California Apple Commission
Annual Report – 2022-23

Project Title:	Evaluation of new biological controls and natural products for management of fire blight caused by *Erwinia amylovora* and postharvest decays of apple
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Introduction

Fire blight, caused by the bacterium *Erwinia amylovora*, is one of the most destructive diseases of pome fruit trees including apples. The disease is indigenous to North America but has spread worldwide. In the spring, flowers are infected through natural openings in nectaries and pistils. From there, the bacterium spreads into the peduncle, spur, and twig where it causes a canker. During warm, humid weather, ooze droplets consisting of new inoculum are exuded from peduncles and other infected tissues. Inoculum is spread by wind, rain, insects, birds, or by contaminated pruning tools. Secondary infections may occur throughout the growing season. The pathogen overwinters in cankers, flower buds, and diseased fruit.

Current chemical control programs for fire blight are based on protective schedules with bactericides. Conventional copper compounds are only effective when disease severity is low to moderate. They may cause fruit russeting and therefore, are labeled 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, Cueva, and MasterCop. Among these, Cueva and MasterCop have been often more effective without causing phytotoxicity. Contributing to the low efficacy of copper is that low to moderate levels of copper insensitivity in pathogen populations have been detected in our surveys. Because only few treatments are permitted for organic apple production, research on OMRI-approved copper and other products needs to be continued.

The antibiotics streptomycin, oxytetracycline, and kasugamycin can only be used in conventional pome fruit production because the first two are used in human medicine but all three are naturally derived compounds. Kasugamycin (Kasumin) is produced by fermentation of the bacterium *Streptomyces kasugaensis* and may potentially become organically approved because it is not used in human or animal medicine. Recently, natamycin, a biofermentation product like kasugamycin that is mostly used as a postharvest treatment of various crops (see below), was OMRI approved without going through the NOSB as a conventional product requesting organic approval. This was done by demonstrating that the formulation was organic including the inerts, and the request was sent directly to the USDA-AMS and OMRI. Resistance in *E. amylovora* to kasugamycin has not been found to date among hundreds of strains evaluated from different pome fruit growing areas in California. Thus, we are pursuing with UPL an organically approved formulation.

Among non-antibiotic treatments for fire blight control, the biocontrols Blight Ban A506 (*Pseudomonas fluorescens* strain A506), Bloomtime Biological (*P. agglomerans* strain E325), Double Nickel 55 (*Bacillus amyloliquifaciens*), and Serenade, a fermentation product of *Bacillus subtilis* (strain QST 713), performed inconsistently over the years in our trials and were most effective at low inoculum levels and less favorable micro-environments. The ASO liquid formulation of Serenade showed higher efficacy in mixtures with copper such as Cueva. Research will need to be continued with new copper products or other additives. 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. Several mechanisms have been described for biocontrol agents that lead to the control of the pathogenic agent including: (1) Competition; (2) Antibiosis or biochemical inhibition; (3) Site exclusion; (4) Parasitism; and (5) Systemic-acquired resistance.

Previous research on apple and pear demonstrated that the non-organic acibenzolar-S-methyl (Actigard) and the OMRI-approved LifeGard (Certis) systemic acquired resistance (SAR) treatments were inconsistent when used alone or in combination with copper. Therefore, we are 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 US-FDA-approved food preservatives. Other compounds under evaluation are capric/caprylic acids in mixtures with different products. In 2021, the plant extract products Thymox and Cinnerate, as well as Alum ($KAl(SO_4)_2 \cdot 12H_2O$) and TD-NC-1 (riboflavin) provided very good blight control on apple and/or pear. All these potentially could qualify as biopesticides with the EPA and ultimately as organic compounds with the NOSB and OMRI. Therefore, we continue to try to improve their efficacy by using selected additives. For ε-poly-L-lysine and nisin, we are currently consulting with formulation chemists of two major registrants. Our goal is to develop effective rotational programs for organic farming practices with the use of copper, biologicals, and innovative strategies such as registering kasugamycin, food preservatives, and OMRI-approved natural products. We also will work on conventional programs with the use of antibiotics alone or in mixtures with copper, biologicals, or natural products during bloom or as cover sprays during early fruit development.

Apples like other pome fruits can be stored for some period of time using the correct storage environments. Still, postharvest decays caused by fungal organisms can cause losses that are economically detrimental to 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, black mold, Mucor decay, and bull's eye rot, respectively. In California, the former three are most common. There is a deficiency of postharvest biocontrols and natural products that are available to prevent decays in storage. BioSave 100 is one of the few materials currently available in the United States, but it is not very effective. Other products like Aspire have been discontinued. Still, new biological products have been registered in other countries.

We demonstrated that the food preservative natamycin is effective against a spectrum of postharvest pathogens including those causing gray mold, Rhizopus rot, Mucor rot, and Alternaria decays, but it was not highly effective against blue mold on pome fruit. Natamycin was registered as the biopesticide BioSpectra 100SC on stone and citrus but not pome fruits. This fungicide has been federally approved by the US-Food and Drug Administration (FDA) as a food additive to prevent mold growth, including *Penicillium* species, on dairy and meat products for many years in the United States. Over all these years of natamycin use, resistance in *Penicillium* species has not occurred. Working with DSM, a producer, and Pace International, the registrant, we submitted a letter of support to the NOSB for approval of natamycin as an organic postharvest treatment of fruit crops. Although the submission was initially denied in 2021, USDA-AMS approved organic use that resulted in OMRI certification of some formulations of natamycin for organic use, and postharvest fruit formulations are pending. Other registrants of natamycin will pursue conventional and organic registrations. Natamycin is 'exempt from tolerance' by the US-EPA. Codex is currently developing a similar category for these types of biopesticides. Therefore, our goal is to continue to evaluate natamycin and other new postharvest fungicides for the management of postharvest decays of apples.

Objectives for 2022-23

Fire blight research

1. Evaluate the efficacy of treatments for managing fire blight.
   - A. Laboratory in vitro tests with zinc products in combination with antibacterial food additives (lactic acid, Ɛ-poly-L-lysine, nisin, and Alum-potassium aluminum sulfate), natural organic acids (Dart-capric/caprylic acid mixtures, natural products (Cinnerate-cinnamon oil/potassium oleate; QAM-agave extract), and other new biologicals (yeast and yeast extract).
   - B. Field trials with protective air-blast spray treatments:
     - i. Kasugamycin and new formulations of oxytetracycline in combination with organic treatments to support organic petition of these products to NOSB or via USDA - AMS.
     - ii. New formulations of copper (e.g., CS-2005, Cueva, MasterCop) and zinc in combination with food additives (poly-L-lysine, nisin), biocontrols (e.g., Serenade ASO, Double Nickel 55), or natural products (Alum, Cinnerate, Thymox, Seican, TDA-NC-1) as new organic antibacterial strategies.
     - iii. Bacterial phage-mixture products in combination with other biological control treatments (i.e., Blossom Protect) to provide an integrated strategy (pending agroindustry cooperation).

Postharvest research

2. Comparative evaluation of new postharvest fungicides
   A. Evaluate Scholar, Penbotec, and Academy (difenoconazole mixed with fludioxonil) compared to organic formulations of natamycin (BioSpectra, Cerafruta), as well as a conventional natamycin formulation (Unigard), and approved organic formulations of polyoxin-D (Oso) at selected rates against gray mold, blue mold, and Alternaria decay.
   B. Evaluate mixtures of these compounds to improve performance of postharvest fungicide treatments.

Procedures

Isolation of E. amylovora, bacterial culturing, and laboratory screening of E. amylovora sensitivity to biologicals/biopesticides. Samples with fire blight symptoms were obtained in the spring/summer of 2022. Infected plant material (fruit, stems, pedicels, twigs) was cut into small sections and incubated in 1 ml of sterile water for 15 to 30 min to allow bacteria to diffuse out of the tissue. Suspensions were streaked onto yeast extract-dextrose-$CaCO_3$ agar (YDC) and single colonies of *E. amylovora* were transferred and placed in storage at -80C.

Using these isolates, we conducted laboratory screening for the sensitivity to alternative conventional and natural toxicants using spiral gradient endpoint and direct exposure assays. A list of materials is shown in **Table 1**. We evaluated cinnamon oil/potassium oleate (Cinnerate), cinnamaldehyde (Seican), potassium aluminum sulfate (Alum), QAM, capric/caprylic acids (Dart), and antibacterial food additives such as poly-L-lysine and nisin for their toxicity to *E. amylovora*. Growth was compared between non-amended and amended media, and the most effective additives were selected for field trials. Additionally, 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) 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.

Laboratory studies on the management of fire blight using protective treatments. Attached flowers on twigs of ornamental pear (*Pyrus calleryana*) from the UCR campus were used in January 2023 to assess the in vivo performance of selected materials identified in in vitro studies described above in protecting blossoms from fire blight. Treatments evaluated included EPL, Jax-1, these treatments in mixture with dodine (Syllit), and Kasumin. After air-drying, flowers were spray-inoculated with *E. amylovora* (10^6 cfu/ml), and disease was evaluated based on the number of diseased flowers per replication (disease incidence).

Field studies on the management of fire blight using protective treatments during the growing season. Air-blast sprayer field studies on the relative efficacy of protective treatments were conducted in experimental apple orchards at KARE and UC Davis. Comparative field studies were also conducted on cv. Bartlett pear and cv. Shinko apple-pear with some overlapping treatments to the apple studies. Three or four applications were done (at pre-bloom, 10-20%, 60-80% full bloom, and petal fall) with timings based on temperature, rainfall, and host development. The relative efficacy of protective treatments were evaluated including new formulations of copper (e.g., CS-2005, Cueva, MasterCop) and zinc in combination with food additives (poly-L-lysine, nisin), biocontrols (e.g., Serenade ASO), or natural products (e.g., Alum, Cinnerate), essential oils (e.g., Thymox, Gargoil), and other products (e.g., TDA-NC-1) as new organic antibacterial strategies. These products were evaluated alone or in selected mixtures to develop integrated programs for resistance management. Incidence of blight infections on blossoms and shoots in addition to potential phytotoxic effects of the treatments (e.g., fruit russeting) were evaluated. Treatments were replicated on four to eight trees. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.4.

Table 1. Bactericides evaluated in field studies on fire blight 2023

Category	FRAC Code	Active ingredient	Trade name/Code
Antibiotics	24	kasugamycin	Kasumin 2L
	41	oxytetracycline	FireLine 45, Mycoshield NUP-17010
	25	streptomycin	FireWall
Natural products	BM 01	agave extract	QAM
	BM 01	bacterial metabolite	RAA-A
	BM 01	bacterial metabolite	RAA-B
	BM 01	capric/caprylic acids	Dart
	BM 01	cinnamaldehyde	Seican
	BM 01	cinnamaldehyde + EPL	Jax
	BM 01	cinnamon oil	Cinnerate
	BM 01	eugenol	ET91
	BM 01	potassium aluminum sulfate	Alum
	BM 01	tea tree oil	Timorex ACT
	BM 01	thyme oil	Thyme Guard
	BM 01	yeast and yeast extract	CWP
Biocontrols	BM 02	*Aureobasidium pullulans*	Blossom Protect
	BM 02	*Bacillus subtilis* QST 713	Serenade ASO
Food preservatives	---	nisin	food additive
	---	KFD-622-NSN (formulated nisin)	food additive
	---	ε-poly-L-lysine	food additive
	---	KFD-623-EPL (formul. ε-poly-L-lysine)	food additive
Other antimicrobials	M01	copper-sulfate pentahydrate	MasterCop
	U12	dodine	Syllit
	---	water-soluble zinc	Manniplex Zn
	---	peroxyacetic acid	Oxidate

Efficacy of new postharvest fungicides for managing apple decays in storage. Cvs. Granny Smith and Fuji apples were treated similar to commercial practices concerning harvest, handling, packing, and temperature management. Fruit were wound-inoculated with conidial suspensions of *P. expansum* of selected fungicide resistance phenotypes and treated after 14-17 h. Potential organic formulations of natamycin (BioSpectra 100SC, Cerafruta, Unigard) were evaluated and in mixtures with fludioxonil or pyrimethanil and compared to fludioxonil, pyrimethanil, or fludioxonil/difenoconazole (Academy) in experimental packingline trials at Kearney Agricultural Center. Four replications of 24 fruit were used for each treatment. Data were analyzed using analysis of variance and averages will be separated using least significant difference mean separation procedures of SAS 9.4.

Results

Laboratory screening of* E. amylovora *sensitivity to biologicals/biopesticides. We continued laboratory screening for the sensitivity of *E. amylovora* to alternative conventional and natural toxicants using spiral gradient endpoint and agar dilution assays. The commercial organic products Timorex ACT and CWP (a yeast extract) were not inhibitory at labeled rates. The food preservatives nisin and Ɛ-poly-L-lysine (EPL) alone and in mixtures with capric/caprylic acid (Dart), Manniplex zinc, or dodine (Syllit) were inhibitory at higher rates. Overall, the two antibiotics streptomycin and oxytetracycline mixed with dodine were the most inhibitory. The commercial product cinnamaldehyde (Seican) was more toxic than cinnamon oil (Cinnerate) but still only moderately toxic to the pathogen (Table 2). However, the mixtures of Seican with EPL or nisin was highly toxic to the pathogen, similar to the antibiotics (Table 2). Overall, the food preservatives in mixtures with Dart, Syllit, or Seican and the mixtures of either of the antibiotics with Syllit (data not shown) had the highest in vitro toxicity.

Streptomycin, oxytetracycline, and kasugamycin were evaluated for their in vitro toxicity to isolates of *E. amylovora* from field surveys using the spiral gradient endpoint method. Thirty-five fire blight samples were made available in 2022 for our annual resistance monitoring, and 77 strains of *E. amylovora* were obtained. 23 strains from 13 orchards in Lake Co. (mostly single strains from each orchard location) were all sensitive to STR, OXY, and kasugamycin (Table 3), similar to previous years' samplings.

Table 2. In vitro toxicity of new bactericides against *E. amylovora* in laboratory amended agar assays

Treatment	Concentration	Growth rating
Control	---	+++
QAM	1000	+++
Timorex Act (tea tree oil)	1000	+++
CWP (yeast extract)	1000	+++
Cinnerate (cinnamon oil)	100	+++
	250	+
	500	-
Seican (cinnamonaldehyde)	50	+++
	100	+
	250	-
Capric/Caprilic acid	1000	-
EPL	500	+++
	1000	-
Nisin	1000	++
EPL + cinnamonaldehyde	500+100	-
Nisin + cinnamonaldehyde	500+100	+

Nutrient agar was amended with selected concentrations of bactericides and a suspension of *E. amylovora* was streaked out. Growth was evaluated after 2 days at 25C. '+++' indicates that growth was similar as on non-amended agar, '+' indicates that growth was inhibited by >80%, and '-' indicates that growth was completely inhibited.

Table 3. Sensitivity of *E. amylovora* strains from pear orchards in California to streptomycin, oxytetracycline, and kasugamycin in 2022

County	Orchard No.	No. isolates	In vitro sensitivity (MIC ppm) Streptomycin	Oxytetracycline	Kasugamycin
Mendocino	1	1	S	S	S
	2	1	S	S	S
	3	1	MR (33.2)	S	S
	4	1	S	S	S
	5	1	S	S	S
	6	1	S	S	S
	7	1	S	S	S
	8	1	S	S	S
	9	3	S	S	S
	10	2	HR (> 40)	S	S
Lake	1	1	S	S	S
	2	1	S	S	S
	3	1	S	S	S
	4	1	S	S	S
	5	1	S	S	S
	6	1	S	S	S
	7	1	S	S	S
	8	1	S	S	S
	9	2	S	S	S
	10	1	S	S	S
	11	5	S	S	S
	12	2	S	S	S
	13	5	S	S	S
Sacramento	1	1	S	S	S
	2	2	S	S	S
	3	1	MR (22.9)	S	S
	4	1	MR (21.5)	S	S
	5	6	S	S	S
	6	2	S	S	S
	7	2	S	S	S
	8	4	S	S	S
	9	3	S	S	S
	10	4	S	S	S
	11	2	S	MR (3.1)	S
	11	4	S	S	S
	12*	9	S	S	S
Total	35	77			

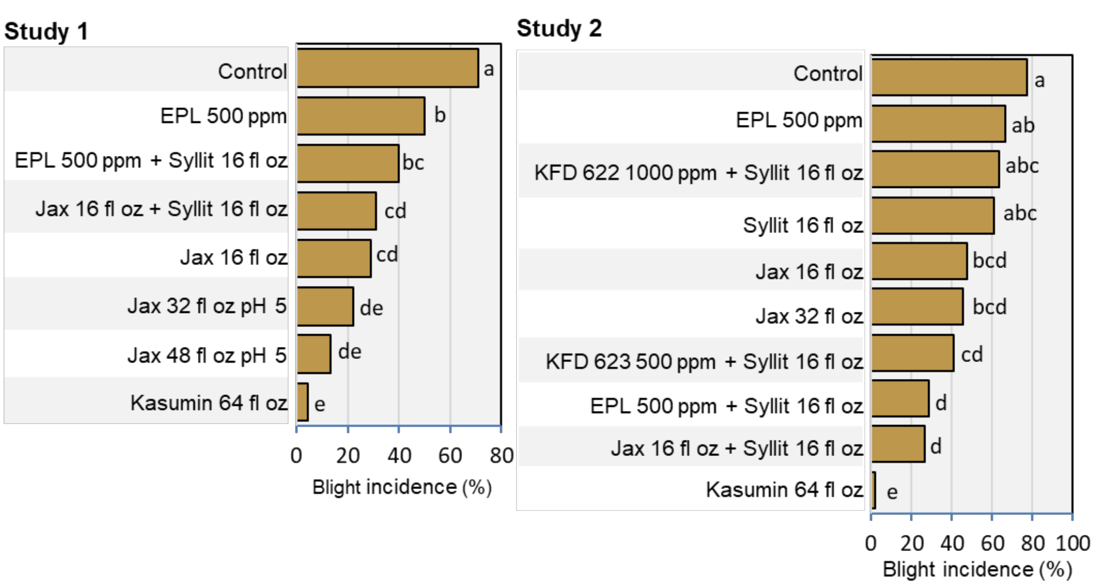
Sensitivity to the three antibiotics was determined using the spiral gradient endpoint method. * Orchard was a Modi apple planting.

Among the 13 strains from 10 orchards in Mendocino Co (again mostly single strains from each location), 10 were sensitive to the three antibiotics. A single strain from one orchard was moderately resistant to STR (MIC = 33.2 ppm), and two strains from another orchard were highly resistant (MIC >100 ppm). Orchards in Mendocino Co. were previously not extensively surveyed by us, but a strain with high resistance to STR was detected several years ago.

All 41 strains from 12 locations in Sacramento Co. were sensitive to KAS (Table 3). Strains from 9 locations were all sensitive to STR and OXY. Moderate resistance to STR (MIC 21.5 – 22.9 ppm) was detected in 2 strains from 2 orchards (Table 3). The incidence of STR resistance in Sacramento Co. has been variable over the years, but often was high, and this has been related to the extent of STR usage. The low incidence in 2022 could be due to improved resistance management. Spray records that were made available to us indicate that rotations of copper, STR, OXY, and KAS were used at some locations. In the 2 orchards with moderate STR resistance, however, 6 of the 10 (including the 5 last treatments) or 7 of the 11 applications (including the 6 last treatments) were done with a FireLine-FireWall mixture.

Starting in 2018, we detected high levels of OXY+STR resistance (MICs >100 ppm) at several locations in Sacramento Co. that was never reported on previously. Different orchards were apparently sampled in 2022, and no high resistance to this antibiotic was identified. Thus, it is not known if these strains persisted at those locations. In 2022, two of the 4 strains from one orchard that had received 4 applications each of FireLine and FireWall, and two applications of Kasumin, however, were moderately resistant to OXY (MIC 3.1 ppm as compared to 0.2 to 1 ppm for sensitive strains) (Table 3). This moderate resistance to OXY in *E. amylovora* also is a new finding.

Fig. 1. Efficacy of bactericides for management of fire blight of ornamental pear flowers in laboratory studies, 2023



Flowering twigs of ornamental pear (*Pyrus calleryana*) were placed into 100-ml Erlenmeyer flasks containing water with 20 ppm gibberellic acid. Flowers were treated using a hand sprayer and were spray-inoculated with a strep[S]/oxy[S] isolate of *E. amylovora*. Twigs were covered with plastic bags, and the incidence of fire blight was determined after 5 days based on the number of blackened flowers of the total number of flowers evaluated

Laboratory studies on the management of fire blight using protective treatments. In two early-season laboratory studies with ornamental pears flowers, the Jax-1 formulation adjusted to pH 5 significantly reduced the incidence of fire blight, similar to Kasumin (Fig. 1, Study 1). Non-formulated EPL, EPL+Syllit, Jax-1+Syllit, Jax-1 without pH adjustment, and KFD 623+Syllit also significantly reduced disease compared to the control (Fig. 1, Studies 1 & 2). The addition of a low rate Syllit to these antibacterial products generally improved efficacy, and this mixture may help to reduce resistance development. These promising results were used to design the treatment lists for our field trials.

Field studies on the management of fire blight using protective treatments during the growing season. A total of four field studies on fire blight management were conducted on cvs. Granny Smith and Fuji apple, and on cvs. Bartlett pear and Shinko apple-pear. In all studies, treatments with the conventional antibiotics STR, OXY, or kasugamycin numerically resulted in the lowest incidence of blight, but several alternative treatments showed promising results with efficacy statistically similar to the three conventional antibiotics.

Fig. 2. Efficacy of bactericides for management of fire blight of Granny Smith apples, Fresno Co. 2023

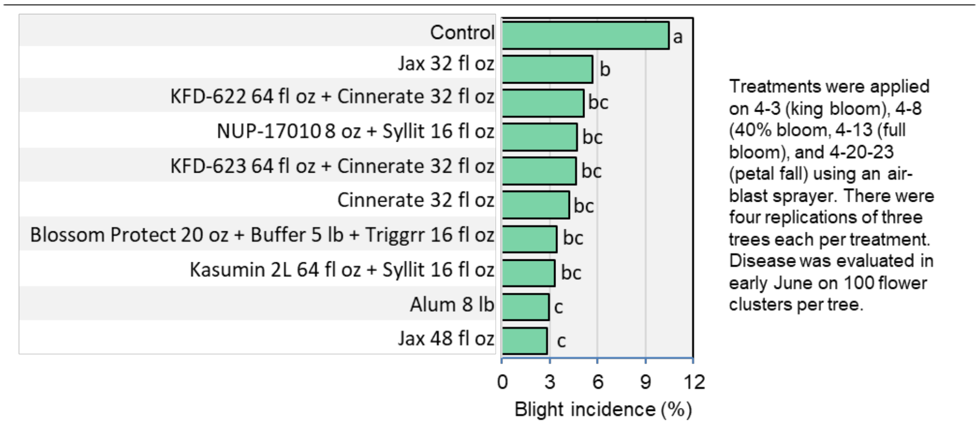
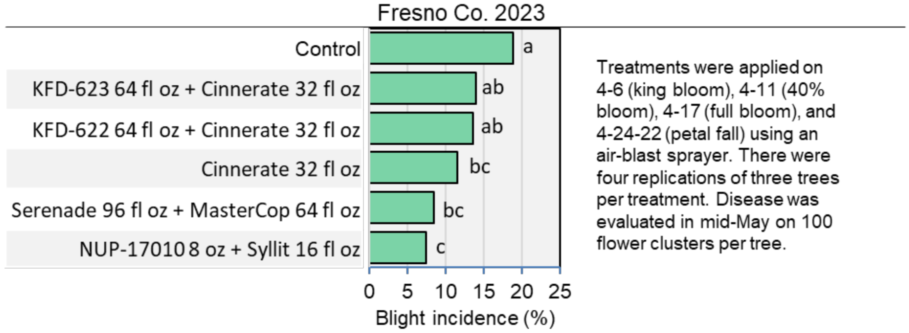


Fig. 3. Efficacy of bactericides for management of fire blight of Fuji Smith apples, Fresno Co. 2023

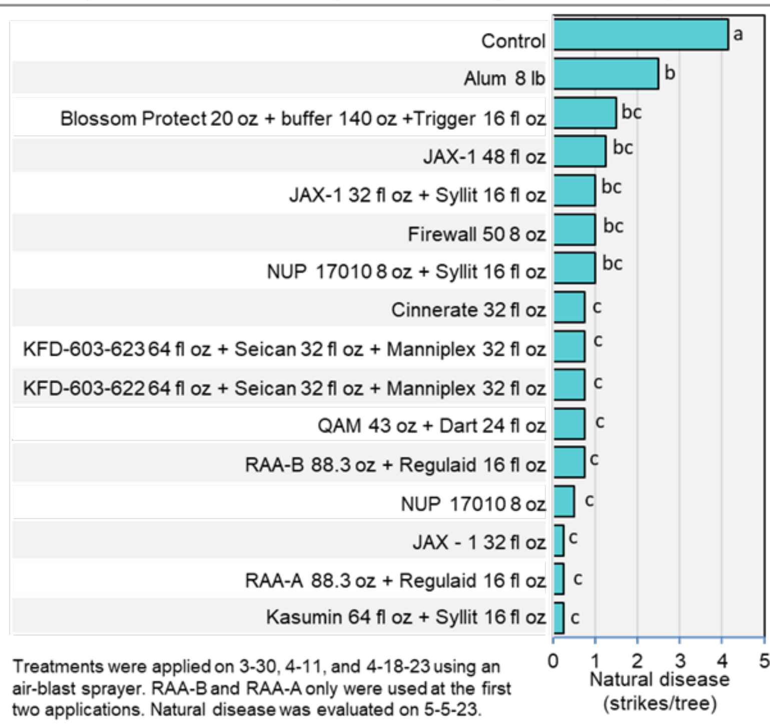


Antibiotics and natural products. On Granny Smith, Kasumin mixed with Syllit was similarly highly effective as Jax-1 and Alum, and the latter two treatments significantly reduced the incidence of blight from 10.4% in the control to 3.3% (Fig. 2). On Fuji apple, NUP17010 (new formulation of Mycoshield - OXY) mixed with Syllit was statistically as effective as Cinnerate and Serenade+MasterCop and significantly reduced blight from 18.9% in the control to between 7.5 and 11.6% (Fig. 3). KFD-623 or -622 mixed with Cinnerate were not significantly different from the control.

In a trial in a commercial Bartlett orchard, an average of 4.2% of flower clusters were affected by fire blight at evaluation date. The most effective treatments reduced disease incidence to 0.75% or less and included mixtures of Kasumin+Syllit, Jax-1 (32 fl oz), RAA-A+Regulaid, RAA-B+Regulaid, NUP17010, QAM+Dart, Cinnerate, and the two KFD formulations mixed with Seican and Manniplex Zinc (Fig. 4). Intermediate treatments that significantly reduced disease from that of the control included Alum, Blossom Protect+buffer+Trigger, Jax-1+Syllit, and NUP17010+Syllit. Thus. the natural products Cinnerate, RAA-A, -B, Seican in mixtures with KFD products (EPL or nisin), QAM+Dart, and Jax-1 were identified as effective treatments that reduced the disease to low levels on Bartlett pears.

In a trial on cv. Shinko apple-pears that were inoculated, however, Serenade ASO, Alum, and Cinnerate did not significantly reduce fire blight from that of the control. Still, Jax-1, Oxidate 5.0, FireWall 50, Blossom Protect+Buffer, and Thyme Guard significantly reduced the disease from 26.7% disease in the control to between 7% and 11%. Overall, in orchards that were not inoculated, the natural products Cinnerate, RAA-A, -B, Seican in mixtures with KFD products (EPL or Nisin), and Jax-1 were identified as effective

Fig. 4. Efficacy of bactericides for management of fire blight of cv. Bartlett pears, Live Oak, 2023

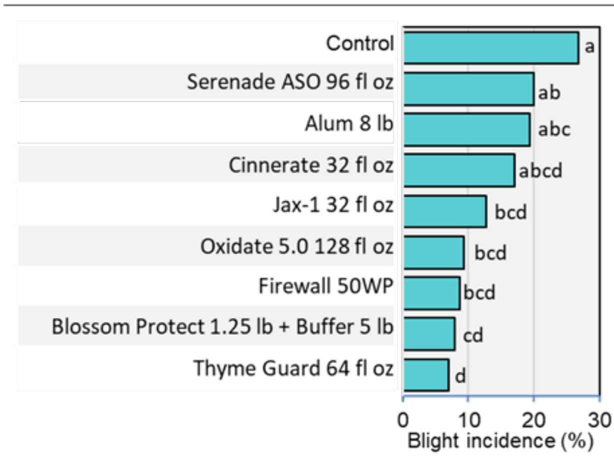


Treatments were applied on 3-30, 4-11, and 4-18-23 using an air-blast sprayer. RAA-B and RAA-A only were used at the first two applications. Natural disease was evaluated on 5-5-23.

treatments that reduced the disease to low levels and were consistently effective on all pome fruit cultivars including apple (cvs. Fuji, Granny Smith), pear (cv. Bartlett), and apple-pear (cv. Shinko). Jax-1 is a mixture of EPL and Seican (an organically approved commercial product) that the registrant is interested in registering as an organic bactericide. KFD products will still have to be mixed or reformulated with additives identified through this research. Alum performed better on apple than on pear or apple pears that were inoculated. Registration of Alum as an organic treatment is planned, however, additional evaluations are needed on non-apple pome fruits.

Conclusion for fire blight management. The efficacy of the three registered antibiotics STR, OXY, and kasugamycin was again high and consistent. Efforts are underway to obtain an organic formulation of Kasumin and to register it in the United States. Among biological treatments, Blossom Protect (based on previous years' studies) and essential oils showed acceptable commercial efficacy, and the latter are the most promising of the new alternative products immediately available. Other biological treatments to be considered are liquid copper formulations such as Cueva and MasterCop, and these can be mixed with biocontrol treatments such as Double Nickel and Serenade. Alum showed good efficacy only in apple studies. Because it does not cause any known

Fig. 5. Efficacy of bactericides for management of fire blight of cv. Shinko apple pears after inoculation, UC Davis, 2023

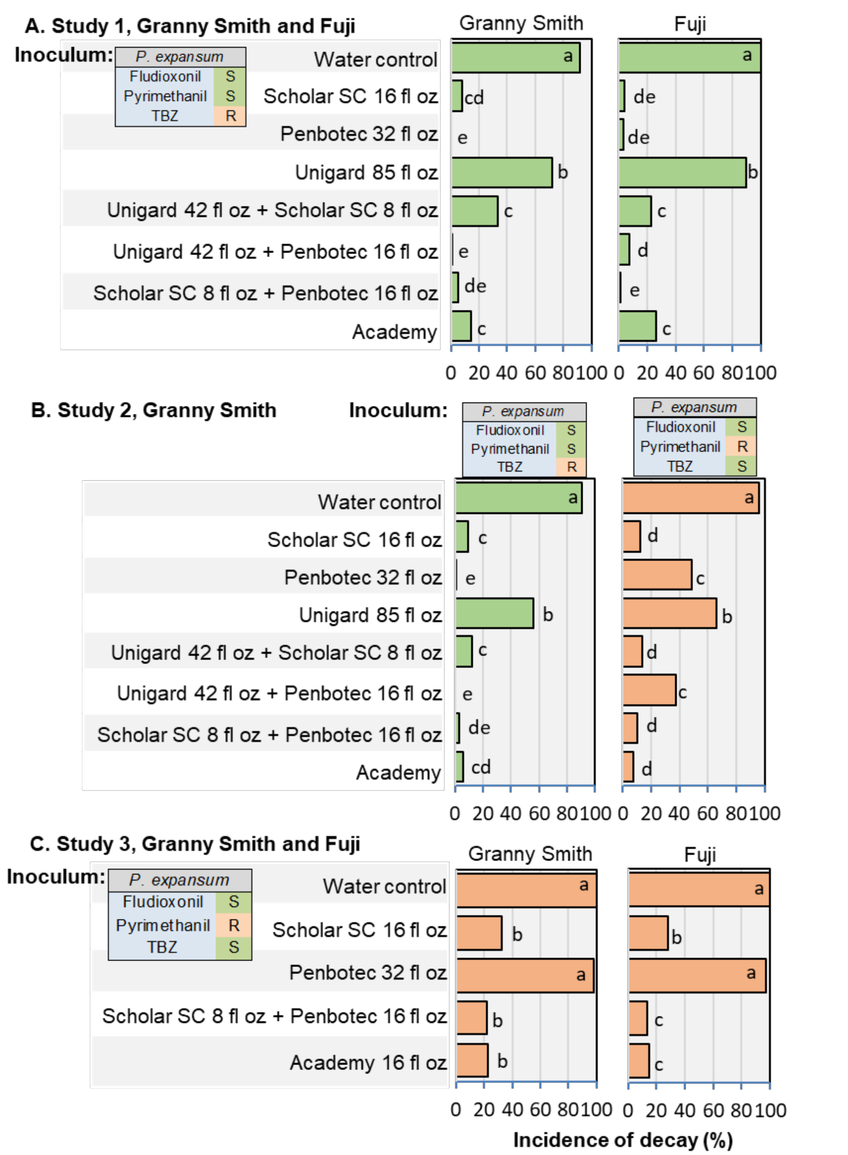


Treatments were applied on 3-23 and 3-28-23 using an air-blast sprayer. Trees were inoculated with *E. amylovora* (1 x 10^6 cfu/ml) on 3-25-23. Disease was evaluated on 4-18-23.

OSHA hazards and is not considered a dangerous substance, it deserves continued evaluation under California conditions. New alternative treatments like Jax-1, Cinnerate, KFD mixtures, and RAA products need to be pursued with registrants in collaboration with us to continue evaluation of their efficacy and consistency as compared to conventional antibiotics with ultimate registrations in the United States as organically approved bactericides.

Evaluation of postharvest treatments using single-fungicides, mixtures, and pre-mixtures. Postharvest studies again focused on the efficacy of the natural compound natamycin that is currently registered as an organic-approved (OMRI certified) biopesticide with tolerance exemption status by the US-EPA. In three experimental packingline studies with inoculated apple fruit, we compared Scholar (fludioxonil), Penbotec (pyrimethanil), Uniguard (natamycin), Academy (fludioxonil + difenoconazole) at full rates and Uniguard at half rate in combination with half rates of Scholar or Penbotec. Uniguard by itself reduced blue mold decay significantly from the untreated control but the level of reduction was not commercially acceptable and was significantly less effective as compared to Scholar, Penbotec, tank mixtures, and Academy treatments (Fig. 6A, B). There was no

Fig. 6. Evaluation of postharvest treatments for managing blue mold of Granny Smith and Fuji apple in experimental packingline studies



Fruit were wound-inoculated with *P. expansum* of different fungicide resistance characteristics (500,000 spores/ml). After 15-17 h, treatments were done by T-jet application that was followed by CDA application with carnauba-based fruit coating. Fruit were then incubated at 20C for 7-8 days.

relationship between apple cultivar used (e.g., Fuji, Granny Smith) and effectiveness. Last year, we obtained similar results and demonstrated that Uniguard was as effective as other postharvest fungicides against gray mold and Alternaria rot. In vitro toxicity of natamycin to mycelial growth of *Penicillium expansum* is similar to other postharvest decay fungi (e.g., *Botrytis cinerea* and *Alternaria alternata*). Thus, pome fruit interfere with the activity of natamycin toward Penicillium but not other decays. This is the first time that we have seen a fungicide perform differently on a certain crop (e.g., pome fruit) as compared to other crops (e.g., stone fruit, citrus) in managing Penicillium decays. We are currently developing an explanation for this unique situation.

In additional studies, we compared the efficacy of treatments against *P. expansum* isolates sensitive or resistant to pyrimethanil. Using the pyrimethanil-resistant isolate for fruit inoculation, Penbotec and Uniguard-Penbotec treatments resulted in significantly higher decay than when a sensitive isolate was used where decay was reduced to near zero incidences (Fig. 6B). In the third study, again using a pyrimethanil-resistant isolate of *P. expansum*, only Scholar, Scholar+Penbotc, and Academy provided high efficacy and significantly reduced decay compared to the control and Penbotec treatments (Fig. 6C). Thus, pyrimethanil (Penbotec)-resistant isolates that are being detected are field resistant and reduce the performance of Penbotec to manage blue mold of apple in California.

Regulatory efforts

Antibiotics. Collaborative efforts are ongoing to work with UPL and the California Apple Commission to develop an organic formulation of kasugamycin. Kaken Chemical and UPL have organic formulations in development or as a laboratory developed product, respectively. Kasugamycin is a biofermentation product similar to natamycin (which received OMRI certification in April 2022). The current formulation of the commercial product Kasumin, however, has inert ingredients that are not on the approved list of materials for organic certification. The goal is to obtain an organic formulation with approved inert ingredients, evaluate the performance, and concurrently submit the organic formulation to the US-EPA, USDA-AMS (Agricultural Marketing Service), and OMRI for organic certification.

Food preservatives. In an effort to improve efficacy of the new formulations of EPL (KFD-623) and nisin (KFD-622), we are requesting updated formulations from UPL as well as mixture formulations of EPL with cinnamaldehyde (Seican) from Summit Agro. We plan to continue to evaluate EPL-Kasumin and Nisin-Kasumin mixtures with UPL. Thus, performance of the two food preservatives will need to be improved in agricultural formulations proposed for registration as biopesticides that may also obtain OMRI-approval. These food preservatives are already FDA approved as food additives and are considered “Generally regarded as Safe” (GRAS); whereas cinnamaldehyde (Seican) is already OMRI-approved.

Other antimicrobials. RAA bacterial fermentation by-products and peroxyacetic acid products (e.g., Oxidate, Perasan) will continue to be evaluated along with Alum for organic growers. We are providing efficacy data for Alum to the registrant for registration on apple. New formulations of copper products that are OMRI-certified and have lower copper content may be less phytotoxic as in-season treatments to apple flowers, fruit, and foliage. Syllit is already registered on apple and thus, continued studies in mixtures with antibiotics and natural products to improve performance is ongoing for conventional growers.