Annual Report – 2018-19

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. Antibiotic and copper resistance surveys for populations of *Erwinia amylovora* in California pear growing areas were continued in 2018.
   a. Kasugamycin: All 70 strains from 13 orchard locations in Sacramento and Lake Co. were sensitive.
   b. Streptomycin: Resistance was detected in all but one location. Forty-two of the resistant strains had plasmid-based moderate resistance (MIC <20 ppm) and 19 strains displayed high resistance that most likely was chromosomal-based. 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 that new alternatives need to be developed.
   c. Oxytetracycline: For the first time, high levels of resistance with growth at >40 ppm were detected at two locations. These resistant strains were also highly resistant to streptomycin. In the location with the highest incidence of Oxy^R, nine applications of the antibiotic were applied between 2017 and 2018. Oxytetracycline resistance in *E. amylovora* has never been reported previously at this level, and this finding is a serious concern. Currently, it is not known if these resistant strains are competitively fit and will persist in the absence of selection pressure.
   d. Copper: Moderate copper resistance was present in strains of *E. amylovora*. Growth was similar to the control using 20 ppm MCE and was reduced at 30 ppm MCE on nutrient agar. Spontaneous mutants growing at high concentrations of copper were also observed. Management failures with the use of copper under high disease pressure have been attributed to highly favorable environments, low rates of copper registered, moderate copper resistance, and spontaneous mutants with high copper resistance.
   e. In 30-min direct exposures of *E. amylovora* suspensions to the food preservatives nisin or ε-poly-L-lysine, the toxicity of both compounds was significantly increased with the addition of ethylenediaminetetraacetic acid (EDTA).
2. Field trials on the management of fire blight were conducted under high disease pressure on cvs. Granny Smith and Fuji apple, as well as under low disease pressure on Bartlett pear.
   a. On Granny Smith apple, Blossom Protect with the newly formulated buffer additive was the most effective treatment. The rotation treatment of Badge, Badge + ProPhyt, followed by two applications of Blossom Protect, however, was somewhat less effective. Statistically similarly effective treatments to Blossom Protect were Kasumin + FireWall, Kasumin + ε-poly-L-lysine + zinc oxide, as well as Kasumin 2L and Kasumin 4L by themselves. Nisin and ε-poly-L-lysine by themselves were only moderately effective, and the addition of EDTA and zinc oxide to nisin or of Dart (28.3% capric and 41.7% caprylic acids) to ε-poly-L-lysine resulted in numerical but not statistical increases in efficacy.
   b. On Fuji apple, Kasumin 2L and 4L, and FireLine + zinc oxide + Dart provided the highest efficacy among treatments evaluated. Among treatments containing the preservatives nisin or ε-poly-L-lysine, ε-poly-L-lysine + zinc oxide + EDTA was most effective.
   c. In small-scale studies, two new experimental biocontrol agents were not effective in reducing fire blight.
   d. 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 on control of blue mold and gray mold of apple, the high-solubility formulation of natamycin was numerically more effective than the BioSpectra formulation. Natamycin again was not effective in reducing blue mold of pears, and there were differences in efficacy also among sources of apple fruit that may be related to the fruit age (time of storage after harvest).
2. In an experimental packingline study using in-line drench applications, BioSpectra significantly reduced blue mold and gray mold of Granny Smith apple, but a treatment with 300 ppm Scholar was significantly more effective.
3. The efficacy of natamycin needs to be improved for apples and other pome fruits. Although efficacy of Scholar or Academy is not improved when BioSpectra is added in mixture treatments, natamycin represents a 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.

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 contact treatments and are not systemic except for the antibiotic streptomycin. 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. New re-formulated copper products that can be used at reduced MCE rates and that cause less phytotoxicity are available. Some products 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, and some were included in our 2018 field studies. In our surveys, however, we detected low to moderate levels of copper insensitivity in pathogen populations.

The antibiotics streptomycin and oxytetracycline can only be used in conventional pome fruit production. The incidence of resistance to streptomycin in California orchards has been fluctuating from very high to low in our surveys between 2006 and 2017. Reduced sensitivity to oxytetracycline has only been found sporadically, and these isolates did not persist. Kasugamycin (Kasumin) is now registered in California. 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 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 inconsistent over the years in their performance 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. A new buffer additive for Blossom Protect that was developed to increase growth of the biocontrol agent became available in 2019 and was included in our field studies. 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 continue to try to improve their efficacy by using selected additives. Our goal 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 postharvest biocontrols and natural products that are available 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. 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 (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 not blue mold. Polyoxin-D was approved as an organic fungicide by the NOSB in April 2018 and is currently pending pre-harvest labeling and postharvest registration on multiple crops. We also demonstrated the efficacy of another bio-fungicide, natamycin (pimaricin). For many years, natamycin 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. 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. In 2018/19, we continued to compare several formulations of natamycin, and we tried to determine the causes for its inconsistency.

OBJECTIVES FOR 2018-2019

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) and experimental compounds (SBH derivatives) that enhance the activity of copper and possibly zinc.
   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) and SBH as a copper activity enhancer in combination or rotation with newly identified antibacterial, food additives (lactic acid, poly-L-lysine, and nisin).
      ii. Biological treatments (Blossom Protect, Serenade) with and without the addition of growth enhancers.
      iii. Blockers of bacterial infection that interfere with Type III secretion systems (e.g., TS products) alone or 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 fludioxonil.
   B. Evaluate mixtures of these compounds.

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 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, 20, 30, or 40 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).

The toxicity of ε-poly-L-lysine and nisin against *E. amylovora* was evaluated in direct contact assays. For this, suspensions of a strain of *E. amylovora* were incubated in final concentrations of 500 ppm of these antimicrobials, and water was used in control treatments. To possibly improve the toxicity of ε-poly-L-lysine and nisin, ethylenediaminetetraacetic acid (EDTA) was added using selected concentrations. Mixtures were incubated for 30 min, diluted 1:1000 with sterile water, and aliquots were then plated onto nutrient agar. After 2 days, bacterial colonies were enumerated, and percent inhibition in colony formation as compared to the control was calculated.

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. 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 approximately 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 disease was evaluated in early May. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.4.

In small-scale field studies at UC Davis, two new experimental biocontrol agents (coded BC250 and T3-07) were evaluated. Treatments were applied to open flowers of Fuji apple or Comice pear using a hand sprayer. Flowers were inoculated with *E. amylovora* after 3.5 h, and peroxyacetic acid (e.g., Oxidate) was applied as a secondary treatment to some of the primary treatments after another hour. Streptomycin was used as standard treatment for comparison. Disease was evaluated after 1 week. Data were statistically analyzed as described above.

Efficacy of new postharvest fungicides for managing apple decays in storage. A new high-solution formulation of natamycin was compared to the BioSpectra formulation using Granny Smith apple inoculated with *P. expansum* or *B. cinerea*. The new formulation was also evaluated for its efficacy to control blue mold of Granny Smith apple, Shinko apple pear, as well as Bartlett, D'Anjou, and Bosc pear in the laboratory. Fruit were treated using an air-nozzle sprayer after 12 h and 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 *B. cinerea* or *P. expansum* and treated after 15 to 16 h with BioSpectra or Scholar by in-line drenches that were followed by a CDA application with a carnauba-based fruit coating (i.e., Decco 230). 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 and copper sensitivity in* E. amylovora *strains from California. In 2018, 70 strains were obtained from 13 orchard locations in Sacramento Co. and tested. All strains were found to be sensitive to **kasugamycin** (Table 1). Resistance to **streptomycin** was detected in all but one location. A low incidence of resistance (2 of 6 isolates) was present in an orchard where only copper and Serenade were applied for fire blight management. Forty-two of the resistant strains from the survey had plasmid-based moderate resistance (MIC <20 ppm) and 19 strains displayed high resistance that most likely was chromosomal-based. For these latter strains streptomycin concentrations of up to 40 ppm were tested, but based on our previous results, these strains typically still grow at >2000 ppm. In one location all 6 resistant strains, and in another location, 6 of the 7 strains were highly resistant. This high incidence of high resistance is interesting because in our surveys several years ago, high-resistance was present only at very low levels. Thus, as we demonstrated previously, the occurrence of streptomycin resistance fluctuates widely among years and probably reflects strain fitness and antibiotic use. Overall, there was no clear correlation between streptomycin usage in 2018 and the incidence and level of streptomycin resistance that was present in the pathogen population (Table 1). However, the previous seasons' applications possibly also need to be considered that will determine the composition of the overwintering pathogen population.

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.

In our evaluations of **oxytetracycline** toxicity against *E. amylovora* strains from the 13 orchard locations, surprisingly we detected high levels of resistance with growth at >40 ppm in the spiral gradient endpoint assay at two locations (6 of 7 strains tested in one orchard and 1 of 8 strains tested in another orchard; Table 1). These resistant strains were also highly resistant to streptomycin. In the location with the highest incidence of Oxy^R, nine applications of the antibiotic were applied between 2017 and 2018. High dependency on one antibiotic in a two-year period may be responsible for the selection of the resistance detected. The strains' identity was verified as *E. amylovora* by specific PCR primers, and their resistance was confirmed by culturing on nutrient agar amended with 40 ppm oxytetracycline (Table 1). 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). We plan to characterize these strains genetically to determine if oxytetracycline resistance genes are similar to those that were previously described from other bacteria (non-plant pathogens).

Regarding **copper** sensitivity, growth of all 70 strains was completely inhibited on CYE (a growth medium with a low copper-binding capacity) agar amended with 20 ppm MCE (Table 1). All strains grew on the nutrient-rich nutrient agar at 20 ppm MCE similar as on non-amended agar. At 30 ppm MCE on nutrient agar, confluent growth of most strains was reduced or inhibited. Thus, as in 2015- 2017, current *E. amylovora* populations are considered moderately copper-resistant. Again, we observed the frequent presence of spontaneous mutant colonies emerging at higher copper concentrations. 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.

Previously, we outlined several factors that likely contributed to the failure of copper applications to control fire blight. Here, we re-summarize this information: 1) Highly conducive disease conditions may allow the pathogen to overcome the suppressive action of copper (copper is bacteriostatic and does not kill the pathogen); 2) Only low rates of copper are registered for fire blight management; 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. Fruit russetting also may occur on some pome fruit varieties with repeated applications of copper. Therefore, there is a need to develop and register products that have different modes of action and that potentially can be registered as organic products.

***In vitro toxicity of ε-poly-L-lysine and nisin against* E. amylovora.** In 30-min direct exposures of *E. amylovora* suspensions, colony formation was reduced by 40 or 50% using nisin or ε-poly-L-lysine, respectively (Fig. 1). The toxicity of both food additives was significantly increased with the addition of 100 or 500 ppm EDTA. Growth was completely inhibited by adding 500 ppm EDTA to either bactericide and by approximately 80 or 100% using 100 ppm EDTA with ε-poly-L-lysine or nisin, respectively; EDTA by itself was only moderately or not inhibitory, depending on the rate used. These results indicated that the toxicity of nisin and ε-poly-L-lysine could be increased, and this was subsequently evaluated in field efficacy studies.

Field studies on fire blight using protective treatments. Fire blight incidence in our research plots in the spring of 2018 was high on apple, i. e., over 40% based on infected flower clusters of untreated control trees. Disease, however, was low on Bartlett pear due to cool temperatures during bloom time. The latter orchard location often had very high disease levels over the years. On Granny Smith apple, Blossom Protect with the newly formulated buffer additive was the most effective treatment, and disease incidence was reduced from 42.4% in the control to 11.1% (Fig. 2). The rotation treatment of Badge, Badge + ProPhyt, followed by two applications of Blossom Protect, however, was somewhat less effective. Statistically similarly effective treatments to Blossom Protect were Kasumin + FireWall, Kasumin + ε-poly-L-lysine + zinc oxide, as well as Kasumin 2L and Kasumin 4L by themselves. Nisin and ε-poly-L-lysine by themselves were only moderately effective, and the addition of EDTA and zinc oxide to nisin or of Dart (28.3% capric and 41.7% caprylic acids) to ε-poly-L-lysine only resulted numerical but not statistically significant increases in efficacy. The three organic copper products Cueva, Mastercop, and CS-2005 also had moderate activity, with Cueva being the most effective. No phytotoxicity was observed using any of the treatments.

On Fuji apple, Kasumin 2L and 4L, and FireLine + zinc oxide + Dart provided the highest efficacy among treatments evaluated (Fig. 3). Among treatments containing the preservatives nisin or ε-poly-L-lysine, ε-poly-L-lysine + zinc oxide + EDTA was most effective. In the latter mixture treatment, ε-poly-L-lysine at the lower rate of 3.5 oz was more effective that at the 13.5-oz rate. Interestingly, in vitro direct exposure studies also indicated that this compound was more toxic at lower rates used. Thus, this needs to be further explored. ε-poly-L-lysine is a very large molecule, and lower concentrations possibly have better access to target sites and prevent auto-binding to itself. Two new potential biocontrol agents that showed activity in vitro were provided to us and were evaluated in small-scale studies on Fuji apple and Comice pear trees. In contrast to treatments with streptomycin, the incidence of blighted flowers as compared with the control was not reduced using these bacteria (Fig. 4). We followed a protocol specified by the provider of the bacteria, and a different treatment-inoculation schedule (e.g., longer time between treatment and inoculation) may improve the effectiveness. These results also stress the difficulty in making potential biocontrol agents that show activity in the lab to be effective treatments in the field.

In a field trial on Bartlett pear, all treatments evaluated significantly reduced the disease from the control, mostly to low levels (Fig. 5). Kasumin + FireWall showed the least amount of blight. With a disease incidence in the control of less than 8%, Serenade + Cueva and Nisin + EDTA + zinc oxide also performed well, however, Nisin + EDTA was the least effective treatment. Three new experimental treatments (NSA, NS1, and NS2) had moderate to good efficacy, and the best one (i.e., NS2) should be evaluated at higher disease pressure).

In our spring 2019 field trials on the management of fire blight, numerous compounds were evaluated that often were used in mixtures. Because it was not possible to test each mixture compound by itself in a field study with trees, it is often difficult to determine which of the mixture components improved efficacy, especially when triple mixtures were used. Some conclusions, however, can be made. ε-poly-L-lysine and nisin have potential as fire blight management treatments, especially considering that they are currently have generally regarded as safe (GRAS) status as food additives by the US Food and Drug Administration (FDA). Zinc oxide did not improve the efficacy of Mycoshield + LI700 at the rate evaluated but improved the efficacy of the Nisin + EDTA treatment (Fig. 5). Formulations are very difficult to develop and require expertise from formulation chemists. As indicated above, lower rates of these components may be better in combination with the active ingredient than higher rates. Thus, we are pursuing development of formulations in cooperation with a potential registrant. 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.

In conclusion, among organic treatments, only Blossom Protect showed acceptable commercial efficacy in the management of fire blight similar to standards. Conventional treatments containing the antibiotics streptomycin or kasugamycin were always very effective. Still, other biological treatments to be considered are the liquid copper formulation Cueva and the preservatives nisin and ε-poly-L-lysine. Formulating these antimicrobial food preservatives to improve their efficacy needs to be done in cooperation with a potential registrant. Nisin and ε-poly-L-lysine are eligible for biopesticide registration with the US-EPA. 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.

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 registered as a biopesticide with tolerance exemption status by the US-EPA. The fungicide is registered as BioSpectra on citrus and stone fruits. In laboratory studies, we compared the efficacy of two formulations for the control of blue mold and gray mold, the commercial BioSpectra and a high-solubility solution formulation. Previously, we determined that the WP formulation is generally less effective. In this year's studies, the high-solubility formulation was numerically more effective as shown in Fig. 6 for a study on Granny Smith apple (and also in other trials on pome and other fruits that are not presented here). For blue mold control, we found natamycin to be highly effective on Granny Smith apple, but not on apple pear and three pear cultivars (Fig. 7). We noted this difference in efficacy among pome fruit cultivars previously, and control of blue mold of pears with natamycin has been a challenge for several years because the fungicide is highly effective on other decays such as gray mold, Alternaria, and Rhizopus rot (even when inoculated on the same fruit that are also inoculated with *P. expansum*). In doing numerous studies over early to late fall, we also noted differences in efficacy of natamycin among sources of apple fruit that likely were related to the fruit age (time of storage after harvest). Thus, late-season tests with Granny Smith apple were generally not very successful, although Scholar still was effective. We are planning to do all our postharvest studies with natamycin soon after harvest in 2019 when commercial postharvest treatments are mostly done in California.

In an experimental packingline study using in-line drench applications, BioSpectra significantly reduced blue mold and gray mold of Granny Smith apple, and the 1000-ppm rate was more effective than the 500-ppm rate for blue mold (Fig. 8). A treatment with 300 ppm Scholar was significantly more effective than those with BioSpectra. Based on the moderate efficacy of natamycin, natamycin may not become registered on pome fruits unless it is developed in a premixture with other fungicides. Still, we will continue to try to improve its efficacy. Moreover, natamycin still has a chance to receive an OMRI listing with our NOSB petition. Mixtures of BioSpectra with Scholar or Academy were evaluated previously by us and were very effective against blue mold, gray mold, Alternaria rot and bull's eye rot. 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.

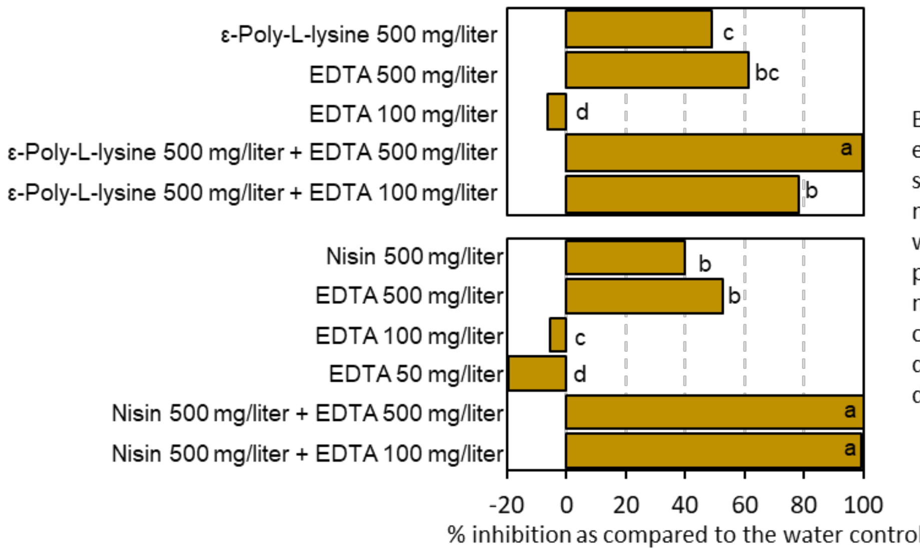
Table 1. Sensitivity of *E. amylovora* strains from pear orchards in Sacramento Co. to streptomycin, oxytetracycline, kasugamycin, and copper in 2018

				Copper				
	Strepto-	Oxytetra-	Kasuga-	CYE	Nutrient agar			
Location	mycin	cycline	mycin	20 ppm	20 ppm	30 ppm	40 ppm	Chemical spray program
1	MR	S	S	--	++	+*	-	Oxy (3)- Strep (1) rotation
	HR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
2	HR	HR	S	--	+++	*	-	
	HR	HR	S	--	+++	*	-	
	HR	HR	S	--	+++	*	-	Oxy (3)- Strep (1) rotation
	HR	HR	S	--	+++	*	-	
	HR	HR	S	--	+++	*	-	
	MR	S	S	--	+++	++*	-	
	HR	HR	S	--	+++	*	-	
3	HR	S	S	--	+++	*	-	Oxy (4)- Strep (1)- PO3 (2)
	HR	S	S	--	+++	*	-	rotation
	HR	S	S	--	+++	*	-	
	MR	S	S	--	+++	++*	-	
	MR	S	S	--	+++	++*	-	
	MR	S	S	--	+++	+*	-	
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
4	MR	S	S	--	+++	+*	-	Oxy (4)- Strep (1)- PO3 (2)
	MR	S	S	--	+++	+*	-	rotation
5	MR	S	S	--	+++	+*	-	Oxy (4)- Strep (1)- PO3 (2)
	MR	S	S	--	+++	*	-	rotation
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
6	S	S	S	--	+++	++	*	Oxy (4)- Strep (1)- PO3 (2)
	S	S	S	--	+++	++	*	rotation
	S	S	S	--	+++	++	*	
	S	S	S	--	+++	++	*	
7	MR	S	S	--	+++	*	-	Copper, Oxy-Strep
	MR	S	S	--	+++	*	-	mixtures, rotated with
	MR	S	S	--	+++	++*	*	Strep, Kasu
8	MR	S	S	--	+++	*	-	Copper, Oxy-Strep
	MR	S	S	--	+++	*	-	mixtures, rotated with
	MR	S	S	--	+++	*	-	Strep, Kasu
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	-	
9	MR	S	S	--	+++	*	-	Copper, Oxy-Strep
	MR	S	S	--	+++	*	-	mixtures, rotated with
	MR	S	S	--	+++	+*	-	Strep, Kasumin
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	++*	-	
	MR	S	S	--	+++	++*	-	
10	HR	S	S	--	+++	*	-	Oxy (3)- Strep (2) rotation
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
	HR	S	S	--	+++	*	-	
11	MR	S	S	--	+++	*	-	Oxy (3)- Strep (2) rotation
	MR	S	S	--	+++	++*	-	
	MR	S	S	--	+++	++*	-	
	MR	S	S	--	+++	*	-	
	MR	S	S	--	+++	*	*	
	MR	S	S	--	+++	*	*	
	MR	S	S	--	+++	++	*	
12	MR	S	S	--	+++	++	*	Oxy-Strep mixtures
	MR	S	S	--	+++	++	*	
	MR	S	S	--	+++	++	*	
	MR	S	S	--	+++	++	*	
	MR	S	S	--	+++	++	*	
	S	S	S	--	+++	++	*	
	HR	HR	S	--	+++	++*	*	
	MR	S	S	--	+++	+++	*	
13	S	S	S	--	+++	++*	*	Copper, Serenade
	S	S	S	--	+++	*	*	
	S	S	S	--	+++	++	*	
	MR	S	S	--	+++	++	*	
	S	S	S	--	+++	+*	*	
	MR	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).

Sensitivity to copper was determined by growth on amended CYE (casitone, yeast extract, glycerol agar) or nutrient agar. Copper ratings: +++ = growth similar to non-amended agar, ++ = reduced growth, + = little growth, - = no growth. * = Spontaneous mutants growing, but no confluent growth.

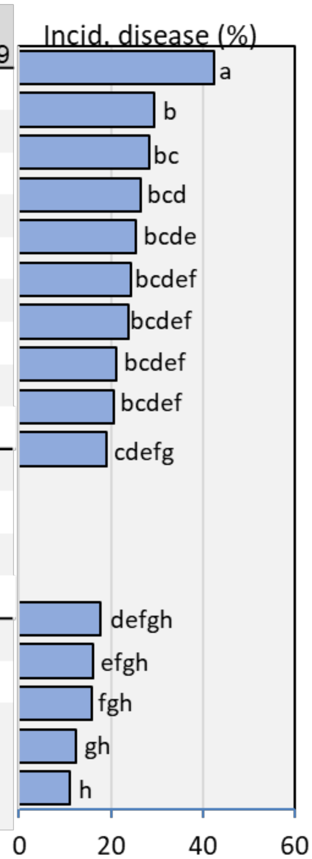
Fig. 1. In vitro toxicity of ε-poly-L-lysin and nisin against *E. amylovora* - Direct exposure studies



Bacteria were exposed to the test substances for 30 min, diluted 1:100 with water, and then plated onto agar media. Bacterial colonies were quantified after 2 days.

Fig. 2. Efficacy of new mostly organic bactericides for management of fire blight of Granny Smith apples, Fresno Co. 2019

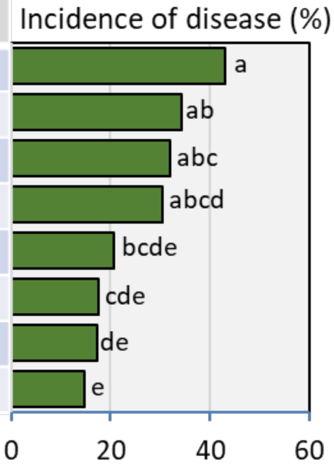
	Treatment	Rate	3-30	4-4	4-10	4-19
	Control	---	---	---	---	---
	ε-Poly-L-lysine	3.5 oz	@	@	@	@
	MasterCop	64 fl oz	@	@	@	@
	CS-2005	25 fl oz	@	@	@	@
	Nisin	13.5 oz	@	@	@	@
	ε-Poly-L-lysine + Dart	3.5 oz + 45 fl oz	@	@	@	@
	Nisin + EDTA + Zinc oxide	13.5 oz + 40 fl oz + 32 oz	@	@	@	@
	"New" Mycoshield	8 oz	@	@	@	@
	Cueva	96 fl oz	@	@	@	@
Rotation	Badge X2	16 oz	@	---	---	---
	Badge X2 + ProPhyt	16 oz + 64 fl oz	---	@	---	---
	Blossom Protect + Buffer	20 oz + 5 lb	---	---	@	---
	Blossom Protect + Buffer	20 oz + 5 lb	---	---	---	@
	Kasumin 4L	32 fl oz	@	@	@	@
	Kasumin 2L	64 fl oz	@	@	@	@
	Kasumin 2L + ε-Poly-L-lysine + Zinc oxide	64 fl oz + 13.5 + 32 oz	@	@	@	@
	Kasumin 2L + FireWall	64 fl oz + 8 oz	@	@	@	@
	Blossom Protect + Buffer	20 oz + 5 lb	@	@	@	@



All trees received a bud break treatment with 6 lb ChampION/A on 3-25-19. In-season treatments were applied on 3-30 (5-10% bloom), 4-4 (20-40% bloom), and 4-10-18 (full bloom), and 4-19 (petal fall) using an air-blast sprayer. Disease was evaluated on 5-21-19. "New" Mycoshield formulation = NUP 17010.

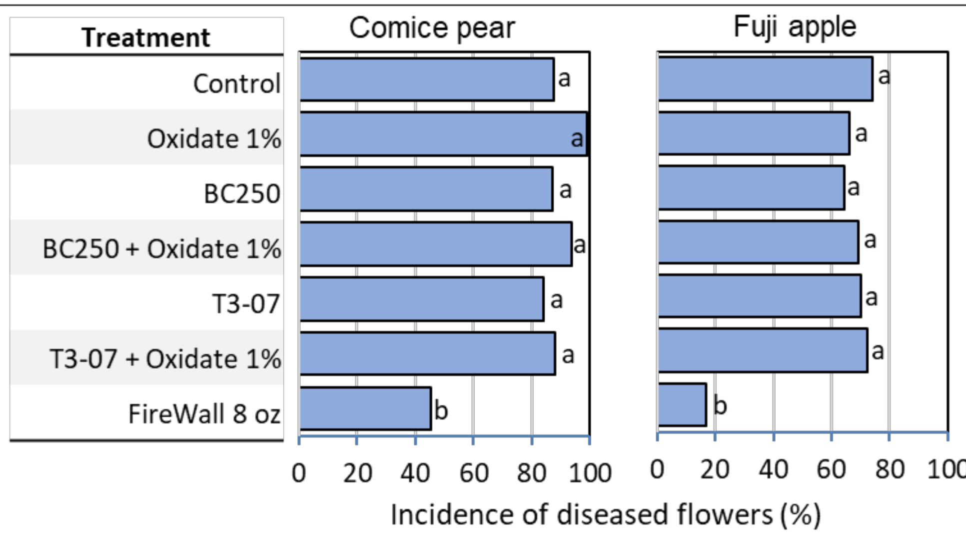
Fig. 3. Efficacy of bactericides for management of fire blight of Fuji apples, Fresno Co. 2019

Treatment	Rate/A
Control	---
Nisin + EDTA + Zinc Oxide	13.5 oz + 40 oz + 32 oz
ε-Poly-L-lysine + Dart	13.5 oz + 45 fl oz
ε-Poly-L-lysine + Zinc Oxide + EDTA	13.5 oz + 32 oz + 40 fl oz
ε-Poly-L-lysine + Zinc Oxide + EDTA	3.5 oz + 32 oz + 40 fl oz
Kasumin 4L	32 fl oz
FireLine + Zinc Oxide + Dart	16 oz + 32 oz + 45 fl oz
Kasumin 2L	64 fl oz



All trees received a bud break treatment with 6 lb ChampION/A on 3-25-19. In-season treatments were applied on 4-2 (5-10% bloom), 4-5 (20-40% bloom), 4-12-18 (full bloom), and 4-22 (petal fall) using an air-blast sprayer at 100 gal/A. Disease was evaluated for 100 flower clusters (spurs) of each tree on 5-20-19. All treatments had four, paired-tree replications (total of 8 trees).

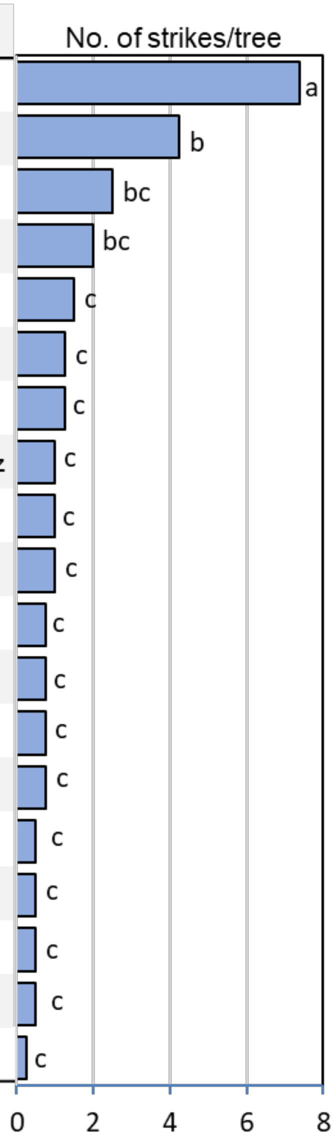
Fig. 4. Efficacy of two new potential bacterial antagonists (BC250, T3-07) in comparison with streptomycin for control of fire blight of Comice pear and Fuji Apple in a small-scale field study at UC Davis 2019



Treatments were applied to open flowers using a hand sprayer. Flowers were inoculated with *E. amylovora* after 3.5 h, and Oxidate was applied after another hour. Disease was evaluated after 1 week.

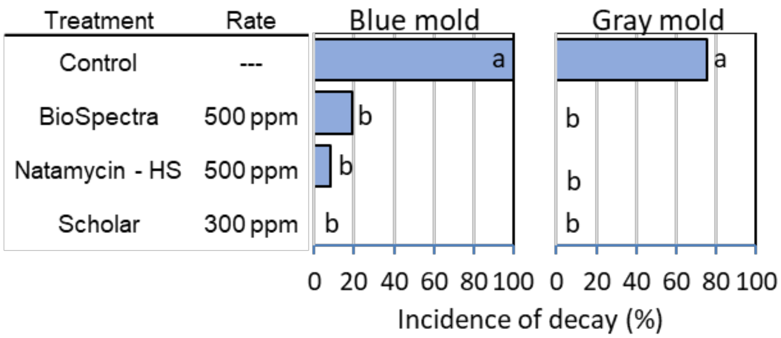
Fig. 5. Efficacy of new bactericides for management of fire blight of Bartlett pear, Sutter Co. 2019

Treatment	Rate
Control	---
Nisin + EDTA	13.5 oz 32 fl oz
NSA + Silco	135 fl oz + 68 fl oz
NS1 + Silco	135 fl oz + 68 fl oz
NS2 + Silco	135 fl oz + 68 fl oz
ε-Poly-L-lysine + ZnNO3	13.5 oz + 50 fl oz
New Mycoshield + ZnNO3 + LI700	8 + 50 fl oz + 8 fl oz
Kasumin + ε-Poly-L-lysine + ZnO	64 fl oz + 13.5 oz + 32 oz
New Mycoshield + LI700	8 oz + 8 fl oz
Cueva	96 fl oz
ε-Poly-L-lysine + DART	13.5 oz + 44 fl oz
Kasumin	64 fl oz
Kasumin + ε-Poly-L-lysine	64 fl oz + 13.5 oz
Blossom Protect + buffer	20 + 80 oz
FireLine + ZnO + LI700	16 oz + 32 oz + 8 fl oz
Serenade ASO + Cueva	64 + 96 fl oz
Nisin + EDTA + ZnO	13.5 + 32 + 32 oz
Kasumin 4L	32 fl oz
Kasumin + Firewall	64 fl oz + 8 oz



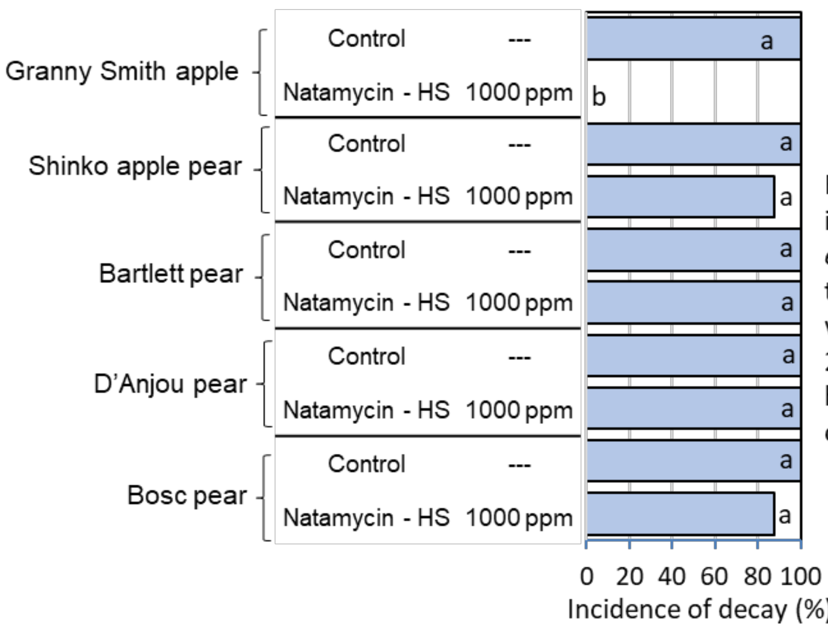
Treatments were applied on 4-4 (5% bloom), 4-12 (full bloom), 4-18 (petal fall), and 4-26-19 (petal fall) using an air-blast sprayer at 100 gal/A. Disease was evaluated on 5-1-19.

Fig. 6. Evaluation of postharvest treatments with two formulations of natamycin for managing postharvest decays of Granny Smith apple in laboratory studies



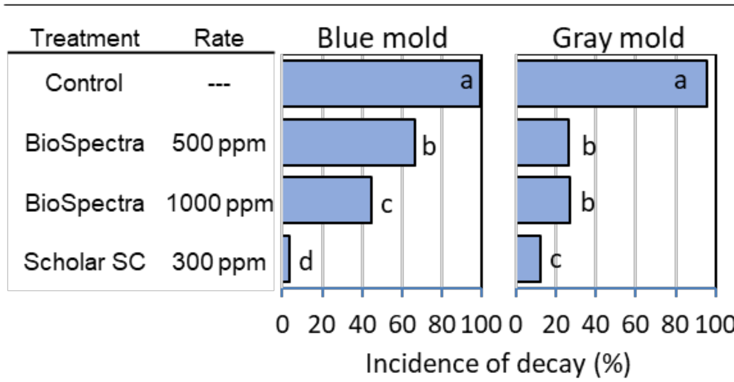
Fruit were wound-inoculated with *P. expansum* or *B. cinerea* and spray-treated after 12 h. Fruit were then incubated at 20C. Natamycin – HS = a high-solution formulation of natamycin.

Fig. 7. Evaluation of postharvest treatments with natamycin for managing postharvest blue mold of pome fruit cultivars in a laboratory study



Fruit were wound-inoculated with *P. expansum* and spray-treated after 12 h. Fruit were then incubated at 20C. Natamycin – HS = a high-solution formulation of natamycin.

Fig. 8. Evaluation of postharvest treatments with natamycin and Scholar for managing postharvest decays of Granny Smith apple in an experimental packingline study



Fruit were wound-inoculated with *P. expansum* or *B. cinerea* and treated by aqueous in-line drench applications with fungicides after 15-16 h. Fruit were sequentially treated with a CDA application with diluted carnauba wax. Fruit were then incubated at 20C for 7 days.