Annual Report - 2009

Prepared for the California Pear Board

Project Title:	Evaluation of new bactericides for control of fire blight of pears caused by *Erwinia amylovora*
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SUMMARY

1. Population studies of the pathogen in 2009 indicated an intermediate (between years 2007 and 2008) incidence of streptomycin resistance (24.3% of the 177 isolates evaluated including 6.2% highly resistant isolates). Resistance was found in 16 of the 36 locations. Isolates of *E. amylovora* highly resistant to streptomycin and intermediately resistant to oxytetracycline were obtained from the same location as in 2007, indicating their competitiveness.
2. Several field trials were conducted on the management of fire blight with the antibiotics kasugamycin (Kasumin), streptomycin, and oxytetracycline (terramycin, Mycoshield), the biocontrol Actinovate (*Streptomyces lydicus*), the natural product Cerebrocide, the fungicides Kocide 3000, Badge, Syllit, and Dithane, as well as selected mixtures of these materials.
    a. In a small-scale trial where branches with blossoms were treated with bactericides and then inoculated with isolates of *E. amylovora* either sensitive to streptomycin and oxytetracycline or highly resistant to streptomycin/intermediately resistant to oxytetracycline, fire blight caused by the sensitive strain was effectively reduced by all three antibiotics, whereas disease caused by the resistant strain was most effectively reduced by kasugamycin or kasugamycin-Dithane.
    b. In another small-scale trial, treatments with streptomycin and kasugamycin were highly effective in controlling the disease when applied before inoculation with a streptomycin-sensitive isolate of the pathogen. Treatments applied 18 h after inoculation significantly reduced disease incidence by > 66% as compared to the untreated control. Oxytetracycline was the least effective of the three antibiotics as a post-infection treatment.
    c. In an air-blast spray field trial, the new antibiotic kasugamycin continued to be highly effective in reducing the natural incidence of fire blight resulting in a numerically lower disease incidence than for streptomycin or terramycin. Kasugamycin was also very effective in mixtures with Dithane or Syllit.
    d. The copper compounds Badge and Kocide 3000, the natural product Cerebrocide, and the biocontrol Actinovate also significantly reduced the incidence of fire blight.
3. Kasugamycin (Kasumin) registration in the US is being pursued on pome fruit with federal registration expected in 2011 or 2012.
4. The extent of *E. amylovora* colonization inside diseased twigs was determined using species-specific primers in PCR amplifications as compared to standard bacterial isolation. The PCR method was generally more sensitive than the isolation method and the pathogen was detected beyond the canker. We will use this method in more detailed studies in 2010 and the results will help to make recommendations for elimination of the pathogen from diseased trees by pruning.

INTRODUCTION

Fire blight, caused by the bacterium *Erwinia amylovora,* is a very destructive disease of pome fruit trees worldwide, especially pears. In addition to cankers, the pathogen overwinters in flower buds, diseased fruit, small twigs, and branches left on the ground after pruning. In the spring, blossoms are infected through natural openings in nectaries and pistils. After destroying the blossoms, the bacteria spread into the peduncles and spurs. During warm, humid weather ooze droplets consisting of new inoculum are exuded from the peduncles. Young fruitlets often become infected, and they also turn black, dry, shrivel, but usually remain attached to the tree. The disease spreads rapidly and the bacteria invade adjacent leaves through stomata, trichomes, hydathodes, but more frequently through wounds caused by hail or wind whipping. Succulent twigs, suckers, sprouts, and shoots are the next tissues infected. Secondary infections may occur throughout the growing season. Inoculum is spread by wind, rain, insects, birds, or by man, e.g. by means of contaminated pruning tools. Primary and secondary infections may spread into the branch. At this time the infection, if walled off, produces a canker or it penetrates further into the branch and then into the trunk. From here the bacteria may move into other branches and finally the trunk. Trunk cankers will eventually girdle the tree and the whole tree will die. The disease can be very severe in some years, causing repeated infections during warm and wet weather.

Control measures. Fire blight is one of the most difficult diseases to manage. Integrated programs that combine sanitation and orchard management with chemical and biological controls are the best approaches available. If the disease is in its early stage and only a few twigs are blighted, it often can be eliminated by pruning. Thus, aggressive and regular scheduled pruning of diseased tissue is essential for keeping inoculum levels low in an orchard. The exact extent of bacterial colonization from the visible infected tissue, however, is not known and this is critical for determining where branches should be excised to eliminate the pathogen. Thus, in 2009 we initiated studies on the molecular detection of *E. amylovora* in woody tissues.

Current chemical control programs for fire blight control are based on protective schedules, because available compounds are contact treatments and are not systemic. Copper compounds have been used since the early 1900s, mostly in the form of copper sulfate plus lime (Bordeaux mixture). Control with copper compounds is only satisfactory when disease severity is low to moderate. On Bartlett (summer) pears, copper treatments are widely used only during dormant and bloom periods because phytotoxic effects commonly occur on fruit as russeting. New formulations of copper, however, allow for reduced rates of metallic copper equivalent (mce) and thus, extended usage past the bloom period may provide an effective rotational treatment without causing russeting. The antibiotic streptomycin came into general commercial use during the late 1950s, followed by the less effective oxytetracycline (terramycin). Because of the lack of alternative control materials, these antibiotics are still being used commercially, although pathogen resistance against the antibiotic streptomycin is widespread. In our antibiotic resistance surveys in recent years, we detected fluctuations in the incidence of streptomycin resistance, correlating with low- (reduced number of antibiotic applications) and high-disease (higher number of antibiotic applications) years. We also detected isolates of *E. amylovora* with reduced sensitivity to oxytetracycline at several locations. At one of these locations field treatments with Mycoshield were reported to be ineffective in controlling the disease and thus, field resistance has occurred in some locations. Furthermore, concerns have been expressed by regulatory agencies regarding the use of antibiotics in agriculture that are also used in human medicine.

New, more effective materials for fire blight control with a different mode of action from currently used bactericides have to be developed to combat this destructive disease. These could then be incorporated into a resistance management program. During the past years we evaluated numerous compounds that either were not effective, were inconsistent in their efficacy, or were effective, but were not further developed because of usage concerns (antibiotic classes that are important in human medicine). The antibiotic kasugamycin (Kasumin) showed very promising results in our 2004-2008 field trials with an efficacy equal to terramycin. The antibiotic kasugamycin is not used in human and animal medicine. Kasugamycin has a different mode of action from streptomycin or terramycin and there is no cross-resistance known to occur. IR-4 residue studies were done with this antibiotic on pear and apple to allow registration on the pome fruit crop group.

In 2009 we conducted additional field experiments for the evaluation of new potential fire blight control treatments including chemical and biological control treatments. We evaluated the antibiotic kasugamycin alone and in mixtures or rotations with other antibiotics or fungicides. We initiated trials with a new formulation copper hydroxide, Kocide 3000 and Badge that use less copper (Kocide: 0.5 lb/A*0.3 mce=0.15 lb mce/A; Badge: 8 fl oz/A*2.27 lb mce/gal= 0.14 lb mce/A). We have been successful in using these materials at reduced rate for the management of walnut blight. Additionally, we are evaluating the bactericidal effects of dodine (Syllit) and mancozeb (e.g., Dithane, Manzate, Penncozeb, etc.) by themselves and in mixtures with kasugamycin. Additive effects of bactericides mixed with EBDC have been reported for other bacterial pathogens. We are also evaluating rotational and mixture programs with copper, fungicides (dodine, mancozeb), and antibiotics to optimize efficacy and prevent resistance developing in pathogen populations by constantly changing the active ingredient during the season.

We also continued to evaluate new biological controls and natural products. Thus, in our 2009 studies we included the biocontrol Actinovate (*Streptomyces lydicus*) and the natural product Cerebrocide.

OBJECTIVES

1. Evaluate the efficacy of the antibiotic kasugamycin (Kasumin) as compared to streptomycin or oxytetracycline (terramycin or Mycoshield) in cooperation with UCCE.
    a. Laboratory in vitro tests to evaluate the bactericidal activity with and without adjuvants: Spiral gradient dilution and direct contact assays.
    b. Small-scale hand-sprayer tests using different treatment-inoculation schedules.
    c. Field trials with protective air-blast spray treatments at several locations. Adjuvants, fungicides (mancozeb, dodine, and new formulations of copper, e.g., Kocide 3000), product rates, timings, and rotations will be evaluated.
2. Determine the distribution of streptomycin- or terramycin-sensitive and –resistant isolates of *E. amylovora* in pear orchards in California (continuation of 2006-08 surveys)
3. Evaluate the efficacy of integrated programs using copper, fungicides, antibiotics and biological controls (e.g., Actinovate, Bloomtime Biological FD Biopesticide, etc.).
    a. Laboratory in vitro tests to evaluate the bactericidal activity with and without adjuvants: Direct contact assays and amended agar assays.
    b. Selected rotations and mixtures to prevent the selection of resistance in pathogen populations.
4. Localize the presence of *E. amylovora* inside woody tissues proximal of cankers and tissue discoloration using molecular methods (*this objective was a late addition from the original proposal*).

MATERIALS AND METHODS

Isolation of* E. amylovora*, bacterial culturing, and verification of species identity. Pear samples (blossoms and twigs) with fire blight symptoms were obtained in the spring and early summer of 2009 from orchards in the main pear-growing areas in central and northern California (i.e., Lake, Sacramento, and Yuba-Sutter Co.). Infected plant tissue was macerated in sterile water and aliquots of the suspension were streaked onto nutrient agar. Single bacterial colonies were transferred. Initially, identification of *E. amylovora* was based on cultural appearance on nutrient agar and yeast extract-dextrose-calcium carbonate (YDC) agar. All isolates were further verified for their identity using primers specific for the ubiquitous *E. amylovora* plasmid pEA29 as described by Bereswill et al. (Appl. Environ. Microbiol. 58:3522-2536). The presence of a 1-kb DNA fragment after gel electrophoresis confirmed a positive identification.

***Laboratory studies on the toxicity of bactericides against* E. amylovora.** Kasugamycin (Kasumin 2L, Arysta Life Sciences, Cary NC), streptomycin (Sigma, St. Louis, MO), and oxytetracycline (Sigma) were evaluated for their in vitro toxicity using the spiral gradient dilution method. For this, a radial bactericidal concentration gradient was established in nutrient agar media in Petri dishes by spirally plating out a stock concentration of each antimicrobial using a spiral plater (Autoplate 4000; Spiral Biotech, Inc., Norwood MA). 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. Minimum inhibitory concentrations (MICs) were obtained from radial distances (measured from the center of the plate) of inhibition using the Spiral Gradient Endpoint computer program (Spiral Biotech, Inc.).

***Field studies using protective treatments during the growing season*.** In small-scale field tests on cvs. Hosui and Shinseki Asian pear at UC Davis, selected treatments (see Results) were applied to run-off to open blossoms using a hand sprayer. After air-drying, blossoms were spray-inoculated with an isolate of *E. amylovora* sensitive to streptomycin, oxytetracycline, and kasugamycin; or with an isolate highly resistant to streptomycin and with reduced sensitivity to oxytetracycline. Branches were bagged overnight and disease was evaluated after 7 days. In another test, branches of cv. Bartlett pear were treated with the three antibiotics 18 h after inoculation with a streptomycin-sensitive isolate of *E. amylovora* (10^6 cfu/ml) to evaluate the post-infection activity of the treatments. Disease incidence was based on the number of infected blossoms per 40-100 blossoms evaluated.

In a field study in a commercial Bartlett orchard in Live Oak, three applications of selected treatments (see Results) were done at 80% bloom (Mar 23), full bloom (Mar 31), or petal fall (April 7) using a back-pack air-blat sprayer at 100 gal/A. The number of shoot infections per tree was evaluated on April 15, 2009, and data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.1.

Localization of* E. amylovora *inside infected woody tissues using molecular methods. Samples were collected from a Bartlett orchard in early June. Twigs (<1 cm in diameter) with fire blight symptoms were first cut between the canker and healthy appearing area. Sections were made in 5-cm increments starting from the canker margin towards the healthy tissue. Tissue sections between these increments were used for analysis. Half of each increment sample was used for bacterial isolation on standard agar media and *E. amylovora* was identified based on cultural morphology. The other half of the sample was used for DNA extraction. Primers specific for *E. amylovora* based on the ubiquitous pEA29 plasmid (Russo et al., 2008) were used in PCR amplifications to detect the pathogen. PCR products were separated by agarose gel electrophoresis and DNA fragments were visualized by UV light after ethidium bromide staining.

RESULTS AND DISCUSSION

***Isolation of* E. amylovora *and studies on the toxicity of antibiotics*.** Isolates of *E. amylovora* were confirmed for species identity by PCR amplification of a 1-kb DNA fragment using specific primers for a plasmid that is ubiquitously found in this bacterium. A total of 177 isolates from 36 California pear orchard locations (1 to 17 isolates per location) were subsequently tested for their sensitivity against the antibiotics streptomycin, oxytetracycline, and kasugamycin. For kasugamycin, there was again a wide sensitivity range among isolates, but all isolates were determined to be sensitive to kasugamycin (Fig. 1).

For streptomycin, among the 170 strains evaluated, 43 (=24.3%) were resistant (Table 1). Among these 43 isolates, 11 isolates from three locations in Yuba-Sutter Co. were highly resistant (minimum inhibitory concentrations >50 ppm as compared to 3.75 - 16.22 ppm for resistant isolates and 0.143 – 0.481 ppm for sensitive isolates). In these same locations, highly resistant isolates were detected in previous years. Spray records from these orchards indicated that streptomycin (after years of intensive use) was applied only once since 2002. Thus, these highly resistant isolates appear to be well adapted and competitive.

Annual fluctuations of the incidence of streptomycin resistance are shown in Table 2. There was a high incidence of resistance during the high-disease seasons of 2006 (49.6% of the isolates, 70.8% of the locations) and 2007 (69.6% of the isolates, 94.7% of the locations), a low incidence of resistance during the low-disease season of 2008 (7.3% of the isolates, 13% of the locations), and an increase in resistance again during the 2009 season (24.3% of the isolates, 44.4% of the locations) when environmental conditions were more favorable for disease development. In high-disease seasons, more antibiotic sprays are being applied, thus, increasing the selection pressure on the pathogen. Our data indicate that most isolates of *E. amylovora* resistant to streptomycin (except the highly resistant ones – *see above*) appear to be less fit as compared to sensitive isolates and the pathogen population is replaced by sensitive isolates in the absence of selection pressure. This information is very useful for the implementation of resistance management strategies. It implies that at locations with a high incidence of resistance against streptomycin, the incidence can possibly be reduced if more rotational treatments are available, making this important management tool more effective again. This emphasizes the need for registration of new bactericides. The apparent difference in competitiveness between streptomycin-resistant and highly resistant isolates is interesting and needs to be further investigated.

All isolates sensitive or resistant to streptomycin were found to be sensitive to oxytetracycline (Table 1) and sensitivity levels were in a similar range as in previous years for sensitive isolates (Fig. 1). Isolates highly resistant to streptomycin, as in previous years, however, had a reduced sensitivity to oxytetracycline. Thus, in these three orchard locations where these latter isolates were collected, the streptomycin-highly resistant/oxytetracycline reduced sensitivity population has been stable for three seasons and apparently has displaced most of the sensitive wild-type population (only resistant isolates were collected from these locations). Thus, new materials for fire blight control have to be developed in order to initiate resistance management practices that ensure that resistance to oxytetracycline will not spread in the pathogen population.

Field studies using pre-infection (protective) or post-infection treatments during the growing season. In a small-scale study on Bartlett pear on the post-infection activity of the three antibiotics, 82.5% disease incidence was observed in the untreated control (Fig. 2). The kasugamycin and streptomycin treatments reduced the disease by > 66%; whereas the terramycin treatment was the least effective. Thus, kasugamycin was similar in post-infection activity as streptomycin (similar to 2008 data) and even under high disease incidence.

In small-scale field tests where blossoms of Asian pear were hand-sprayed with bactericides and then inoculated, streptomycin, terramycin, kasugamycin, and a mixture of kasugamycin with Dithane were equally highly effective on cv. Hosui when inoculation was done with an isolate of *E. amylovora* that was sensitive to streptomycin and oxytetracycline (Fig. 3A). On cv. Shinseki, Mycoshield resulted in the lowest disease incidence, followed by kasugamycin-Dithane, kasugamycin, and then streptomycin (Fig. 3B).

When inoculation was done with an isolate highly resistant to streptomycin and intermediately resistant to oxytetracycline, on both cultivars the incidence of disease was similar for the control and the streptomycin treatment. Kasugamycin and kasugamycin-Dithane resulted in the lowest disease incidence. Mycoshield had an intermediate efficacy and was less effective than when inoculation was done with the sensitive isolate of the pathogen. This indicates that under field conditions treatments with Mycoshield are less effective in the presence of isolates intermediately resistant to oxytetracycline. This correlates with observations from the location where these less sensitive isolates originated from, that Mycoshield treatments were not effective in managing fire blight. Thus, this study demonstrates the high efficacy of kasugamycin in controlling fire blight caused by diverse pathogen populations.

In another small-scale trial on cv. Shinko, treatments with streptomycin were highly effective (no disease observed) in controlling the disease when applied before inoculation with a streptomycin-sensitive isolate of the pathogen. Treatments applied 24 h after inoculation reduced disease incidence by 71% as compared to the untreated control (100%). Thus, the post-infection activity of this antibiotic was demonstrated and we will conduct similar studies with the other two antibiotics in 2010.

In an air-blast spray field trial on Bartlett pear, all treatments significantly reduced the incidence of disease from that of the control and the efficacy of most treatments could not be statistically separated (Fig. 4). Treatments with kasugamycin, kasugamycin-Dithane, or kasugamycin-Syllit numerically resulted in the lowest disease incidence. the rotation of Mycoshield-Syllit, kasugamycin-Dithane, and streptomycin-Dithane numerically was not as effective, possibly because the most effective of the three rotation treatments (i.e., Kasugamycin-Dithane) was not applied at the time when most infections occurred. The copper compounds Badge and Kocide 3000, the natural product Cerebrocide, and the biocontrol Actinovate also significantly reduced the incidence of fire blight. Overall, numerically Actinovate had the highest disease incidence in this trial.

In summary, our field trials in 2009 again indicate that kasugamycin is a highly effective treatment against fire blight of pear that can be used in resistance management programs with mixtures and rotations. No phytotoxicity was observed after three consecutive applications at 100 ppm. In our previous studies, phytotoxicity was very low after 5 to 6 applications and it was negligible when kasugamycin was used in rotation with other compounds. Arysta Life Sciences Corp. is supporting registration of the material for agricultural use in the United States and we are working closely with this company to proceed with the process. In Sept. 2005 the US-EPA granted an import tolerance for kasugamycin on some agricultural crops and IR-4 residue studies were done on pear in 2006 and on apple in 2007. Federal registration of kasugamycin for management of fire blight is expected in 2011 and is timely because with the first occurrence of populations of *E. amylovora* with reduced sensitivity to oxytetracycline further selection of the pathogen population and spread of resistance has to be prevented. Mixture partners for kasugamycin and the registered antibiotics need to be continued to be evaluated to maximize the efficacy of treatments and as part of a resistance management program. This should especially be initiated from the onset of introduction of kasugamycin. Thus, identification of integrated fire blight programs with copper, fungicides, and antibiotics is successfully being pursued for the California pome fruit industries.

Localization of* E. amylovora *inside infected woody tissues using molecular methods. DNA was extracted from sections of infected pear twigs and *E. amylovora* was localized by PCR amplifications using species-specific primers. The sensitivity of detection overall was higher using this molecular method than the bacterial isolation (Fig. 5). Of the ten twigs sampled, the pathogen was detected by isolation only in one sample. For three samples, the same level of detection was obtained using both methods. For six samples, the pathogen was detected further into the healthy appearing tissue by the molecular method (thus, this method was more sensitive). The extent of pathogen colonization from the canker margin that could be detected was 15 cm. We will use this method in more detailed studies in 2010 using branches of different sizes. The results will help to make recommendations for elimination of the pathogen from diseased trees by pruning.

Table 1. Distribution of isolates of *Erwinia amylovora* sensitive or less sensitive to streptomycin or oxytetetracycline in a survey of 36 California pear orchards in 2009

No.	County	No. isolates	Incidence Steptomycin Resistance (%)	Incidence Oxyteracycline Resistance (%)
1	Yuba-Sutter	4	100*	100*
2	Yuba-Sutter	6	100*	100*
3	Yuba-Sutter	1	NA,*,**	NA
4	Yuba-Sutter	2	0	0
5	Yuba-Sutter	5	0	0
6	Yuba-Sutter	2	0	0
7	Yuba-Sutter	4	0	0
8	Lake	1	NA	0
9	Lake	1	NA	0
10	Lake	1	NA	0
11	Lake	1	NA	0
12	Lake	1	NA	0
13	Lake	1	NA	0
14	Lake	1	NA	0
15	Sac	5	100	0
16	Sac	6	83.3	0
17	Sac	17	58.8	0
18	Sac	4	50	0
19	Sac	9	44.4	0
20	Sac	10	40	0
21	Sac	8	37.5	0
22	Sac	9	28.6	0
23	Sac	8	28.6	0
24	Sac	4	25	0
25	Sac?	9	22.2	0
26	Sac	5	20	0
27	Sac	13	15.4	0
28	Sac	3	0	0
29	Sac	8	0	0
30	Sac	7	0	0
31	Sac?	7	0	0
32	Sac?	3	0	0
33	Sac	1	NA	0
34	Sac	1	NA	0
35	Sac	1	NA	0
36	unknown	8	0	0
Total		177		

* - Inhibitory concentrations were determined on nutrient agar using the SGD method. Minimum inhibitory concentrations (MIC) of isolates sensitive to streptomycin were 0.143-0.481 ppm; whereas MIC of isolates resistant to streptomycin were 3.75-16.22 ppm.

**- Minimum inhibitory concentrations of isolates highly resistant to streptomycin was >50 ppm; whereas for oxytracycline was > 0.51 (0.82-1.03).

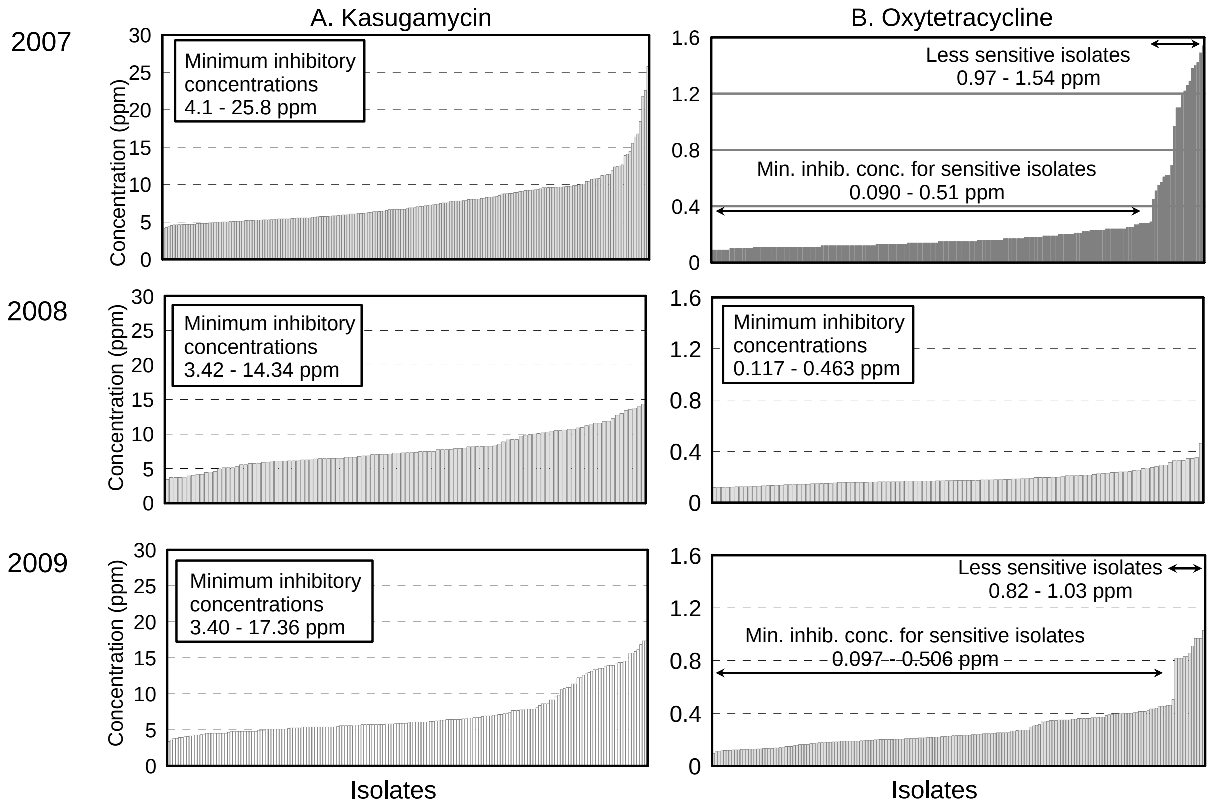
** - NA or not applicable. The incidence of streptomycin or oxytetracycline resistance is only indicated for samplings with more than 1 isolate.

Table 2. Incidence of streptomycin resistance among isolates of *Erwinia amylovora* in surveys of California pear orchards 2006-2009*

Year	Incidence (%) (based on total number of isolates)	Incidence (%) (based on number of orchards sampled)
2006	49.6	70.8
2007	69.6	94.7
2008	7.3	13
2009	24.3	44.4

* - Inhibitory concentrations were determined on nutrient agar using the SGD method.

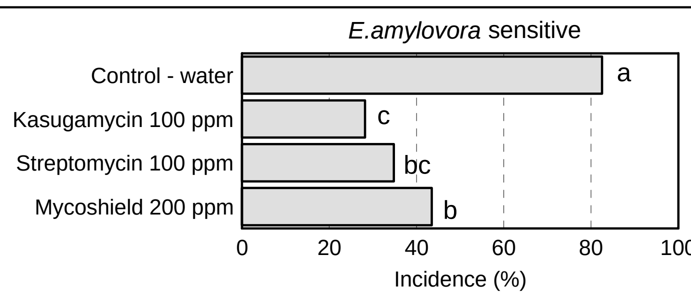
Fig. 1. *In vitro* sensitivity of isolates of *Erwinia amylovora* to kasugamycin and oxytetracycline 2007 - 2009



Inhibitory concentrations were determined on nutrient agar using the SGD method. The minimum inhibitory concentration is the lowest concentration of bactericide where a reduction of bacterial growth is observed. The total number of isolates was 190, 109, and 177 for 2007, 2008, and 2009, respectively.

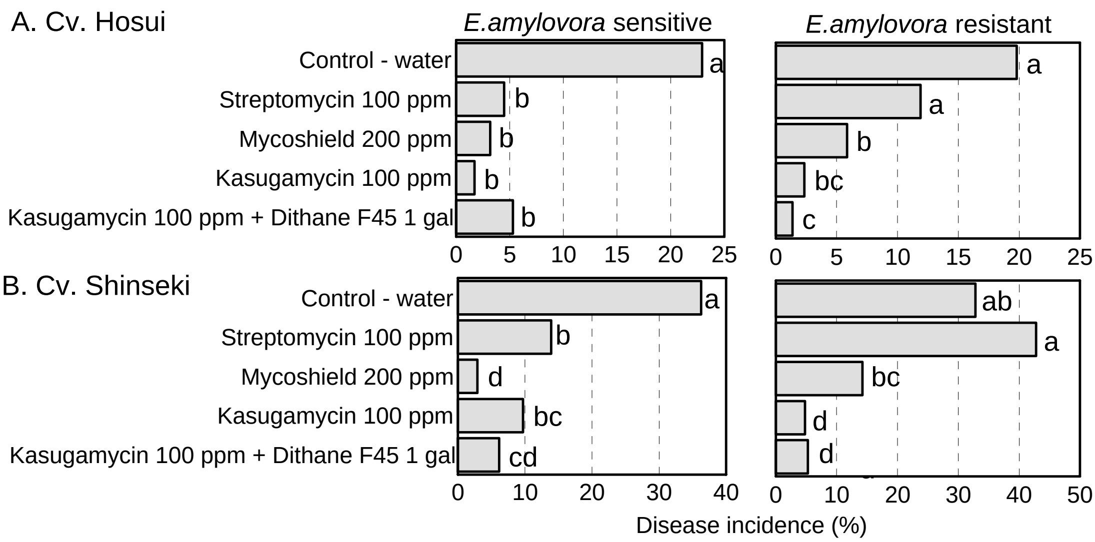
Fig. 2. Efficacy of three antibiotics for fire blight management onBartlett pear in a small-scale field test at UC Davis 2009

- Post-infection activity of treatments -



Blossoms were sprayed inoculated with a streptomycin-sensitive isolate of*E. amylovora*. Treatments were applied after 18 h using a hand-sprayer. Disease incidence was based on the number of infected blossoms per 50-100 blossoms evaluated.

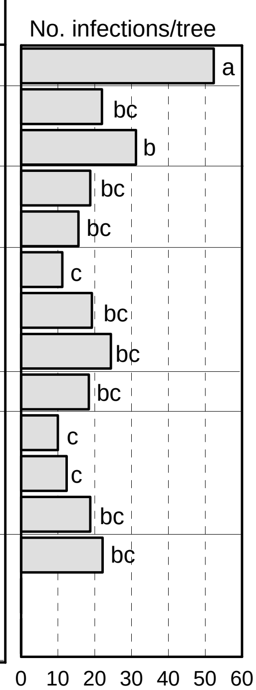
Fig. 3. Efficacy of three antibiotics for fire blight management on Asian pear in a small-scale field test at UC Davis 2009



Treatments were applied to run-off to clusters of blossoms using a hand sprayer. After air-drying (ca. 1 h), blossoms were then spray-inoculated with an isolate of *E. amylovora* sensitive to streptomycin, oxytetracycline, and kasugamycin; or with an isolate highly resistant to streptomycin and with reduced sensitivity to oxytetracycline. Branches were bagged overnight and disease was evaluated after 7 days. Disease incidence was based on the number of infected blossoms per 40-100 blossoms evaluated.

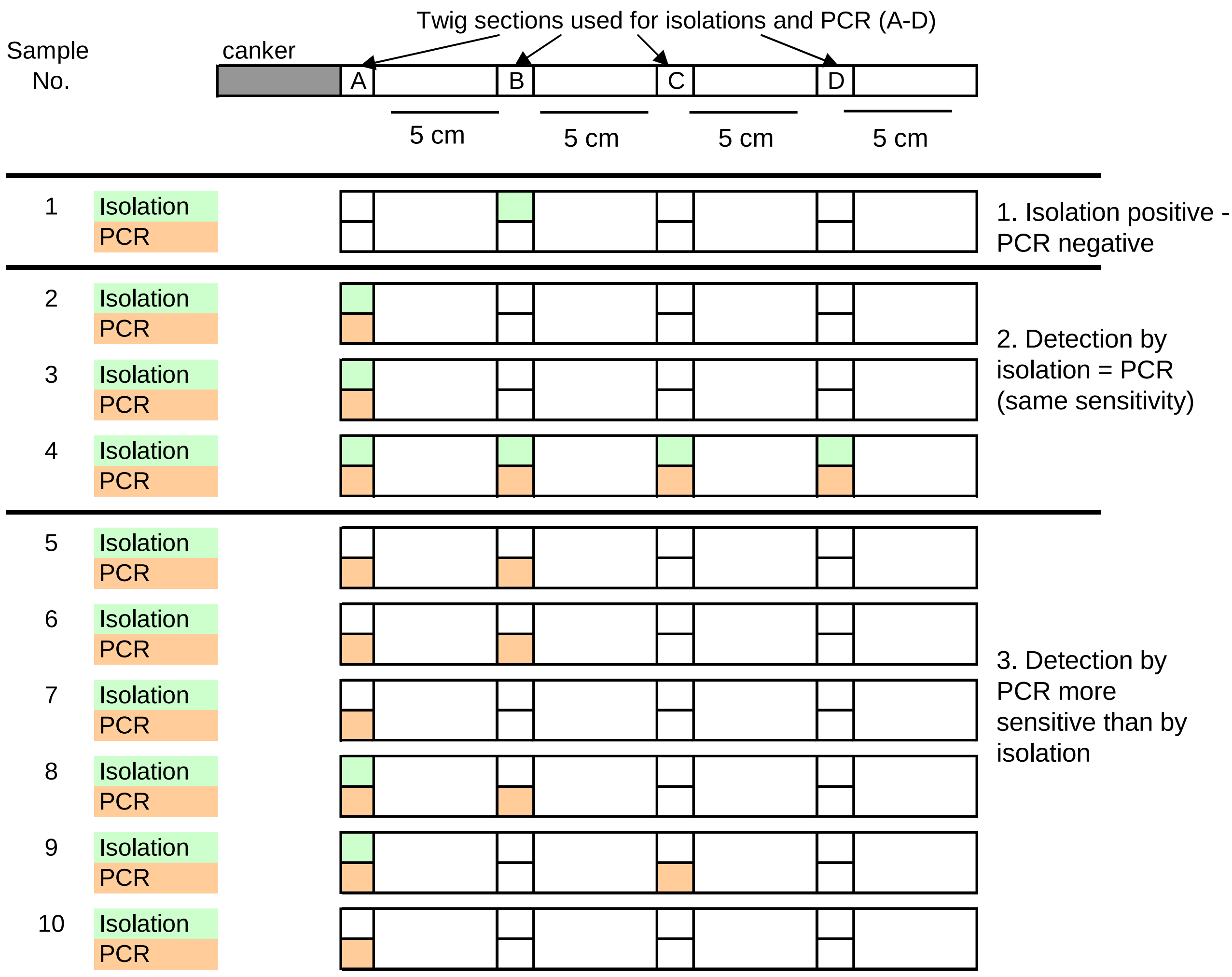
Fig. 4. Evaluation of new bactericides for fireblight management on Bartlett pears in a field trial in Live Oak CA - 2009

	Treatment	80% bloom (3-23)	Full Bloom (3-31)	Petal Fall (4-7)
	Control	---	---	---
Natural product and biocontrol	Cerebrocide 0.5%	@	@	@
	Actinovate 12 oz + Breakthru 2 fl oz	@	@	@
Coppers	Kocide 3000 8 lb	@	@	@
	Badge 8 fl oz	@	@	@
Antibiotics	Kasumin 2L 100 ppm	@	@	@
	Streptomycin 100 ppm	@	@	@
	Mycoshield 200 ppm	@	@	@
Fungicide	Dithane 75DF 6 lb	@	@	@
Mixtures	Kasumin 2L 100 ppm + Dithane 6 lb	@	@	@
	Kasumin 2L 100 ppm + Syllit 2 pts	@	@	@
	Kasumin 100 ppm + Streptomycin 100 ppm	@	@	@
Rotation of mixtures	Mycoshield 200 ppm + Syllit 2 pts	@		
	Kasumin 2L 100 ppm + Dithane 6 lb		@	
	Streptomycin 100 ppm + Dithane 6 lb			@



Treatments were applied using an air-blast sprayer at 100 gal/A. Disease was evaluated on 4-15-09 and the number of fireblight strikes for each of the 6-7 single-tree replications was counted.

Fig. 5. Localization of *E. amylovora* in infected Bartlett pear twigs based on bacterial isolation and using species-specific PCR primers



Samples were collected from a Bartlett orchard in early June. Twigs (<1 cm in diameter) with fire blight symptoms were first cut between the canker and healthy appearing area. Sections were made in 5-cm increments starting from the canker margin to the healthy tissue. Tissue sections between these increments were used for bacterial isolation and DNA extraction.