Annual Report - 2022

EVALUATION OF NEW BACTERICIDES FOR CONTROL OF FIRE BLIGHT OF PEARS CAUSED BY *ERWINIA AMYLOVORA*

J. E. Adaskaveg, Department of Microbiology and Plant Pathology, University of California, Riverside CA 92521

Collaborators: H. Förster and D. Thompson (UC Riverside), L. Wade (Grow West), and R. Elkins (UCCE, Lake Co.)

ABSTRACT

1. **Antibiotic resistance**
   a. **Surveys** of *Erwinia amylovora* in California pear orchards were continued with 77 strains obtained from 35 orchards in Lake, Mendocino, and Sacramento Co. The incidence of resistance was low in 2022. Among 13 strains from locations in Mendocino Co., one with moderate resistance and two with high resistance to streptomycin were recovered. Among 41 strains from Sacramento Co., two were moderately resistant to streptomycin and two were moderately resistant to oxytetracycline. All strains were sensitive to kasugamycin.
2. **Efficacy studies with new bactericides for management of fire blight.**
   a. In **in vitro studies**, a mixture of EPL and cinnamaldehyde (Seican) was found to be synergistic: EPL at 500 ppm was not inhibitory, but when mixed with cinnamaldehyde at 100 ppm, growth of *E. amylovora* was completely inhibited.
   b. In **laboratory studies with ornamental pear flowers**, non-formulated EPL and the KFD 623-01 formulation significantly reduced the incidence of fire blight to similar levels as using FireWall or FireLine.
3. **Field trials on the management of fire blight** were conducted under moderate natural disease pressure at a commercial Bartlett pear site or high disease pressure in field inoculation studies with Comice pear and apple pear at UC Davis.
   a. In the Bartlett study, the most effective treatments included mixtures of Kasumin with FireWall or Syllit, as well as of the NUP17010 formulation of oxytetracycline with Dart. A mixture of 32 fl oz Kasumin with the KFD 623-01 formulation of EPL and ManniPlex Zn was similarly effective. These Kasumin mixtures were significantly more effective than Kasumin by itself. Kasumin use is now restricted to a maximum of four applications per season (no more than two consecutive applications) and is recommended in mixture with another bactericide to help minimize the risk for resistance development.
   b. In the Bartlett study, the natural product cinnamon oil (Cinnerate) was identified as an effective treatment that reduced the disease to low levels. After inoculation of Comice pear, only FireLine+Dart and KFD 622-01+Kasumin significantly reduced

the incidence of blight from the control. In the apple pear study, FireWall, Cinnerate, and ThymeGard resulted in significant reductions of disease as compared to the control. Additional other new experimental bactericides, including QAM, Alum, Ninja, CWP, and Agriphage lacked acceptable and consistent efficacy based on current commercial standards.

INTRODUCTION

Fire blight, caused by the bacterium *Erwinia amylovora*, is the most destructive disease of pome fruit trees worldwide, especially pears. In California, prolonged rat-tail bloom contributes to a long infection period. Fire blight is very difficult to manage, and few effective treatments are available. Integrated programs with sanitation practices and applications with chemical and biological controls are the best approaches. If the disease occurs at low incidence, it may be eliminated by pruning. Thus, aggressive and regular pruning of diseased tissue is essential for keeping inoculum levels low.

Current chemical control programs for fire blight are based on protective treatments with antibiotics or copper. On Bartlett pears, copper treatments traditionally have been used only during the dormant and bloom periods because they cause fruit russeting. Some newer formulations of copper, however, are labeled at lower metallic copper equivalent (MCE) and can be used after bloom without causing russeting. At low disease pressure, copper compounds can provide satisfactory disease control, and they can be an effective rotational or mix partner. At high disease pressure, however, copper applications generally fail to control fire blight at satisfactory levels. Therefore, copper is ranked as '+/++' in our UCIPM ratings indicating inconsistent performance depending on environmental conditions. In addition, we reported reduced copper sensitivity in strains of *E. amylovora* from pear that can also explain the moderate and inconsistent performance of copper. Lack of systemic action and low registered rates are other factors contributing to low efficacy.

Treatments with the antibiotics streptomycin (STR) and oxytetracycline (OXY) have been employed for many years to manage fire blight. Continued use for many seasons and lack of alternative control materials resulted in resistance to develop against STR at many locations in California, mostly in Sacramento Co. Strains with moderate plasmid-based resistance and strains with chromosome-based high resistance have been identified. The incidence of STR resistance has been fluctuating widely among years from very low to very high levels, and this has been attributed to disease pressure and the intensity of STR use. Strains of *E. amylovora* with reduced sensitivity to OXY were found several times during our surveys. Starting in 2018, however, we detected strains with high resistance to this antibiotic for the first time at several locations in Sacramento Co. These strains were found to be similarly virulent and competitive as wildtype strains.

Surveys on antibiotic resistance monitoring were continued in 2022 in collaboration with farm advisors and PCAs, and samples were obtained from Mendocino, Lake, and Sacramento Co. Resistance to kasugamycin (Kasumin) has never been found in our surveys. Kasumin became available for use in 2018 in California. Concerns have been expressed by regulatory agencies regarding the use of antibiotics in agriculture, but kasugamycin is not used in human or animal medicine and has a different mode of action from STR or OXY (no cross-resistance). In 2020, after 7 years of environmental resistance monitoring with no detected shifts in sensitivity among non-target bacteria, the EPA has suspended this requirement for the kasugamycin registration.

With the current emphasis on identifying alternative bactericides, we continued our evaluations of exempt-from-tolerance and potential organic compounds in 2022. Thus, we continued to evaluate the food preservatives ε-poly-L-lysine (EPL) and nisin as non-formulated technical compounds and agricultural formulations. Other products evaluated in 2022 field trials include the biocontrols Blossom Protect, Serenade, Double Nickel, a yeast-based experimental product, and a phage formulation; the natural products Thyme Gard, Cinnerate, and Dart; the FDA GRAS bactericide TDA-NC1, the natural antibiotic ningnanmycin (Ninja), the experimental QAM, the fungicide Syllit, and Alum (Table 1). The essential oil product cinnamaldehyde (Seican) was only evaluated in in vitro studies.

Table 1. Bactericides and additives evaluated in studies on fire blight 2022

Category	**Active ingredient**	**Trade name/Code**
Antibiotics	kasugamycin	Kasumin 2L
	ningnanmycin	Ninja
	oxytetracycline	FireLine 45, NUP-17010
	streptomycin	FireWall 34%, 50%
Natural products	agave extract	QAM
	capric/caprylic acids	Dart
	cinnamon oil	Cinnerate
	essential oils	Thyme Gard
	nisin	food additive
	ε-poly-L-lysine	food additive
	KFD-622-01-NSN (nisin)	formulated food additive
	KFD-623-01-EPL (ε-poly-L-lysine)	formulated food additive
Other bactericides	aluminum potassium sulfate	Alum
	copper octanoate	Cueva
	dodine	Syllit
	riboflavin	TDA-NC1
	peroxyacetic acid	Oxidate 5.0
Biocontrols	*Aureobasidium pullulans*	Blossom Protect
	Bacillus amyloliquefaciens	Double Nickel 55
	Bacillus subtilis	Serenade ASO
	phage	Agriphage
	yeast and yeast extract	CWP
Additives	citric acid, disodium phosphate	Buffer Protect
	nitrate and urea nitrogen, zinc	ManniPlex Zn
	pinene polymers and others	NuFilm 17

OBJECTIVES

1. Continue resistance surveys for streptomycin, oxytetracycline, and kasugamycin in *E. amylovora* populations from pear orchards in California.
   a) Determine the antibiotic sensitivity of strains of *E. amylovora* provided by farm advisors, PCAs, and growers
   b) Characterize antibiotic resistance on a molecular basis.
2. Evaluate and optimize the performance of kasugamycin (Kasumin), streptomycin (e.g., Agrimycin-17, FireWall), oxytetracycline (e.g., Mycoshield, FireLine), ningnanmycin (Ninja), and new copper formulations in field trials.
   a) Kasumin in combination with exempt-from-tolerance antimicrobials (see below).
   b) Oxytetracycline – new formulations of Mycoshield and FireLine and trials using oxytetracycline with selected adjuvants or stabilizers to increase persistence of residues and overall efficacy.
   c) Ningnanmycin at higher rates and in combinations.
   d) Low concentrations of copper sulfate in combination with antibiotics.
3. Evaluate new natural products, GRAS food additives, and biocontrols.
   a) New formulations of ε-poly-L-lysine and nisin in combination with capric/caprylic acids (Dart). Treatments identified as effective in small-scale studies will be included in field studies.
   b) The new FDA GRAS bactericide TDA-NC-1, the biocontrol Reju-Agro A, aluminum sulfate (Alum), and new essential plant oils (ET-91, EF-400, BacStop) and extracts (QAM).
   c) Registered biologicals including Blossom Protect and Serenade ASO, will be compared to other experimental compounds.

MATERIALS AND METHODS

Isolation of *E. amylovora* and bacterial culturing. Samples with fire blight symptoms were obtained in the spring and early summer of 2022 from 10 pear orchards in Mendocino Co., 13 orchards in Lake Co., and 12 orchards (including one apple orchard) in Sacramento Co. Infected plant material (fruit, peduncles, 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, and single colonies of *E. amylovora* were transferred. A total of 77 strains were obtained and evaluated for their sensitivity to antibiotics.

Laboratory studies on the toxicity of bactericides against *E. amylovora*. STR, OXY, 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.

Several of the new bactericides including Timorex ACT, a yeast-yeast extract formulation, cinnamaldehyde, and mixtures of cinnamaldehyde with EPL or nisin were also evaluated for their in vitro toxicity against *E. amylovora* in amended agar studies where the pathogen was in continuous contact with the test substance. A suspension of *E. amylovora* was streaked on the agar surface, and growth was rated after 2 days of incubation at 25C as '+++' = growth similar as on non-amended agar, '++' = some growth inhibition, '+' = growth inhibited by >80%, and '-' = growth completely inhibited.

Molecular analysis of high STR-OXY resistant strains of *E. amylovora*. High molecular-weight genomic DNA was submitted to long- and short-read whole-genome sequencing. Genomes were assembled and annotated, and sequences were compared with known plasmids.

Studies on the evaluation of new bactericide treatments. Preliminary early-season studies focusing on EPL and nisin were conducted in the laboratory on flowering ornamental pear (*Pyrus calleryana*). Twigs were placed into 100-ml Erlenmeyer flasks containing water with 20 ppm gibberellic acid (to delay senescence), treated using an air-nozzle hand sprayer, allowed to air-dry, and were spray-inoculated with *E. amylovora* ($5x10^6$ cfu/ml). The incidence of fire blight was determined after 7 days based on the number of blackened flowers of the total number of flowers evaluated.

In a field trial in a commercial cv. Bartlett orchard in Live Oak, treatments (see Fig. 2) were done using an air-blast sprayer at 100 gal/A on 3-16 (white tip, 5% bloom), 3-22, 3-29, and 4-7-22. There were four single-tree replications per treatment. The natural incidence of fire blight was evaluated on 4-12-22.

New bactericide alternatives were evaluated on Comice pear and Shinko apple pear at UC Davis. On Comice pear, treatments (see Fig. 3) were applied using an air-blast sprayer on 3-15-22 (20-50% bloom) and 3-22-22. Trees were inoculated with *E. amylovora* (10^6 cfu/ml) after the second application. Blight incidence on 4-6-22 was based on the number of flower/fruit clusters with disease of the total number of clusters evaluated. In the trial on Shinko apple pears, treatments (see Fig. 4) were applied using an air-blast sprayer on 3-15, 3-16, and 3-22-22. Trees were inoculated with *E. amylovora* (10^6 cfu/ml and $2x10^6$ cfu/ml, respectively) after the first and third applications. Blight incidence on 3-30-22 was based on the number of flower clusters with at least one infection of the total number of clusters evaluated. All data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.4.

Table 2. 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.

RESULTS AND DISCUSSION

Survey of antibiotic sensitivity in *E. amylovora* strains from pear in California in 2022. Thirty-five fire blight samples were made available in 2022 for our annual resistance monitoring, and 77 strains of *E. amylovora* were obtained. The 23 strains from 13 orchards in Lake Co. (mostly single isolates from each orchard location) were all sensitive to STR, OXY, and kasugamycin (Table 2), similar to previous years' samplings. Among the 13 strains from 10 orchards in Mendocino Co (again mostly single isolates from each location), 10 were sensitive to the three antibiotics. A single isolate from one orchard was moderately resistant to STR (MIC = 33.2 ppm), and two isolates 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 previously.

All 41 strains from 12 locations in Sacramento Co. were sensitive to KAS. 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. 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 better 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 isolates). This moderate resistance to OXY in *E. amylovora* also is a new finding.

Molecular analysis of high STR-OXY resistant strains of *E. amylovora*. We are currently concluding the molecular characterization of high OXY resistance in California strains of *E. amylovora*. Long- and short-read whole-genome sequencing demonstrated that *tet, strA,* and *strB* genes were acquired by the novel conjugative IncX plasmid pX11-7. The molecular mechanism of moderate resistant to OXY remains to be investigated.

Our previous detections of STR and OXY resistance and the need to protect the efficacy of kasugamycin stress the high importance of resistance management. Antibiotics should only be used in mixtures or rotations, the number of applications of each per season should be limited to two if possible, and new alternatives (e.g., early-season copper, biologicals) should be used, and new options should be developed.

In vitro toxicity of new bactericides. In amended agar studies, the tea tree (i.e., Timorex ACT) and yeast/yeast extract (i.e., CWP) products did not inhibit growth of *E. amylovora* at 1000 ppm (Table 3). Cinnamon oil (i.e., Cinnerate), cinnamaldehyde (i.e., Seican), and EPL completely inhibited growth at 500, 250 ppm, and 1000 ppm respectively. EPL at 500 ppm was not inhibitory, but when mixed with cinnamaldehyde at 100 ppm, no growth occurred.

Thus, this EPL-cinnamaldehyde mixture is synergistic and needs to be included in field studies in 2023. Both components potentially could be approved for organic production.

Table 3. Table 3. In vitro toxicity of new bactericides against *E. amylovora* in laboratory amended agar tests

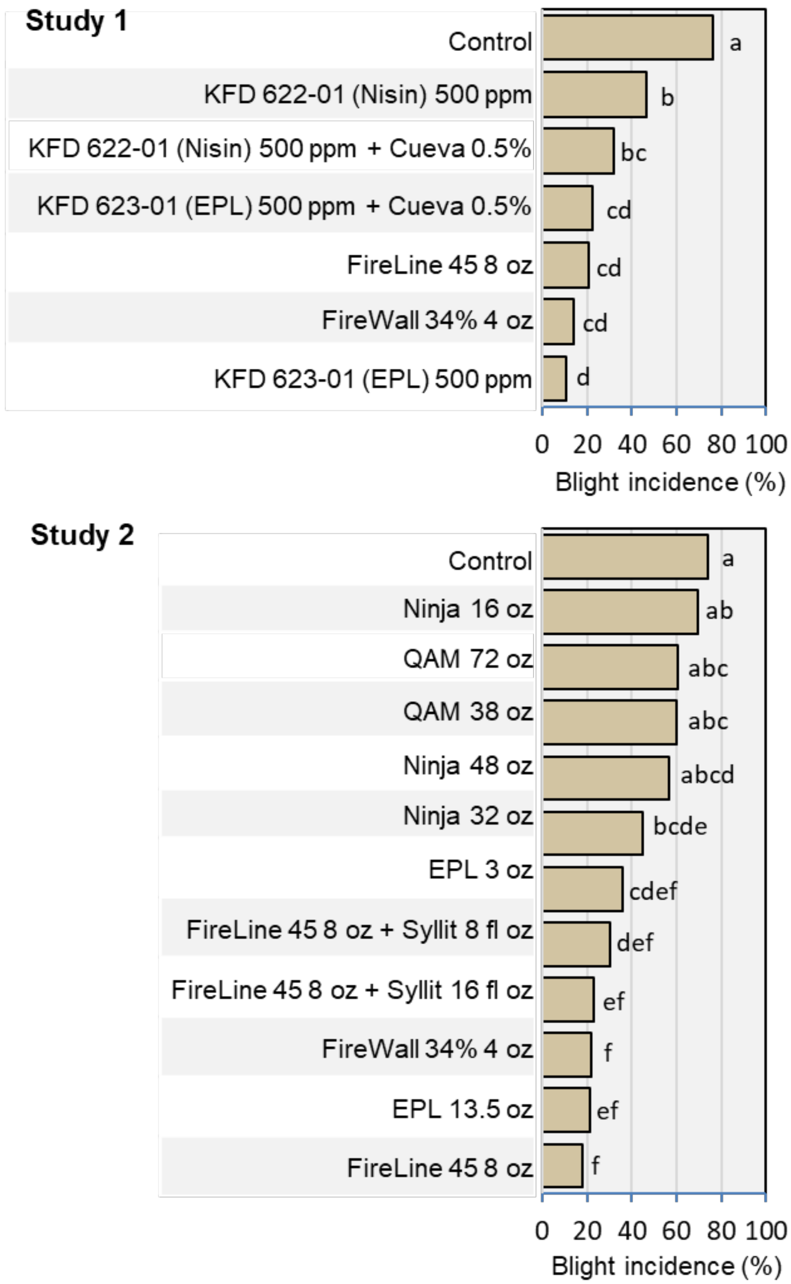
Treatment	Concentration (ppm)	Growth rating
Control	---	+++
Timorex ACT - tea tree oil	1000	+++
CWP - yeast + yeast extract	1000	+++
Cinnerate - cinnamon oil	100	+++
	250	+
	500	-
Seican - cinnamaldehyde	50	+++
	100	+
	250	-
EPL	500	+++
	1000	-
Nisin	1000	+++
EPL + cinneraldehyde	500 + 100	-
Nisin + cinneraldehyde	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.

Field studies on the evaluation of new bactericide treatments. In two early-season laboratory studies with ornamental pears flowers, non-formulated EPL and the KFD 623-01 formulation significantly reduced the incidence of fire blight, similar to FireWall or FireLine (Fig. 1). The two food preservatives EPL and nisin previously were not very effective in field studies. They possibly do not persist and degrade quickly in the environment, emphasizing the importance of designing agricultural formulations. The addition of Cueva to KFD 623-01 did not increase performance, and the nisin formulation KFD 622-01 was less effective than KFD 623-01 (Fig. 1, study 1). The natural antibiotic Ninja showed little efficacy, and the natural product QAM was not effective even when used at high rates (Fig. 1, study 2). Additionally, the addition of Syllit to FireLine did not improve the antibiotic's efficacy (Fig. 1, study 2), but this mixture may still help to reduce resistance development. These promising results were used to design the treatment lists for our field trials.

In a trial in a commercial Bartlett orchard, an average of 25.2% of flower clusters were affected by fire blight at evaluation date. The most effective treatments included mixtures of Kasumin and FireWall, Kasumin and Syllit, as well as of the NUP17010 formulation of OXY and Dart (Fig. 2). A mixture of a reduced rate of Kasumin (32 fl oz) with KFD 623-01 and ManniPlex Zn was similarly effective. These Kasumin mixtures were significantly more effective than Kasumin by itself. A mixture of Kasumin (32 fl oz) with the nisin formulation KFD 622-01 and ManniPlex Zn, however, was significantly less effective than the other mixtures. The natural product Cinnerate was identified as an effective treatment that reduced the disease to low levels. The incidence of fire blight using other treatments in this study such asTDA-NC-1, Blossom Protect, Ninja, Alum, QAM, and the Cueva-Double Nickel mixture was not significantly different from the control, and blight incidence was only numerically reduced (Fig. 2). Thyme Gard was not effective.

Fig. 1. Efficacy of bactericides for management of fire blight of ornamental pear flowers in laboratory studies, 2022



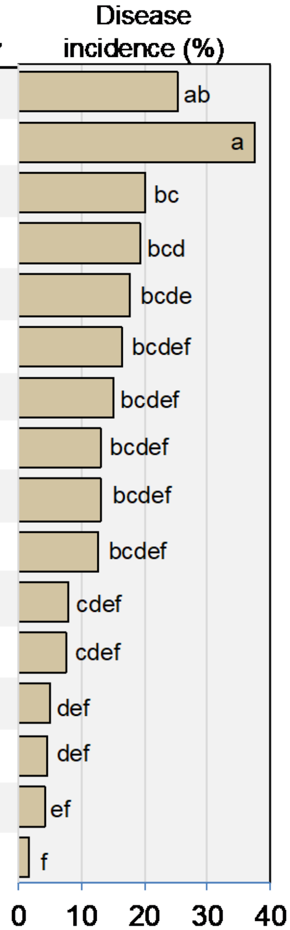
Flowering twigs of ornamental pear (*Pyrus calleryana*) were spray-treated, allowed to air-dry, and were spray-inoculated with a strep^S/oxy^S isolate of *E. amylovora*. The incidence of fire blight was determined after 7 days based on the number of blackened flowers of the total number of flowers evaluated.

In the two UC Davis inoculation studies, 32.8% of flower/fruitlet clusters developed disease on Comice pear and 42% on Shinko apple pear. In the Comice study, only FireLine+Dart and KFD 622-01+Kasumin significantly reduced the incidence of blight from the control (Fig.

Fig. 2. Efficacy of bactericides for management of fire blight of Bartlett pears, Live Oak, 2021

Natural incidence of disease

Treatment	3-16	3-22	3-29	4-7
Control	---	---	---	---
Thyme Gard 64 fl oz	@	@	@	@
Cueva 64 fl oz + Double Nickel 55 128 fl oz	@	@	@	@
QAM 43 fl oz + Dart 24 fl oz	@	@	@	@
Alum 8 lb	@	@	@	@
Ninja 64 oz + Dart 24 fl oz	@	@	@	@
Blossom Protect 20 oz + buffer 140 oz	@	@	@	@
TDA -NC-1 20 oz + NuFilm 17 8 fl oz	@	@	@	@
Kasumin 64 fl oz	@	@	@	@
KFD 622-01 64 fl oz + Kas. 32 fl oz + Manniplex 32 fl oz	@	@	@	@
FireLine 45 8 oz + Syllit 16 fl oz	@	@	@	@
Cinnerate 32 fl oz	@	@	@	@
KFD 623-01 64 fl oz + Kas. 32 fl oz + Manniplex 32 fl oz	@	@	@	@
NUP-17010 8 oz + Dart 24 fl oz	@	@	@	@
Kasumin 64 fl oz + Syllit 16 fl oz	@	@	@	@
Kasumin 64 fl oz + Firewall 50 8 oz	@	@	@	@



Treatments were applied using an air-blast sprayer. Disease was evaluated on 4-12-22. Incidence was based on the number of infected flower clusters of the total number of clusters evaluated.

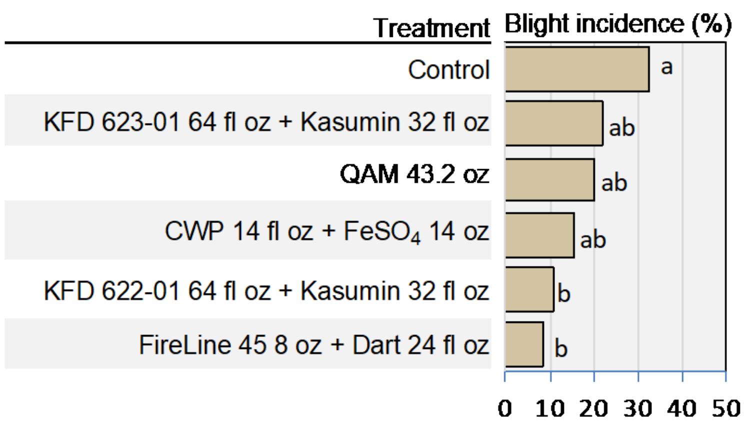
3). CWP, QAM, and the KFD 623-01+Kasumin mixture only numerically, but not significantly reduced the incidence. In the Shinko study, FireWall, Cinnerate, and ThymeGard resulted in significant reductions of disease as compared to the control (Fig. 4). Blossom Protect, Alum, Agriphage, and Serenade ASO were not effective. Inoculations with *E. amylovora* in the field can create a high disease pressure, and only highly effective, persistent treatments are able to reduce disease. The biocontrol treatments Serenade, Agriphage, and Blossom Protect probably could not build up high enough populations before inoculations were done and therefore failed to provide good efficacy. Oxidate was not effective as a pre-inoculation treatment because it is not persistent but has the potential to reduce inoculum in an orchard when applied as an early-season treatment.

In summary, none of the new experimental natural product and biocontrol bactericides was as consistently effective as the registered antibiotics. The efficacy of Kasumin was significantly improved in 2022 with the addition of Syllit, and a mixture of Kasumin and

FireWall is one of the top treatments. The restricted use of Kasumin (no more than four applications per season) and recommended use in mixture with another bactericide will help to minimize the risk for resistance development. New formulations of oxytetracycline such as FireLine 45 and NUP-17010 are also providing a high level of efficacy.

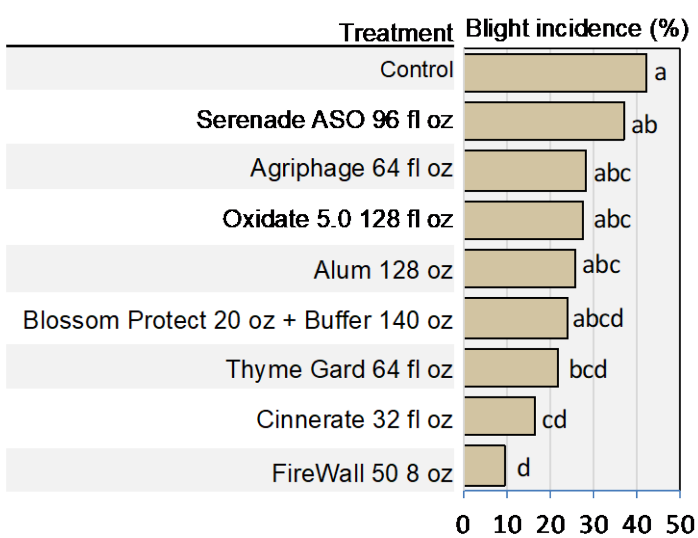
Among new bactericides evaluated, the natural product Cinnerate was the most promising material evaluated. The KFD 623-01 formulation of EPL used by itself was only included in the laboratory study with ornamental pear where it was highly effective. In the Bartlett field study, it was highly effective when mixed with low rates of Kasumin and ManniPlex Zn, and therefore should be continued to be evaluated. Moreover, based on our in vitro studies, a mixture of EPL and cinnamaldehyde has the potential to be highly effective in the field. Additionally, we are collaborating with another agrochemical company in the development of new field formulations for the two food preservatives EPL and nisin.

Fig. 3. Efficacy of new bactericides for management of fire blight of Comice pear after inoculation in a field study at UC Davis 2022



Treatments were applied using an air-blast sprayer on 3-15-22 (20-50% bloom) and 3-22-22. Trees were inoculated with *E. amylovora* after the second application. Blight incidence on 4-6-22 was based on the number of flower/fruit clusters with disease of the total number of clusters evaluated.

Fig. 4. Efficacy of new bactericides for management of fire blight of Shinko apple pears in a field study at UC Davis 2022



Treatments were applied on 3-15, 3-16, and 3-22-22 using an air-blast sprayer. Trees were inoculated with *E. amylovora* after the first and third applications. Blight incidence on 3-30-22 was based on the number of flower clusters with at least one infection of the total number of clusters evaluated.

As in 2021, the new natural antibiotic ningnanmycin (Ninja) showed little efficacy in 2022 although the rate was increased in 2022. Additionally, phytotoxicity developed as marginal leaf burn. The natural product QAM and the FDA GRAS bactericide TDA-NC1 were not effective or were inconsistent in 2021 and 2022 field studies, and therefore are unlikely to be of use under California conditions. Similarly, we also obtained inconsistent results with Alum in several years of trials. The biocontrol Blossom Protect has shown moderate to good efficacy in studies conducted over numerous years. Although it was not very effective in 2022, it still represents one of the best treatments in organically managed orchards and can be used in rotation with Serenade. We will continue to evaluate new experimental bactericides and biocontrols in future studies to potentially identify additional effective options for fire blight management and to provide rotation alternatives for effective anti-resistance programs in conventionally and organically managed orchards.