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

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SUMMARY

1. Antibiotic and copper resistance surveys for populations of *Erwinia amylovora* in California pear growing areas were continued.
   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.
2. FDA-approved food preservatives (e.g., ε-poly-L-lysine, nisin, and lactic acid) were highly toxic to *E. amylovora* in direct-exposure laboratory studies.
3. Field trials on the management of fire blight were conducted under moderate to high disease pressure.

a. The food preservatives ε-poly-L-lysine and nisin by themselves were moderately effective and alginate formulations did not improve efficacy. Citric acid did not improve the efficacy of Kasumin.
b. Among three inhibitors of the type III secretion system of the pathogen, only one resulted in a moderate reduction in the incidence of infected spurs.
c. The foliar fertilizer zinc nitrate resulted in a moderate reduction in fire blight incidence. The biocontrol treatments Serenade ASO and Blossom Protect resulted in a similar moderate, but significant, reduction in disease, but Serifel was not effective. The copper product Cueva was moderately effective.
d. Kasumin by itself and the other two antibiotics FireWall, Mycoshield/FireLine were again the most effective treatments, and the mixture of Kasumin with FireWall resulted in the lowest disease level.

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 often can be eliminated by pruning. Thus, aggressive and regular scheduled 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 phytotoxicity commonly occurs on fruit as russeting. With some newer formulations of copper, however, reduced rates based on metallic copper equivalent (MCE) can be used past the bloom period without causing russeting. Under low disease pressure, copper compounds can provide satisfactory disease control and they can be an effective rotational or mix-partner. In years with high disease pressure, however, copper applications generally fail to control the disease at satisfactory levels. Therefore, in our UCIPM ratings, copper is ranked as a +/++ treatment indicating inconsistent performance depending on environmental conditions. In 2015 and 2016, we also reported reduced sensitivity to copper in strains of *E. amylovora* with growth occurring at 20 to 30 ppm MCE on nutrient agar and 10 to 20 ppm on the low copper-binding CYE agar. These levels indicate moderate copper resistance and can explain the moderate and inconsistent performance of copper. Lack of systemic action and low registered rates are other factors for low efficacy. In 2018, we continued to evaluate copper sensitivity in strains of *E. amylovora,* and copper was included in our efficacy trials.

Treatments with the antibiotics streptomycin and oxytetracycline have been employed for many years to manage fire blight. Continued usage for many seasons and lack of alternative control materials caused resistance against streptomycin to develop at high incidence at many locations in California, mostly in Sacramento Co. starting in 2006. Since then, the incidence of streptomycin resistance has been fluctuating widely among years from very low to very high levels, and this was attributed to disease pressure and the intensity of streptomycin use. Previously, we also detected isolates of *E. amylovora* with reduced sensitivity to oxytetracycline at several locations. At one of these locations, field treatments with oxytetracycline (e.g., Mycoshield) were reported to be ineffective in controlling the disease. These less-sensitive populations were not persistent and were not detected in successive samplings at the same locations. Surveys on antibiotic resistance monitoring were continued in 2018 in collaboration with farm advisors. Resistance monitoring was also done for kasugamycin (Kasumin). After many years of field evaluations and regulatory battles, this third antibiotic became available for use in the 2018 season in California. Concerns have been expressed by regulatory agencies regarding the use of antibiotics in agriculture, but kasugamycin is not used in human and animal medicine and has a different mode of action from streptomycin or oxytetracycline (no cross-resistance).

The incidence of fire blight was variable among California growing regions in the 2018 spring season and was moderate to high at our field trial locations. Our evaluations focused on new biological products that potentially could qualify for organic production for which is a growing interest. Thus, we continued to evaluate ε-poly-L-lysine and nisin. ε-poly-L-lysine is used commercially as a food preservative in Japan, Korea and in imported items in the United States. It has demonstrated antimicrobial activity against yeasts, fungi, Gram-positive, and Gram-negative bacteria (J. Antibacterial Antifungal Agents 1995, 23:349–354). Nisin (Niprosin) is used as a preservative in some processed foods. ε-poly-L-lysine and nisin showed promising results in our fire blight trials in 2017. In 2018, we tried to improve their efficacy by encapsulation in alginate. In the literature (Carbohydr. Polym. 2014, 103:573-80), nisin-alginate encapsulation was reported to improve stability and long-term effectiveness. We also added the UV-protectant zinc oxide to some of these treatments.

In 2018, we also evaluated three experimental inhibitors for the type III secretion system of *E. amylovora* in cooperation with other researchers. We evaluated if the performance of oxytetracycline can be improved by adding a registrant-recommended adjuvant, and if Kasumin efficacy can be enhanced by adding citric acid that has antimicrobial activity. The foliar fertilizer zinc nitrate (Brandt) that was reported to have antibacterial activity was also included in our field studies. Additionally, we continued to evaluate DAS1 (a potential enhancer of copper activity), the natural product 1552, the new biocontrol Serifel (*Bacillus amyloliquifaciens* strain MBI 600), as well as Serenade ASO (a liquid formulation) and Blossom Protect.

OBJECTIVES

1. Determine the sensitivity of *E. amylovora* populations from pear orchards in California to streptomycin, oxytetracycline, kasugamycin, and copper

2. Evaluate new anti-microbials for their toxicity against *E. amylovora* in in-vitro laboratory studies.

a) Zinkicide
b) New SBH derivatives (using selected ratios) in copper mixtures
c) ε-poly-L-lysine, nisin, citric acid, and/or lactic acid

4. Evaluate and optimize the performance of kasugamycin (Kasumin), streptomycin (e.g., Agrimycin-17, Firewall), oxytetracycline (e.g., Mycoshield, Fireline), and new antimicrobials in field trials.
   a) Kasumin in combination with exempt-from-tolerance antimicrobials and other bactericides such as Zinkicide and citric acid.
   b) Large-scale field trials with Kasumin after registration.
   c) Type III secretion system inhibitors
   d) Oxytetracycline formulations in combination with registrant adjuvants.

5. Assist in fungicide sensitivity testing of the pear scab pathogen (*no samples were provided in 2018*).

MATERIALS AND METHODS

Isolation of *E. amylovora* and bacterial culturing. Pear samples with fire blight symptoms were obtained in the spring and early summer of 2018 from 13 orchards in Sacramento Co. 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. A total of 70 strains were obtained and evaluated for their sensitivity to antibiotics and copper.

Laboratory studies on the toxicity of bactericides against *E. amylovora*. Streptomycin, oxytetracycline, and kasugamycin were evaluated for their in vitro toxicity using the spiral gradient endpoint method. For this, a radial bactericidal concentration gradient was established in nutrient agar in Petri dishes by spirally plating a stock concentration of each antimicrobial using a spiral plater. After radially streaking out suspensions of the test bacteria (10 µl of 10^8 cfu/ml as determined by measurement of optical density at 600 nm) along the concentration gradient, plates were incubated for 2 days at 25ºC. Measurements were taken visually for two inhibitory concentrations: i) the lowest inhibitory concentration (LIC; the lowest concentration where inhibition of bacterial growth was observed, i.e., where the bacterial streak became less dense visually), and ii) 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. Sensitivity to oxytetracycline for selected isolates was also evaluated using nutrient agar amended with 0, 20, or 40 ppm of the antibiotic.

Copper sensitivity was determined by streaking bacterial suspensions (70% transmission at 600 nm) on CYE (casitone-yeast extract-glycerol agar) or nutrient agar amended with 0, 10, 20, or 30 ppm MCE. Growth was recorded after 2 days of incubation at 25°C and was rated as ++ (growth not inhibited, similar to control), + (growth inhibited as compared to the control), or - (no growth). The presence of spontaneous mutants that developed as distinct colonies along the streak was noted.

The toxicity of ε-poly-L-lysine, nisin, citric acid, and lactic acid against *E. amylovora* was evaluated in direct contact assays. For this, suspensions of the standard strain E2 were incubated in final concentrations of 1000 ppm of these antimicrobials. ε-poly-L-lysine was also evaluated at 100 ppm, and water was used in control treatments. Mixtures were incubated for 1 h, diluted 1:1000 with sterile water, and aliquots were then plated onto nutrient agar. After 2 days, bacterial colonies were enumerated, and percent reduction as compared to the control was calculated.

Field studies using protective treatments during the growing season. In two trials in a commercial cv. Bartlett orchard in Live Oak, three applications of selected treatments (see Results) were done using a back-pack air-blast sprayer at 100 gal/A between March 28 and April 11. Natural incidence of disease (i.e., infected spurs of 90 spurs evaluated for each tree) was evaluated on April 18. Data were analyzed using analysis of variance and LSD 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 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, possibly the previous seasons' applications 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. 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.

Laboratory studies on the toxicity of bactericides against *E. amylovora*. Direct contact studies with the antimicrobials ε-poly-L-lysine, nisin, and lactic acid demonstrated high toxicity against *E. amylovora* (Fig. 1). Growth of the bacterium was inhibited by $\geq$95% following a 1-h incubation in 100 or 1000 ppm ε-poly-L-lysine, or 1000 ppm nisin or lactic acid. These results support testing these compounds in field efficacy studies.

Field studies using protective treatments during the growing season. Fire blight incidence was moderate to high at our field study sites in 2018. In evaluation of food preservatives, **ε-poly-L-lysine** (Fig. 2) or **nisin** (Fig. 3) by themselves were not highly effective, but still significantly reduced the incidence of infected spurs from that of untreated control trees. In an attempt to improve their efficacy, alginate formulations were prepared. These, however, did not improve efficacy. Due to the highly promising in vitro toxicity results with ε-poly-L-lysine and nisin described above, we will continue our efforts to increase the performance. There are reports that nisin becomes more toxic to Gram-negative bacteria such as *E. amylovora* in the presence of the chelator EDTA, and this will be investigated in 2019. **Citric acid**, another food grade antimicrobial was used in combination with Kasumin, slightly improved the efficacy of this newly registered bactericide (Fig. 2).

The other novel approach to fire blight management that we investigated in 2018 was the use of **inhibitors of the type III secretion system** of the pathogen. Three of these compounds were evaluated in our field studies, and only one of them (TS153) resulted in a moderate reduction of the incidence of infected spurs (Fig. 3). Possibly other inhibitor types are more effective, and therefore will be evaluated if they become available.

In both trials with **zinc nitrate**, a moderate reduction in fire blight incidence was observed (Figs. 2,3). Thus, claims of antibacterial activity of this foliar fertilizer were validated. The biocontrol treatments **Serenade ASO** and **Blossom Protect** resulted in a similar moderate, but significant, reduction in disease, but **Serifel** was not statistically effective compared to the control (Fig. 3). The addition of copper (i.e., **Cueva**) to Serenade did not increase performance of Serenade; Cueva by itself was moderately effective (Fig. 2). Addition of the potential **copper enhancer DAS1**, as in 2017, had no significant effect on copper (i.e., Cueva) efficacy. In our studies in 2017, the copper activity-enhancing compound **ZTD** was a very promising treatment, but current no US-registrant has been identified that would support its registration as a conventional pesticide that will require extensive toxicological and environmental fate testing. For the small size of the bactericide market, this financial investment may not be justified and the registrant in China is not willing to register their product in the United States.

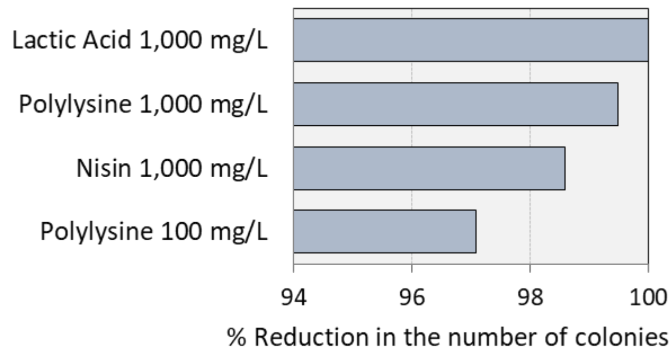
A rotation program with the natural product **1552** and Kasumin was not effective (Fig. 2), although 1552 by itself showed good efficacy in previous years' studies. Kasumin by itself and the other two antibiotics **FireWall, Mycoshield/FireLine** were again the most effective treatments, and the mixture of **Kasumin with FireWall** resulted in the lowest disease level (Fig. 2). The registrant-recommended adjuvant Tactic did not improve the performance of FireLine (Fig. 3), but this does not rule out potential benefits on other cops and diseases. We will continue to investigate ways to improve oxytetracycline and other antibiotics.

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

Location	Strepto-mycin	Oxytetra-cycline	Kasuga-mycin	Copper				Chemical spray program
				CYE	Nutrient agar			
				20 ppm	20 ppm	30 ppm	40 ppm	
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.

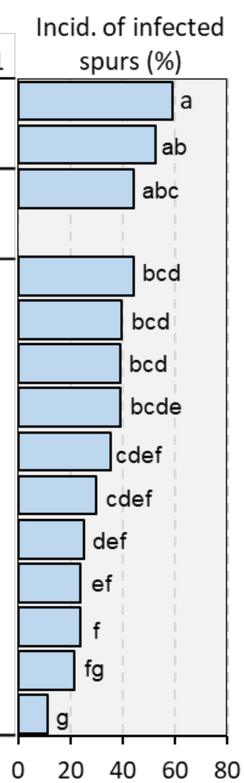
Fig. 1. In vitro toxicity of lactic acid, ε-poly-L-lysine, and nisin against *E. amylovora* in direct contact studies



Suspensions of *E. amylovora* were incubated in the presence of the antimicrobials or water for 1 h. Mixtures were diluted 1:1000 with sterile water, and aliquots were plated onto nutrient agar. After 2 days, bacterial colonies were enumerated, and percent reduction as compared to the control was calculated.

Fig. 2. Evaluation of antibiotics, copper, and biologicals for management of fire blight of Bartlett pear in a field trial in Live Oak, CA 2018

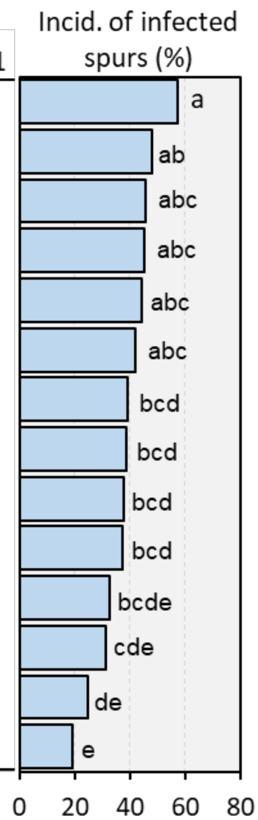
Treatment	Rate	Adjuvant	Applications 3-28	4-3	4-11
Control	---	---	---	---	---
PolyLysine/Alginate	13.5 oz	NuFilmP	@	@	@
Rotation: 1552	28 oz	---	@	---	@
Rotation: Kasumin	64 fl oz	---	---	@	---
Brandt ZnNO3	64 fl oz	NuFilmP	@	@	@
PolyLysine/Alginate + ZnO	13.5 + 32 oz	NuFilmP	@	@	@
Cueva	96 fl oz	---	@	@	@
PolyLysine	13.5 oz	NuFilmP	@	@	@
DAS1 + Cueva	27 + 96 fl oz	---	@	@	@
Kasumin + Polylysine	64 fl oz + 13.5 oz	---	@	@	@
Kasumin	64 fl oz	---	@	@	@
Kasumin + Citric Acid	64 fl oz + 26.5 oz	---	@	@	@
Mycoshield	16 oz	Tactic	@	@	@
FireWall	8 oz	---	@	@	@
Kasumin + FireWall	64 fl oz + 8 oz	---	@	@	@



Applications were done using an air-blast sprayer at 100 gal/A on 3-28 (5% bloom), 4-3 (full bloom), and 4-1-18 (petal fall). NuFilm-P was used at 5 fl oz and Tactic at 8 fl oz/A. There were four single-tree replications per treatment. Disease was evaluated on April 18, 2018.

Fig. 3. Evaluation of biologicals in comparison to an antibiotic for management of fire blight of Bartlett pear in a field trial in Live Oak, CA 2018

Treatment	Rate	Adjuvant	Applications 3-28	Applications 4-3	Applications 4-11
Control	---	---	---	---	---
TS28 10% SL	107 fl oz	Regulaid	@	@	@
Nisin/Alginate	13.5 oz	NuFilmP	@	@	@
Serifel	4 oz	NuFilmP	@	@	@
TS108 20% SL	62.5 fl oz	Regulaid	@	@	@
Nisin/Alginate + Brandt ZnO	13.5 oz + 32 oz	NuFilmP	@	@	@
Serenade ASO + Cueva	64 + 96 fl oz	---	@	@	@
Brandt ZnNO3	64 fl oz	NuFilmP	@	@	@
Serenade ASO	64 fl oz	NuFilmP	@	@	@
Blossom Protect + buffer	20 + 143 oz	---	@	@	@
TS153 10% SL	101 fl oz	Regulaid	@	@	@
Nisin	13.5 oz	NuFilmP	@	@	@
FireLine	16 oz	Tactic	@	@	@
FireLine	16 oz	---	@	@	@



Applications were done using an air-blast sprayer at 100 gal/A on 3-28 (5% bloom), 4-3 (full bloom), and 4-1-18 (petal fall). NuFilm-P was used at 5 fl oz, Tactic at 8 fl oz, and Regulaid at 16 fl oz/A. There were four single-tree replications per treatment. Disease was evaluated on April 18, 2018.