending multiple phages to overcome specificity and to improve performance in different regions of apple production.

The antibiotics streptomycin, oxytetracycline, and kasugamycin can only be used in conventional pome fruit production and are currently the mainstay in conventional fire blight management. The incidence of resistance to streptomycin in California orchards has been fluctuating from very high to low in our surveys between 2006 and 2018. Reduced sensitivity to oxytetracycline has only been found sporadically, and these isolates did not persist. In 2018 and again in 2019 at two locations, however, we detected for the first strains of *E. amylovora* that were highly resistant to this antibiotic. Resistance to kasugamycin in *E. amylovora* has not been found to date. Efforts are ongoing to differentiate kasugamycin from other bactericides and allow certification as an organic treatment by the National Organic Standards Board and OMRI. The goal of our field evaluations of new bactericides is to develop effective rotational programs for organic farming practices with the use of copper and biologicals, as well as conventional programs with the use of antibiotics, copper, biologicals, and other bactericidal compounds for use during bloom and early fruit development.

Management of postharvest decays. Apples like other pome fruits can be stored for some period of time in optimum fruit storage environments. Still, postharvest decays caused by fungal organisms can result in economic crop losses. The major postharvest pathogens of apples are *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 organically-approved postharvest treatments for preventing these decays in storage. BioSave 100 is one of the few materials currently available in the United States, but its efficacy is limited. Other biological products are registered in other countries and these potentially could be evaluated for California conditions if registrants decide to market their products (e.g., Shemer - *Metschnikowia fructicola*, Candifruit - *Candida sake*, Nexy - *Candida oleophila*, Boni-Protect - *Aureobasidium pullulans*) in the U.S.

We previously showed that the bio-fungicide polyoxin-D (Ph-D, Oso, Tavano) is very effective in reducing gray mold and Alternaria rot, but it is not effective against blue mold. Polyoxin-D is labeled as a conventional fungicide on pome and other crops and is now an approved organic fungicide that is pending pre-harvest and postharvest organic labeling on multiple crops. We also demonstrated the efficacy of another bio-fungicide, natamycin (pimaricin). For many years, natamycin has been a federal-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. This compound was registered in late 2016 as BioSpectra for postharvest treatment of citrus and stone fruits. Natamycin has an exempt registration status and has been submitted to the NOSB for organic registration. In our evaluations, natamycin showed very good and consistent efficacy against gray mold and Mucor rot. Efficacy against blue mold, however, has been very variable over the years ranging from excellent to unsatisfactory. Therefore, our goal is to improve its performance so it potentially can be made available to the pome fruit industry.

OBJECTIVES FOR 2019-2020

Fire blight research

1. Evaluate the efficacy of treatments for managing fire blight.
   - A. Evaluate growth enhancers (e.g., buffers) of biological control agents in lab and field trials.
   - B. Laboratory in vitro tests on copper and zinc products (registered copper products) with newly identified antibacterial, food additives (lactic acid, poly-L-lysine, and nisin), new biologicals, and experimental compounds.
   - D. Field trials with protective air-blast spray treatments:
     - i. Kasugamycin in combination with organic treatments to support organic petition to NOSB.
     - ii. New formulations of copper (e.g., Badge X2, CS-2005, Cueva), zinc, food additives (lactic acid, poly-L-lysine, and nisin), and biologicals (e.g., Serenade ASO) in combination as new antibacterial strategies.
     - ii. Biological treatments (Blossom Protect, Serenade) with and without the addition of growth enhancers and copper.
     - iii. Bacterial phage-mixture products with other biological control treatments (i.e., Blossom Protect) to provide an integrated strategy.

Postharvest research

1. 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 fludioxonil.
   - B. Evaluate mixtures of these compounds and new formulations of natamycin to improve performance of the fungicide.

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 2019 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), and single colonies were cultured. Streptomycin, oxytetracycline, and kasugamycin were evaluated for their in vitro toxicity using the spiral gradient endpoint method. For this, a radial bactericidal concentration gradient was established in nutrient agar in Petri dishes by spirally plating a stock concentration of each antimicrobial using a spiral plater. After radially streaking out suspensions of the test bacteria (10 µl of 10^8 cfu/ml as determined by measurement of optical density at 600 nm) along the concentration gradient, plates were incubated for 2 days at 25ºC. Measurements were taken visually for 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 computer program.

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). Four applications were done starting at pink bud stage and followed by three phenology-based treatments until petal fall. Treatments included single treatments, mixtures, and a rotation. Incidence of blight was assessed in late May based on the number of infected flower clusters of 100-200 clusters evaluated for each of the four two-tree replications. Additionally, potential phytotoxic effects of the treatments (e.g., fruit russeting and leaf burn) were evaluated. For comparison, field studies were also conducted on Bartlett pear with some overlapping treatments to the apple studies. Four applications were done, and one branch with flowers per tree was inoculated with *E. amylovora* after the second application. Disease was evaluated in April. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.4.

In a study on Comic pear at UC Davis, three applications with new biological treatments were compared to a non-treated control and to FireWall. Branches with flowers were inoculated after the last application, and the incidence of infected flowers was determined after one week.

In a small-scale field study at UC Davis on Fuji apple, six new experimental biological treatments were compared to FireWall, the organically approved Alum, as well as an untreated control. Treatments were applied to open flowers using a hand sprayer. Flowers were inoculated with *E. amylovora* after 3 h and were bagged overnight. Disease was evaluated after 1 week. Data were statistically analyzed using least significant difference mean separation procedures of SAS 9.4.

Efficacy of new postharvest fungicides for managing apple decays in storage. In an experimental packingline study at KARE, BioSpectra by itself or in mixtures with Scholar or Inspire (i.e., difenoconazole), as well as Academy and a new experimental fungicide (i.e., EXP-ADA) were applied to Granny Smith apple by aqueous in-line drenches that were followed by a CDA application with a carnauba-based fruit coating (i.e., Decco 230). For efficacy evaluation against blue mold, fruit were wound-inoculated 15 to 16 h before treatment with *P. expansum* (i.e., post-infection activity of treatments); and for evaluation against gray mold, inoculation with *B. cinerea* was done after treatment (i.e., pre-infection activity of treatments). Fruit were evaluated for the presence of decay after incubation for 7 days at 20C. 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

Survey of antibiotic sensitivity in E. amylovora strains from pear in California in 2019. In 2019, 65 strains were obtained from 14 orchard locations in Sacramento Co. and 52 strains from 38 locations in Lake Co. All 117 strains were found to be sensitive to kasugamycin (Table 1).

In Sacramento Co., resistance to streptomycin was detected at eight of the locations with an incidence of 33.3% to 100%. At all eight locations, moderately resistant strains (MIC <20 ppm) with plasmid-based resistance were present, but 6 locations also had strains with high-resistance (MIC >100 ppm) that most likely was chromosomal-based. Strains with high-resistance that once used to be common had declined in recent years; but in in 2018, a high incidence of high-resistance was detected in several locations. One of these locations was re-sampled in 2019, and 41.7% of the strains recovered were highly resistant to streptomycin. In another orchard with a low incidence of high resistance in 2018, one strain evaluated in 2019 also was highly resistant. In some of the orchards with low- and high-resistance, a rotation of copper – streptomycin + oxytetracycline + mancozeb – Actigard – Kasumin was applied in the 2019 spring season. Thus, resistant strains persisted under this relatively low selection pressure. In three orchards where the same rotation was done in 2019, however, no streptomycin resistance was detected. No resistance was also detected at two locations with organic programs. Over the years, there has been no clear correlation between streptomycin usage in a specific year and the incidence and level of streptomycin resistance present in the pathogen population in the spring season of the respective years. The previous seasons' applications also may need to be considered because they will affect the composition of the overwintering pathogen population. Thus, spray schedules from multiple years will need to be examined. Our current recommendation is to use streptomycin only once a year to reduce selection pressure on the pathogen. Because this was followed in the orchards with high levels of resistance in 2019, it would also be interesting to know if fire blight was successfully managed with the copper – streptomycin + oxytetracycline + mancozeb – Actigard – Kasumin rotation.

High-resistance (>40 ppm) to oxytetracycline in *E. amylovora* was detected for the first time at two locations in 2018, and all resistant strains were also highly resistant to streptomycin. These two orchards were re-sampled in 2019, and oxytetracycline resistance was again detected. At the first location, 6 out of 7 strains were resistant in 2018, whereas in 2019, 4 out of 12 strains were resistant. At the other location, 1 of 8 or 12 strains was resistant in 2018 and 2019, respectively. The resistant strains' identity was verified as *E. amylovora* by specific PCR primers. Thus, these resistant strains persisted. Additionally, 1 of 8 strains was resistant to oxytetracycline in a third orchard in 2019. As in 2018, strains resistant to oxytetracycline were also highly resistant to streptomycin. At the location with the highest incidence of oxytetracycline resistance, nine applications of the antibiotic were applied between 2017 and 2019. High dependency on one antibiotic in a two-year period may be responsible for the selection of the resistance detected.

Oxytetracycline resistance in *E. amylovora* has never been reported previously at this high level, and this finding is of serious concern. Considering the wide fluctuations in streptomycin resistance in California pear orchards and the previously described non-persistent population of the pathogen with reduced sensitivity to oxytetracycline, it is currently not known if these new resistant strains are competitively fit and will persist in the absence of selection pressure (i.e., applications with oxytetracycline and streptomycin). In preliminary studies we characterized these strains genetically and determined that the oxytetracycline resistance genes are similar to those that were previously described from non-plant pathogenic epiphytic bacteria. Apparently, these genes have jumped between bacterial species. It will also be interesting to determine if there is a molecular association between high-streptomycin and high-oxytetracycline resistance.

In Lake Co., most of the 52 strains evaluated were sensitive to streptomycin and oxytetracycline (Table 1). Moderate resistance to streptomycin (MIC <20 ppm), however, was found at 3 locations where streptomycin-oxytetracycline mixtures were applied for fire blight management. This is of interest because except for one strain collected numerous years ago, only sensitive strains have been recovered in our surveys in this California pear growing area.

Field studies on fire blight using protective treatments. Fire blight incidence in our research plots in the spring of 2020 was low on apple with 16-20% infected flower clusters on untreated control trees and on Bartlett pear with an average of 33 strikes per tree. With this low disease pressure, all treatments on Granny Smith apple significantly reduced the disease from the control to an incidence of 4.6 to 7.9%, and all treatments performed statistically similar (Fig. 1). Numerically, Kasumin-FireWall and Blossom Protect-Buffer had the lowest incidence of disease, and Cueva-Serenade had the highest incidence. Intermediate treatments included copper products (MasterCop, CS2005 - MagnaBon), selected mixtures containing nisin and ε-poly-L-lysine, the natural product BacStop, as well as Kasumin and Mycoshield. The addition of zinc nitrate to Mycoshield-Dart did not improve efficacy. On Fuji apple, all treatments were also statistically similarly effective and reduced the incidence of blight to between 3.3% (i.e., ε-poly-L-lysine + Dart) and 5.8% (i.e., ε-poly-L-lysine + $ZnNO_3$) (Fig. 2). Intermediate treatments included nisin-mixtures, BacStop and Kasumin.

Disease pressure was very high in a small-scale, hand-sprayer study on Fuji apple at UC Davis where 97% of flowers became diseased after inoculation (Fig. 3). Treatments that performed well under low disease pressure in the two studies with natural infection discussed above (e.g., BacStop, nisin and ε-poly-L-lysine treatments) but also Gargoil and ET91, did not significantly reduce the incidence from the untreated control. Alum (potassium aluminum sulfate) significantly reduced the disease to an incidence of 43.3%, and FireWall was the best treatment with an incidence of 26%. Therefore, under these high-disease pressure conditions with inoculations, none of the new biological treatments provided control against fire blight.

In field studies on Bartlett pears in 2020, the natural incidence of fire blight was most effectively reduced using oxytetracycline (formulated as FireWall or Mycoshield) or Kasumin (Fig. 4). Some of the new biological compounds under evaluation, however, performed statistically similarly, and among these were nisin + zinc nitrate, ε-poly-L-lysine + Dart (a mixture of 28.3% capric and 41.7% caprylic acids), and TDA-NC-1. When flowers were inoculated with *E. amylovora*, only FireWall resulted in a very low incidence of disease, whereas the other treatments showed reduced or no efficacy. Nisin + Dart and BacStop + EF400 were not effective in both evaluations. In contrast to our previous studies and to the study on apple discussed above, Blossom Protect did not significantly reduce the amount of disease on Bartlett pear from that of the control after inoculation and occurring naturally. This biocontrol product has been effective to very effective in most field trials that we conducted in previous years.

In a study at UC Davis on Comice pear where flowers were inoculated, FireWall was again the most effective treatment, BacStop + EF400 and nisin + ε-poly-L-lysine + zinc nitrate were less effective, whereas ET91 and TDA-NC-1 were not effective (Fig. 5). In contrast to the inoculation study on Bartlett pear, in the studies on Comice pear and Fuji apple at UC Davis, flowers were bagged overnight, and this created extremely high favorable conditions for infection. Thus, in future experiments, flowers should not be bagged after inoculation.

Summary on the evaluation of new potential treatments against fire blight. The GRAS ('generally regarded as safe') compounds nisin and ε-poly-L-lysine have been evaluated by us over several years. Their performance on apple and pear has been variable and was good under natural disease conditions in the field in 2020. Their variable efficacy in mixtures with different additives indicates that there likely is more room for improvement, and additives need to be continued to be evaluated. Based on our results, Dart appears to be a beneficial additive for ε-poly-L-lysine, and zinc nitrate for nisin. We are pursuing development of formulations in cooperation with a potential registrant. Nisin and ε-poly-L-lysine should be continued to be evaluated because they show promise, and they are eligible for biopesticide registration with the US-EPA.

Among other new treatments, TDA-NC-1 showed promise in the trial on Bartlett pear, and BacStop in the two apple studies. Alum significantly reduced the disease in the inoculation study on Fuji apple. This organically-approved compound has been used with some success in other pome fruit production areas in the United States as well as in other countries, and its performance under California conditions warrants additional field evaluation. In a study conducted in Switzerland (Int. J. Environ. Res. Public Health 2015, 12, 11422-11447), the environmental impact of this aluminum compound was rated similar as for copper, but additional studies also on human health were recommended. Developing these new modes of action is critical in providing safe, effective alternatives to current products registered and for reducing the risk of resistance development to existing registered products as rotational or mixture treatments.

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. This is an ongoing process with UPL and the California Apple Commission and therefore, an organic registration seems plausible.

Evaluation of postharvest treatments using single-fungicides, mixtures, and pre-mixtures. A postharvest study with Granny Smith apple on an experimental packingline using in-line drench applications focused on the efficacy of the biopesticide BioSpectra and the new experimental EXP-ADA. Treatments of inoculated fruit with BioSpectra by itself or mixed with Scholar or Inspire as well as Academy provided excellent control of blue mold, whereas EXP-ADA was only moderately effective (Fig. 6). When fruit were wounded and inoculated with *B. cinerea* after treatment, only Academy and BioSpectra mixed with Scholar were highly effective. In our previous studies, BioSpectra treatments of fruit after wound inoculations with *B. cinerea* consistently provided good to very good control and the low effectiveness in this year's pre-inoculation treatments indicates that BioSpectra does not have locally systemic activity, unlike Scholar. In contrast to gray mold, the efficacy of BioSpectra against blue mold has been variable over the years especially when different types of pome fruits were used. Therefore, we will continue to try to improve its effectiveness. Still, based on the moderate performance of natamycin, natamycin may not become registered on pome fruits unless it is developed in a premixture with other fungicides. Although efficacy is not improved as compared to using the two registered fungicides by themselves, adding natamycin 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 pear orchards in Sacramento and Lake Co. to streptomycin, oxytetracycline, and kasugamycin in 2019

Sacramento Co.

Orchard No.	Streptomycin	Oxytetracycline	Kasugamycin
1	MR	S	S
	MR	S	S
	MR	S	S
2	S	S	S
	S	S	S
3	HR	S	S
	MR	S	S
	MR	S	S
4	MR	S	S
	MR	S	S
	HR	HR	S
	HR	S	S
	MR	S	S
	MR	S	S
	MR	S	S
	HR	S	S
5	MR	S	S
	HR	S	S
	MR	S	S
6	HR	S	S
	MR	S	S
	HR	S	S
	HR	S	S
	MR	S	S
	MR	S	S
7	S	S	S
	S	S	S
	S	S	S
8	S	S	S
	S	S	S
	S	S	S
	S	S	S
9	S	S	S
	S	S	S
	S	S	S
	S	S	S
	S	S	S
	S	S	S
	MR	S	S
	MR	S	S
	HR	HR	S
	S	S	S
	S	S	S
	MR	S	S
10	HR	HR	S
	MR	S	S
	HR	HR	S
	MR	S	S
	HR	S	S
	MR	S	S
	HR	HR	S
	MR	S	S
	MR	S	S
	S	S	S
	MR	S	S
	HR	HR	S
11	MR	S	S
12	S	S	S
	S	S	S
13	S	S	S
14	S	S	S
	S	S	S
	S	S	S
	S	S	S
	S	S	S

Lake Co.

Orchard No.	Streptomycin	Oxytetracycline	Kasugamycin
1	S	S	S
	S	S	S
	S	S	S
2	S	S	S
3	S	S	S
4	S	S	S
5	S	S	S
	S	S	S
6	S	S	S
	S	S	S
7	S	S	S
8	S	S	S
9	S	S	S
10	S	S	S
11	S	S	S
12	S	S	S
	S	S	S
13	S	S	S
14	S	S	S
15	S	S	S
16	S	S	S
17	S	S	S
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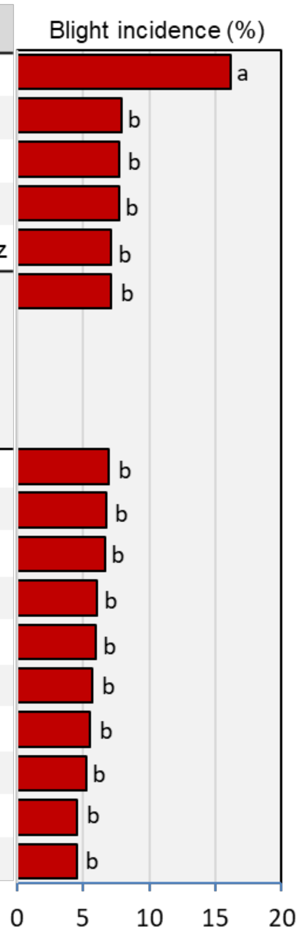
	S	S	S
	S	S	S
18	S	S	S
19	S	S	S
	S	S	S
20	S	S	S
	S	S	S
21	S	S	S
22	S	S	S
23	MR	S	S
24	S	S	S
25	S	S	S
26	S	S	S
27	S	S	S
28	S	S	S
29	S	S	S
30	S	S	S
31	S	S	S
32	S	S	S
33	S	S	S
34	S	S	S
35	S	S	S
	S	S	S
36	MR	S	S
37	MR	S	S
	MR	S	S
38	S	S	S
	S	S	S

Sensitivity to streptomycin, oxytetracycline, and kasugamycin was determined using the spiral gradient endpoint method. S = sensitive, MR = moderately resistant (MIC = <20 ppm), HR = highly resistant (MIC = >40 ppm).

Fig. 1. Efficacy of bactericides for management of fire blight of Granny Smith apples, Fresno Co. 2020

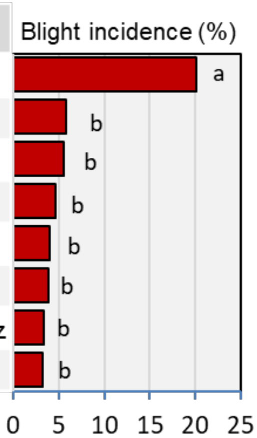
	Treatment	Rate
	Control	---
	Cueva + Serenade ASO	96 fl oz + 64 fl oz
	ε-poly-L-lysine + $ZnNO_3$	3.5 oz + 32 fl oz
	Nisin + Dart	13.5 oz + 24 fl oz
	Kasumin 2L + Poly-L-lysine + $ZnNO_3$	64 fl oz + 3.5 oz + 32 fl oz
Rotation	BacStop	64 fl oz
	Double Nickel	128 fl oz
	Blossom Protect + Buffer	20 oz + 5 lbs
	Blossom Protect + Buffer	20 oz + 5 lbs
	Mycoshield (NUP 17010) + Dart + $ZnNO_3$	8 oz + 24 fl oz + 32 fl oz
	BacStop	64 fl oz
	Nisin + $ZnNO_3$	13.5 oz + 32 fl oz
	MasterCop	64 fl oz
	CS2005	25 fl oz
	Kasumin 2L	64 fl oz
	ε-poly-L-lysine + Dart	3.5 oz + 24 fl oz
	Mycoshield (NUP 17010) + Dart	8 oz + 24 fl oz
	Blossom Protect + Buffer	20 oz + 5 lbs
	Kasumin 2L + FireWall	64 fl oz + 8 oz



Treatments were applied on 3-11 (pink bud), 3-15 (20% bloom), 3-21 (80-90% bloom), and 3-31-20 (petal fall) using an air-blast sprayer. There were four replications of two trees each per treatment. Disease was evaluated in late May on 100 to 200 flower clusters per tree.

Fig. 2. Efficacy of new bactericides for management of fire blight of Fuji apples, Fresno Co. 2020

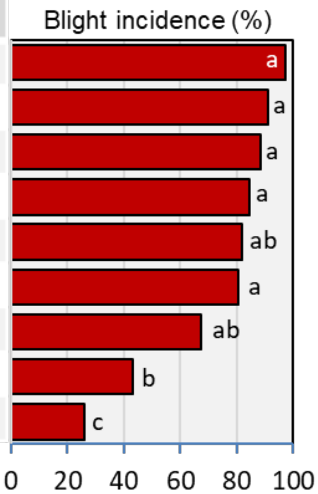
Treatment	Rate/A
Control	---
ε-poly-L-lysine + $ZnNO_3$	13.5 oz + 32 fl oz
BacStop	64 fl oz
Kasumin 2L	64 fl oz
Nisin + $ZnNO_3$	13.5 oz + 32 fl oz
Nisin + Dart	13.5 oz + 32 fl oz
FireLine + $ZnNO_3$ + Dart	16 oz + 32 fl oz + 32 fl oz
ε-poly-L-lysine + Dart	13.5 oz + 32 fl oz



Treatments were applied on 3-13 (pink bud), 3-19 (20% bloom), 3-26 (80-90% bloom), and 4-3 (petal fall) using an air-blast sprayer at 100 gal/A. There were four replications of two trees each per treatment. Disease was evaluated on 100-200 flower clusters (spurs) of each tree in late May 2020.

Fig. 3. Efficacy of new bactericides for management of fire blight of Fuji apple in a small-scale field study at UC Davis 2020

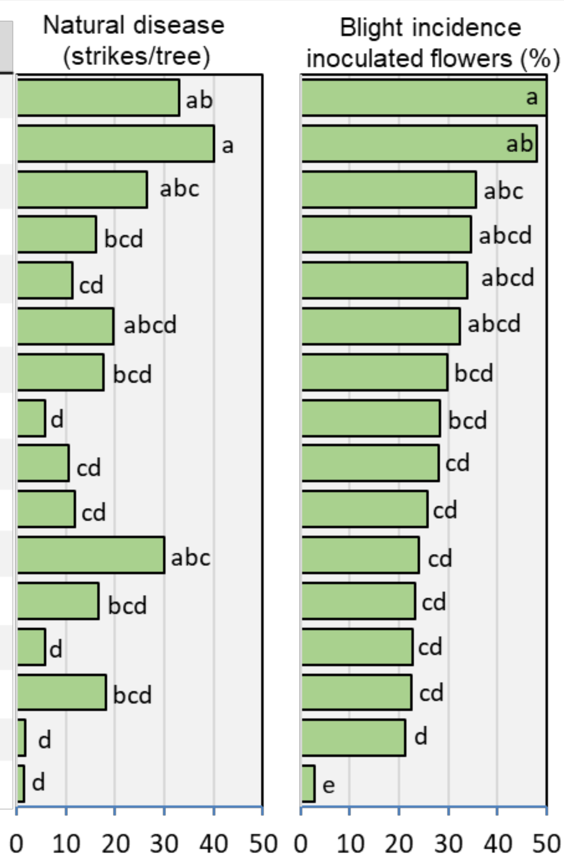
Treatment	Rate/A
Control	---
Gargoil	96 fl oz
BacStop	64 fl oz
TDA-NC-1 + Silwet	40 oz + 1.6 fl oz
ε-poly-L-lysine + $ZnNO_3$	13.5 oz + 48 fl oz
ET91	1.28 fl oz
Nisin + ZnO_3	13.5 oz + 48 fl oz
Alum + Regulaid	128 oz + 32 fl oz
FireWall	8 oz



Treatments were applied to open flowers using a hand sprayer. Flowers were inoculated with *E. amylovora* after 3 h and were bagged overnight. Disease was evaluated after 2 weeks.

Fig. 4. Efficacy of bactericides for management of fire blight of Bartlett pears, Live Oak, 2020

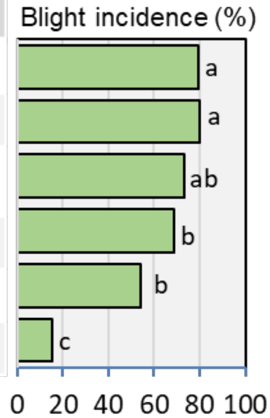
Treatment	Rate
Control	---
Nisin + Dart	13.5 oz + 48 fl oz
BacStop + EF400	38 + 38 fl oz
Cueva	64 fl oz
TDA-NC-1 + Silwet	60 oz + 1.6 fl oz
Blossom Protect - new buffer	20 oz + 128 oz
Gargoil	96 fl oz
Mycoshield (NUP 17010) + Dart	8 oz + 32 fl oz
ε-poly-L-lysine + Dart	13.5 oz + 48 fl oz
Nisin + $ZnNO_3$	13.5 oz + 32 fl oz
Nisin + Humectin	13.5 oz + 32 ml
ε-poly-L-lysine + Humectin	13.5 oz + 32 ml
Kasumin 4L	32 fl oz
ε-poly-L-lysine + $ZnNO_3$	3.5 oz + 32 fl oz
Mycoshield (NUP 17010)	8 oz
FireWall	16 oz



Treatments were applied on 3-18, 3-24, 4-1, and 4-7-20 using an air-blast sprayer. One branch with flowers per tree was inoculated with *E. amylovora* on 3-24-20 after the application. Inoculated flowers were evaluated on 4-7-20, and natural incidence was determined on 4-22-20.

Fig. 5. Efficacy of new bactericides for management of fire blight of Comice pear in a small-scale field study at UC Davis 2020

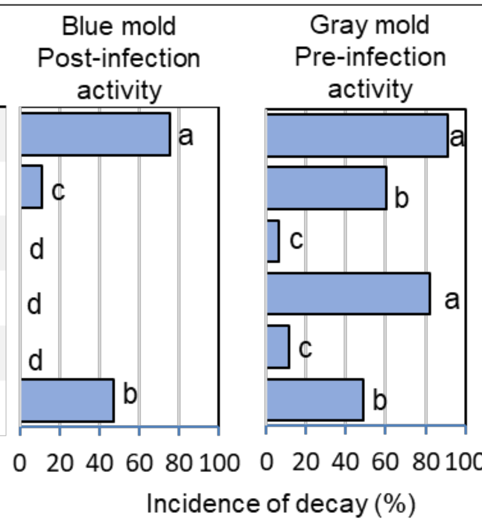
Treatment	Rate	3-24	3-31	4-7
Control	---	---	---	---
ET91	0.5% fl oz	---	@	@
TDA-NC-1 + Silwet	40 oz + 1.6 fl oz	@	@	@
BacStop + EF400	38 + 38 fl oz	@	@	@
Nisin + poly-L-lysine + $ZnNO_3$	13.5 + 3.5 oz + 32 fl oz	@	@	@
FireWall	16 oz	@	@	@



Treatments were applied using an air-blast sprayer. Flowers of one branch per tree were inoculated with *E. amylovora* after the third application. Disease was evaluated after one week.

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

Treatment	Rate
Control (water)	---
BioSpectra	128 fl oz
BioSpectra + Scholar	64 fl oz + 16 fl oz
BioSpectra + Inspire	64 fl oz + 16 fl oz
Academy	16 fl oz
EXP-ADA	40 fl oz



Fruit were wound-inoculated with *P. expansum* 15-16 h before treatment or with *B. cinerea* after treatment. Treatments were done by aqueous in-line drenches that were followed by a CDA application with carnauba wax. Fruit were then incubated at 20C for 7 days.