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

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ABSTRACT

1. Antibiotic resistance surveys for populations of *Erwinia amylovora* in California pear orchards were continued with samples submitted from Lake county.
   a. Kasugamycin: 17 strains from 15 orchards were all sensitive.
   b. Streptomycin: 10 strains obtained from 8 locations were sensitive; moderate resistance (MIC $\leq$25 ppm) was found at 4 locations; and high resistance (MIC>100 ppm) at 3 locations. Resistance to streptomycin was found previously in Lake Co. in our surveys in 2006 and 2019. Thus, in 2020 moderate and high resistance has been confirmed in Lake county for the first time. Streptomycin should be used strategically, and our findings stress the importance of resistance management with mixtures or rotations, limiting the number of applications per season (ideally one), and the development of new alternatives.
   c. Oxytetracycline: Strains with high resistance levels ($\geq$100 ppm) were detected for the first time in Sacramento Co. in 2018 and 2019. Two highly resistant strains were detected in Lake Co. in 2020, and these were also highly resistant to streptomycin. Oxytetracycline resistance at this level in *E. amylovora* has never been reported from other locations, and this finding is a serious concern. In pear flower inoculation studies, these resistant strains were virulent, and they were competitive in the presence of sensitive strains in co-inoculations. We initiated studies to characterize the molecular mechanism of resistance.
2. Field trials on the management of fire blight were conducted under moderate natural disease pressure.
   a. The food preservatives ε-poly-L-lysine mixed with Dart and nisin mixed with ManniPlex Zn reduced the natural incidence of blight from 33.1% in the control to 10.5% and 11.8%, respectively. Significant reductions in disease were also obtained after flower inoculations.
   b. The FDA GRAS bactericide TDA-NC1 significantly reduced the natural incidence of disease as compared to the control but was not as effective as the antibiotics. The biocontrol treatment Blossom Protect and the copper product Cueva, however, only numerically reduced the disease in evaluations of natural incidence and incidence after inoculation. The essential oil product Gargoil significantly reduced

the incidence of blight after inoculation, but only numerically reduced the natural incidence.

c. Kasumin continued to perform very well, but streptomycin (FireWall) was significantly more effective after flower inoculation with a streptomycin-sensitive strain. Its restricted use (maximum of two applications per season) and recommended use in mixture with another bactericide will help to minimize the risk for resistance development. In previous studies, the mixture of Kasumin with FireWall generally resulted in the lowest disease incidence.

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 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 fire blight at satisfactory levels. Therefore, in our UCIPM ratings, copper is ranked as a +/++ treatment indicating inconsistent performance depending on environmental conditions. In 2015 - 2018, we 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 also explain the moderate and inconsistent performance of copper. Lack of systemic action and low registered rates are other factors for low efficacy.

Treatments with the antibiotics streptomycin (STR) and oxytetracycline (OXY) have been employed for many years to manage fire blight. Continued usage for many seasons and lack of alternative control materials caused resistance against STR to develop at high incidence 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 streptomycin 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. In 2018 and 2019, however, we detected strains highly resistant to this antibiotic for the first time at three locations in Sacramento Co. In 2020, we characterized these strains for their virulence and competitiveness, and research on the molecular resistance mechanism is ongoing. Surveys on antibiotic resistance monitoring were continued in 2020 in collaboration with farm advisors and PCAs in Lake Co. Resistance monitoring was also done for kasugamycin (Kasumin) for numerous years, but no resistance has been found. This third antibiotic 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 and 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.

Our treatment evaluations in 2019 focused on new natural products that potentially could qualify for organic production for which is a growing interest. We continued to evaluate ε-poly-L-lysine and nisin as non-formulated technical compounds. 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. 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 previously, and we continued our efforts to improve their efficacy using different mixture partners. Other natural products evaluated in 2020 include BacStop, EF400, Gargoil, and ET91, as well as the FDA GRAS bactericide TDA-NC1 that generates chlorine dioxide.

OBJECTIVES

1. Continue antibiotic resistance surveys in *E. amylovora* populations from pear orchards in California for streptomycin, oxytetracycline, and kasugamycin.
2. Evaluate and optimize the performance of kasugamycin (Kasumin), streptomycin (e.g., Agrimycin-17, FireWall), oxytetracycline (e.g., Mycoshield, FireLine), and new copper formulations in field trials.
   a) Kasumin in combination with exempt-from-tolerance antimicrobials (see below).
   b) Oxytetracycline – new formulations (e.g., new Mycoshield) and trials using Fireline with selected adjuvants or stabilizers to increase persistence of residues and overall efficacy.
   c) New natural products, GRAS food additives, and biocontrols
      i. ε-polylysine, nisin, lactic acid in combination with capric/caprylic acids (Dart).
      ii. The new FDA GRAS bactericide TDA-NC and a new plant extract based on clove oil
      iii. 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 of 2020 from 15 pear orchards in Lake 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 17 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. Selected resistant strains were also tested in an agar dilution test using nutrient agar amended with 100 ppm STR or OXY.

Virulence and competitiveness of STR-OXY double-resistant strains of *E. amylovora*. Ornamental pear flowers on the UCR campus were inoculated with single strains or with 1:1 mixtures of sensitive and highly STR-OXY-resistant strains. The incidence of infected flowers was determined after 7 days. *E. amylovora* was re-isolated from 15 flowers of each of three replications. Three isolates per flower were then tested for their sensitivity to OXY, and the percentage of recovery of resistant isolates was calculated.

Molecular characterization of STR-OXY double-resistant strains of *E. amylovora*. The presence of *strA-StrB* genes was evaluated in PCR amplifications using published primers (Appl. Environ. Microbiol. 63:4604,1997) at an annealing temperature of 58C. Primers for tetracycline resistance genes Tet1-F and Tet1-R (Schnabel and Jones, Appl. Environ. Microbiol. 65:4898, 1999) were used at an annealing temperature of 55ºC. Amplification products were separated by agarose gel electrophoresis.

Field studies on the evaluation of new bactericide treatments. In a trial in a commercial cv. Bartlett orchard in Live Oak, treatments (see Fig. 3) were done using an air-blast sprayer at 100 gal/A on 3-18, 3-24, 4-18 (petal fall), and 4-7-20. There were four single-tree replications per treatment. One branch with flowers per tree was inoculated with a STR-sensitive strain of *E. amylovora* on 3-24-20 after the application. Inoculated flowers were evaluated on 4-7-20, and natural incidence was determined on 4-22-20.

New non-antibiotic alternatives were evaluated on cv. Shinko apple pears at UC Davis. Applications were done using an air-blast sprayer at 100 gal/A on 3-18, 3-24, and 4-1-20. One branch with flowers of each tree was spray-inoculated with a STR-sensitive strain of *E. amylovora* (2×10^6 cfu/ml) on 4-2-20. Disease was evaluated periodically, and a final evaluation was done on 4-21-20. For this, the number of spurs with disease (blighted flowers or shoot blight) of the total number of spurs on each inoculated branch was assessed. Natural incidence of fire blight on the rest of the tree and any phytotoxicity were also assessed.

In another study on cv. Comice pear at UC Davis, treatments were applied using an air-blast sprayer on 3-24, 3-31, and 4-7-20. Flowers of one branch per tree were inoculated with a STR-sensitive strain of *E. amylovora* after the third application. Disease was evaluated after 7 days based on the number of diseased flowers. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.4.

RESULTS AND DISCUSSION

Survey of antibiotic sensitivity in *E. amylovora* strains from pear in California in 2020. Samples for our annual resistance monitoring in *E. amylovora* were only made available from Lake Co. in 2020, and most of them consisted of a single infected twig. Thus, only 17 strains were obtained, however, our results are still very important.

For **STR**, 10 strains obtained from 8 orchards were sensitive (Table 1). **Moderate resistance** (MIC $\leq$25 ppm) was found at 4 locations and **high resistance** (MIC>100 ppm) at 3 locations. Resistance to STR was found previously in Lake Co. in our surveys in 2006 and 2019. Thus, in 2020 moderate and high STR resistance was confirmed in Lake county for the first time. Whether this was introduced or newly acquired is currently not know but could be investigated using molecular fingerprinting off isolates. Generally, less favorable environmental conditions for blight and the common practice of using STR-OXY in mixtures, may have delayed the development of resistance in Lake Co. In future surveys in this county, a larger number of samples should be collected (more than one sample per orchard) to determine the extent of resistance more accurately. Our findings indicate that STR should be used strategically in management programs. The importance of resistance management cannot be overstressed. Antibiotics should only be used in mixtures or rotations, the number of applications of each per season should be limited to two, and new alternatives (e.g., early season copper, biologicals) should be used and developed.

Strains with **OXY resistance** levels of >100 ppm were detected for the first time in Sacramento Co. in 2018 and 2019. Two highly resistant strains were detected in Lake Co. in 2020, and these were also highly resistant to STR, similar to the OXY-resistant strains we described from Sacramento Co. Spray programs in these orchards consisted of STR, OXY, and kasugamycin. OXY resistance at this level in *E. amylovora* has never been reported from other locations worldwide, and this finding is a serious concern. Resistance development to oxytetracycline in pathogen populations was considered a low risk due to the ephemeral residues of the antibiotic. Over-reliance on OXY combined with frequent, alternate row applications in short intervals may have contributed to high resistance.

In pear flower inoculation studies, two strains highly resistant to STR and OXY were statistically similarly virulent as a sensitive strain and resulted in 62.1% or 47.1% infected flowers as compared to 56.1% for the sensitive strain (Table 2A). These resistant strains also competed well in the presence of a sensitive strain. Thus, in mixed inoculations, 61.5% or 63.8% of strains recovered from diseased flowers had the high STR-OXY resistance phenotype (Table 2B). Still, it is currently not known if these new resistant strains will persist in the absence of selection pressures (i.e., applications with oxytetracycline and streptomycin).

We started to characterize the molecular mechanism of high STR-OXY resistance. PCR amplifications using primers specific for the previously described *Str*A and *Str*B genes expectedly amplified a fragment from strains moderately resistant to STR (Fig. 1). For these strains, we previously were able to locate these genes on a new plasmid. These primers also amplified a fragment for strains with high STR-OXY resistance, but not for strains that were only highly resistant to STR. High resistance to STR in California is considered to be based on a chromosomal mutation in the ribosomal *rpsL* gene. Thus, a different mechanism of STR resistance appears to be present in the high STR-OXY resistant strains that may be linked to OXY resistance.

Using published primers for tetracycline resistance genes tetA, tetB and tetC, a DNA fragment with the expected length of 293 bp was amplified using primers Tet1-F/Tet1-R in highly STR-OXY-resistant strains of *E. amylovora* (Fig. 2). Sequencing of this fragment resulted in 98% homology with a *tetA or tetB* gene from *Pantoea agglomerans* and other members of the family Enterobacteriacae that was also described from phylloplane bacteria in Michigan apple orchards (Schnabel and Jones1999). Thus, OXY resistance in *E. amylovora* is based on the presence of a previously described *tet* gene. Our next steps will be to localize this gene as well as *StrA-StrB* in our highly STR-OXY-resistant strains. Additional molecular characterization of resistant strains from different areas may provide information if this new resistance evolved several times or if resistant strains were spread among growing areas.

Field studies on the evaluation of new bactericide treatments. In a trial in a commercial Bartlett orchard, 33.1% of flowers of untreated control trees developed fire blight by 4-22-20 (Fig. 3). Environmental conditions were very favorable for disease development. Efficacy differed widely among treatments ranging from not effective to highly effective in evaluations for natural incidence of fire blight. In inoculation studies, 50% of flowers of non-treated trees became blighted, and many treatments were less effective as compared to natural blight incidence evaluations. It is possible that inoculum levels used were too high, or alternatively, orchard environmental conditions were highly favorable after inoculation.

The **<u>food preservatives</u>** ε-poly-L-lysine mixed with Dart (capric and caprylic acids) and nisin mixed with ManniPlex Zn reduced the natural incidence of blight to 10.5% and 11.8%, respectively. ε-poly-L-lysine-Dart and nisin-zinc oxide had shown very good efficacy in 2019 under lower disease pressure. Significant reductions in disease were also obtained after flower inoculations. The **FDA GRAS bactericide TDA-NC1** significantly reduced the natural incidence of disease to 11.3% but was not effective after inoculation. The **<u>biocontrol</u> treatment Blossom Protect** and the **<u>copper product</u> Cueva**, however, only numerically reduced the disease in both evaluations. The **essential oil product Gargoil** numerically reduced the natural incidence of blight but resulted in a significant reduction from the control after inoculation. FireWall, a new formulation of Mycoshield, and Kasumin were the most effective in reducing natural disease incidence.

In a comparative study of biological treatments on 'Shinko' apple-pear, no natural disease developed likely due to low temperatures during the trial period. In evaluation of inoculated branches, 25.7% of spurs of untreated trees developed disease (Table 3). A rotation of Cueva and Blossom Protect resulted in the lowest incidence of blight (i.e., 2.1%). Other treatments that significantly reduced the disease from that of the control included the type III secretion inhibitor TS108 and the chlorine dioxide treatment Aqua-Clear Organic. The non-organic Aqua-Clear formulation was statistically not significantly different from the control. TDA-NC-1, FireWall, Alum, the essential oil product ET91, and TS-28 (another type III secretion inhibitor) were statistical intermediate groups with no significant differences to the control. Phytotoxicity was evident as brown flower petals soon after application for the ET91 treatment. In both Aqua-Clear treatments and TS28, petal browning, early fruit drop, and/or leaf discoloration was present. Phytotoxicity in the FireWall treatment was evident as black streaking on leaves. This study was compromised by cold, dry, and windy environmental conditions during treatment and inoculation times. These treatments should be re-evaluated in another study under more favorable environmental conditions.

In a study on cv. Comice, no natural disease developed, likely due to low springtime temperatures at UC Davis (similar to the apple-pear study). Flowers were inoculated after the third air-blast treatment, and new biological treatments were compared to FireWall. Firewall was highly effective (Fig. 4). The only other treatment that significantly reduced blight incidence was a mixture of Nisin, ε-poly-L-lysine, and ManniPlex Zn. Inoculum levels may have been too high to obtain efficacy of other treatments

In summary, ε-poly-L-lysine and nisin continue to be promising bactericides for management of fire blight. These antimicrobials were evaluated as technical compounds, and this is the reason for our evaluation these materials in combination with multiple adjuvants. In collaboration with an agrochemical company, we are trying to develop agricultural formulations for these compounds to improve their field performance, and we hope to have these available in spring 2021. The recently registered Kasumin continues to perform very well. Its restricted use (maximum of two applications per season) and recommended use in mixture with another bactericide will help to minimize the risk for resistance development. TDA-NC-1 was evaluated for the first time with some promising results. Blossom Protect has performed consistently well over numerous years of our studies but was not effective in 2020. Cool early-spring temperatures may have slowed flower colonization by the biocontrol agent *Aureobasidium pullulans*. We will continue to evaluate new experimental bactericides and biocontrols in future studies to potentially offer additional effective options for fire blight management and to provide rotation alternatives for effective anti-resistance programs.

Table 1. Sensitivity of *E. amylovora* strains from pear orchards in Lake Co. to streptomycin, oxytetracycline, and kasugamycin in 2020

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

Sensitivity to streptomycin, oxytetracycline, and kasugamycin was determined using the spiral gradient endpoint method. S = sensitive, MR = moderately resistant (MIC = <20 ppm), HR = highly resistant (MIC = >100 ppm).

Table 2. Virulence and competitiveness of oxytetracycline-resistant strains of *E. amylovora*

A. Virulence

Inoculation with	Incidence of flower infections (%)
STR^S/OXY^S	56.1 a
STR^R/OXY^R -1	62.1 a
STR^R/OXY^R -2	47.1 a

B. Competitiveness

Inoculation with		Recovery of resistant phenotype (%)
Single strains	STR^S/OXY^S	0
	STR^R/OXY^R -1	94.5
	STR^R/OXY^R -2	100
Mixtures	STR^S/OXY^S + STR^R/OXY^R -1	61.5
	STR^S/OXY^S + STR^R/OXY^R -2	63.8

Ornamental pear flowers were inoculated with single strains or with 1:1 mixtures of sensitive and resistant strains. The incidence of infected flowers was determined after 7 days. *E. amylovora* was re-isolated from flowers, and sensitivity to oxytetracycline was determined.

Table 3. Efficacy of new bactericide treatments for managing fire blight of 'Shinko' apple pears (Asian pears) at UC Davis 2020

No.	Treatment	Rate/A	Applications* 3-18	3-24	4-1	Incidence** %	LSD^	Phyto-toxicity***
1	Control	—	---	—	—	25.7	a	-
2	Aqua-Clear/SureFlow non-org	40 ppm	@	@	@	33.5	a	+
3	TDA-NC-1 + Silwet	20 oz + 1.6 fl oz	@	@	@	23.1	ab	-
4	Firewall	16 oz	@	@	@	16.8	ab	(+)
5	Alum + Regulaid	128 oz + 32 fl oz	@	@	@	15.3	ab	-
6	ET91	0.50%	@	@	@	14.6	ab	+
7	TS-28	25 fl oz	@	@	@	13.5	ab	+
8	Aqua-Clear/Activator CH Org	40 ppm	@	@	@	11.1	b	+
9	TS-108	29 fl oz	@	@	@	10.2	b	-
10 (rotation)	Cueva	128 fl oz	@	—	—	2.1	b	-
	Blossom Protect/Buffer	20 oz + 64 oz	---	@	@			

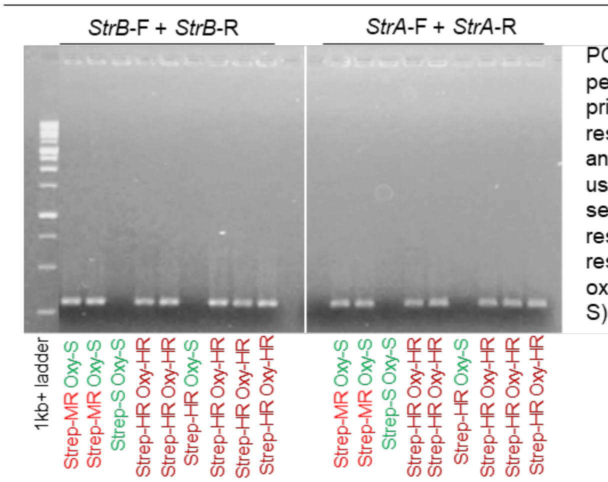
* - Applications were done using an air-blast sprayer at 100 gal/A. One branch with flowers of each tree was spray-inoculated with *E. amylovora* (2 x 10^6 cfu/ml) on 4-2-20.

** - Disease was evaluated on April 21, 2020. The number of spurs with disease (blighted flowers or shoot blight) of the total number of spurs on each inoculated branch was assessed. Natural disease did not develop.

*** - Phytotoxicity in the ET-91 treatments was evident as brown flower petals soon after application. In other treatments, petal browning, early fruit drop, and/or leaf discoloration was present.

^ - Values followed by the same letter are not significantly different based on an analysis of variance and least significant difference (LSD) mean separation (P > 0.05) procedures.

Fig. 1. PCR amplification of *StrA*-*StrB* genes in *E. amylovora* strains with different resistance profiles to streptomycin and oxytetracycline



PCR amplifications were performed using published primers for the two streptomycin resistance genes with a 58C annealing temperature. Isolates used were either streptomycin-sensitive (Strep-S), moderately resistant (Strep-MR), or highly resistant (Strep-HR), and oxytetracycline-sensitive (Oxy-S) or highly resistant (Oxy-HR).

Fig. 2. PCR amplification of a tetracycline resistance gene in *E. amylovora* strains sensitive or highly resistant to oxytetracycline

Amplification using degenerate primers Tet1-F and Tet1-R with a 55ºC annealing temperature (Schnabel and Jones1999)

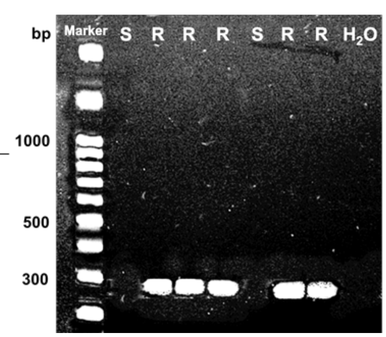
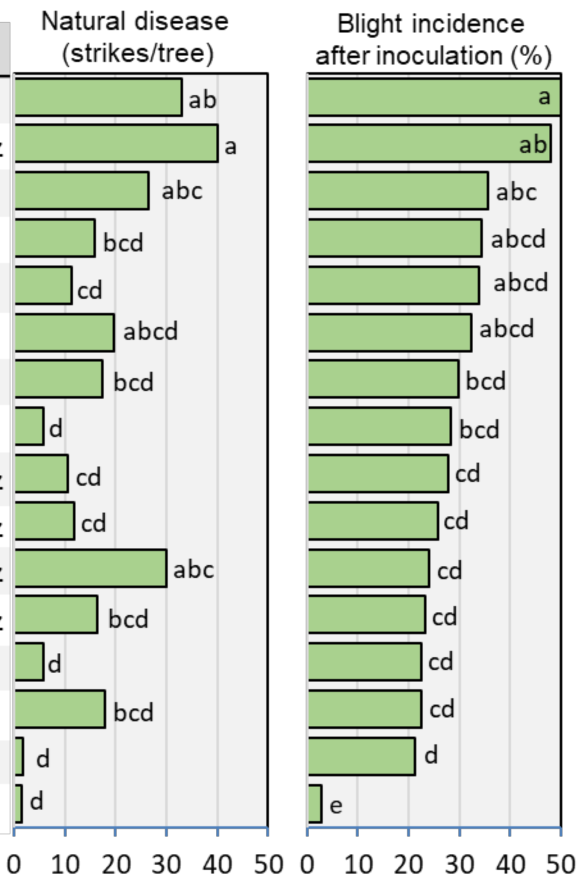


Fig. 3. Efficacy of bactericides for management of fire blight of Bartlett pears, Live Oak, 2020

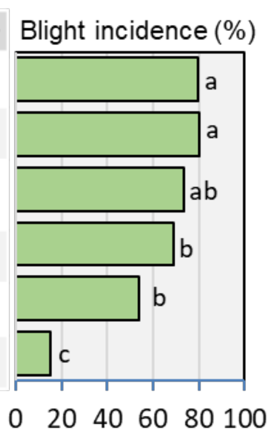
Treatment	Rate/A
Control	---
Nisin + Dart	13.5 oz + 48 fl oz
BacStop + EF400	38 + 38 fl oz
Cueva	64 fl oz
TDA-NC-1 + Silwet	60 oz + 1.6 fl oz
Blossom Protect - buffer	20 oz + 128 oz
Gargoil	96 fl oz
Mycoshield (NUP 17010) + Dart	8 oz + 32 fl oz
ε-poly-L-lysine + Dart	13.5 oz + 48 fl oz
Nisin + ManniPlex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



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

Fig. 4. Efficacy of new bactericides for management of fire blight of Comice pear in a field study at UC Davis 2020

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



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