Annual Report - 2011

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. The incidence of fire blight was low at most locations in 2011 due to cool springtime temperatures and low rainfall during bloom. Population studies of the pathogen indicated an intermediate incidence of streptomycin resistance with resistance present in 5 of 10 locations. Only moderately resistant strains of *E. amylovora* were found. No strains less sensitive to oxytetracycline or kasugamycin were obtained in the 2011 survey.
2. Two air-blast field trials were conducted on the management of fire blight. The following products were evaluated: the antibiotics kasugamycin (Kasumin, two formulations), streptomycin (Firewall), and oxytetracycline (Fireline, Mycoshield); copper hydroxide/copper oxychloride (Badge X2); the fungicide Quintec; the fermentation product polyoxin-D (Ph-D); the biocontrols *Streptomyces lydicus* (Actinovate) and *Aureobasidium pullulans* (Blossom Protect); and the natural products Cerebrocide, Proalexin, and Citrox.
    a. The new antibiotic kasugamycin continued to be highly effective in reducing the incidence of fire blight after inoculation and the natural incidence resulting in a numerically lower disease incidence than streptomycin or oxytetracyline. Kasugamycin was also very effective in mixtures with streptomycin, Quintec, or Actinovate.
    b. Badge, Cerebrocide, Blossom Protect, Actinovate, and Ph-D also significantly reduced the incidence of disease after inoculation.
    c. In laboratory studies, the inhibition of growth of *E. amylovora* was additive for mixtures of kasugamycin with captan, mancozeb, or dodine.
3. Kasugamycin (Kasumin) registration in the United States is being pursued on pome fruit with a California registration expected in 2012.
4. Studies on the molecular mechanism of streptomycin resistance in *E. amylovora* revealed a point mutation in the chromosomal *rps*L gene in highly resistant isolates. Moderately resistant isolates contained the *strA-strB* genes on transposon Tn*5393* as previously shown for isolates from other locations. Preliminary studies indicated that the resistance genes of California isolates were not located on plasmids previously shown to harbor resistance (i.e., pEa29, pEa34), but on another plasmid, pEU30.

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 infecting the blossoms, the bacteria grow into the peduncles and spurs. During warm and 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. After invading blossoms, the bacterial pathogen can invade adjacent leaves through stomata, trichomes, hydathodes, and 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 develop 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.

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 based on the metallic copper equivalent (MCE) and thus, extended usage past the bloom period may provide an effective rotational treatment or mix-partner without causing russeting. The antibiotic streptomycin came into general commercial use during the late 1950s, followed by the less effective oxytetracycline (terramycin). Because of lack of alternative control materials, these antibiotics are still being used commercially, although pathogen resistance against 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. In follow-up experiments we demonstrated that these strains could not be effectively controlled using oxytetracycline. Thus, documented field resistance has occurred in some locations.

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 where compounds are rotated and mixed. The most effective alternative treatment that we identified during the past years with an efficacy equal to streptomycin and oxytetracycline is the antibiotic kasugamycin (Kasumin). Although concerns have been expressed by regulatory agencies regarding the use of antibiotics in agriculture that are also used in human medicine, kasugamycin is not used in human and animal medicine and has a different mode of action from streptomycin or oxytetracycline (no cross-resistance). Through our efforts, registration of Kasumin in California is expected in 2012 and we have recommended an emergency Section 18 registration to allow usage in the 2012 growing season.

A high efficacy of kasugamycin was again observed in our field trials in 2011. We tested a new formulation of the compound (i.e., 8L) that is more concentrated than the previous 2L formulation (easier to use on a commercial base) and that has better storability that the 10L formulation that we evaluated last year. Kasumin was applied by itself in 2011 and in mixtures with selected other materials, including biological treatments. We also included additional new materials in our field evaluations in 2011 to find new alternatives that can be used in rotation programs. Among these materials were the biocontrols Actinovate (*Streptomyces lydicus*) and Blossom Protect (*Aureobasidium pullulans*), the natural products Cerebrocide and Citrox + ProAlexin, the fermentation product polyoxin-D (Ph-D), as well as the fungicide quinoxyfen (Quintec) that was shown to have antibacterial activity by us in the management of bacterial spot of tomato and by others for selected bacterial diseases. Additionally, we evaluated the reduced MCE copper compound Badge in a program with four consecutive sprays.

In previous years' field trials we demonstrated that some fungicides (e.g., captan, mancozeb, and dodine) can significantly reduce the incidence of fire blight. In mixtures with kasugamycin, the efficacy was often increased. Laboratory studies were conducted in 2011 to find out if this increase in efficacy is based on synergism or additive activity.

In another objective of our project we are investigating the molecular mechanism of streptomycin resistance in California isolates of *E. amylovora*. Several mechanisms have been described for isolates of the pathogen from various locations. For California isolates, the main mechanism reported to date is a point mutation in the chromosomal *rps*L gene that confers a high level of resistance. For three isolates with moderate levels of resistance, the streptomycin resistance genes *StrA* and *StrB* were found to be located on plasmid pEa8.7 that closely resembles the broad-host-range plasmid RSF1010 (Palmer et al., Appl. Envir. Microbiol. 63:4604-4607, 1997). Among streptomycin-resistant isolates collected in Michigan, the chromosomal point mutation was rare and for the majority of isolates, *StrA* and *StrB* were found to be on transposon Tn*5393* that is integrated into either plasmid pEa34, or more recently, in plasmid pEa29 (the ubiquitous nonconjugative plasmid). Less commonly, the genes were found to be integrated in the bacterial chromosome. In 2011, we continued to analyze streptomycin resistance mechanisms in California isolates of the pathogen. This will help to better understand the biology of the pathogen, how it responds to selection pressures, and this may lead to improved management strategies.

OBJECTIVES

1. Evaluate and optimize the performance of the antibiotic kasugamycin (Kasumin) as compared to streptomycin, oxytetracycline (Mycoshield) in cooperation with UCCE.
    a. Laboratory in vitro tests to evaluate the bactericidal activity with and without pH buffering using spiral gradient dilution assays.
    b. Small-scale hand-sprayer tests using different treatment-inoculation schedules.
    c. Field trials with protective air-blast spray treatments at several locations: mix with buffering adjuvants to adjust the pH of spray solutions, fungicides (mancozeb, dodine), other antibiotics (streptomycin, oxytetracycline), new formulations of copper (e.g., Kocide 3000, Badge X2), and plant defense activators (e.g, ProAlexin, Citrox). Evaluate product rates, timings, and rotations.
    d. Evaluate the efficacy of integrated programs using copper, fungicides, antibiotics and biological controls (e.g., Actinovate, Blossom Protect) and natural products (e.g, ProAlexin, Citrox).
2. Determine the distribution of streptomycin- or oxytetracycline -sensitive and -resistant strains of *E. amylovora* in pear orchards in California (continuation of 2006-10 surveys)
    a. Laboratory in vitro tests to evaluate the bactericidal activity with and without additives in amended agar assays.
    b. Characterization of streptomycin- and oxytetracycline-resistant strains using molecular approaches.
3. Localize the presence of *E. amylovora* inside woody tissues proximal of cankers and tissue discoloration using molecular methods.
    a. Compare the efficiency of molecular detection of the pathogen with bacterial isolations on agar media
    b. Determine the extent of tissue colonization beyond blight cankers.

MATERIALS AND METHODS

***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 as determined by measurement of optical density at 600 nm) along the concentration gradient, plates were incubated for 2 days at 25ºC. Measurements were visually taken 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 (Spiral Biotech, Inc.).

To investigate the interaction between kasugamycin and captan, mancozeb, or dodine, a microtiter plate assay was used. *E. amylovora* was grown in nutrient broth in the absence (control) or presence of kasugamycin or any of the three fungicides, or in mixtures of kasugamycin with the fungicides. Rates were chosen where growth of *E. amylovora* was only partially inhibited to be able to see interaction effects. Plates

were shaken for 20 h, and the optical density at 600 nm was determined as a measurement of growth. Reduction of growth by the treatments was compared to the untreated control.

Isolation of* E. amylovora*, bacterial culturing, and verification of species identity. Pear samples with fire blight symptoms were obtained in the spring and early summer of 2011 from orchards in Sacramento Co. A total of 47 isolates of *E. amylovora* from ten orchard locations were obtained. Infected plant material (flowers, fruit, stems, and pedicels) was surface-disinfested for 1 min using 400 mg/L sodium hypochlorite, rinsed with sterile water, cut into small sections, and incubated in 1 ml of sterile water for 15 to 30 min to allow bacteria to stream out of the tissue. Suspensions were streaked onto yeast extract-dextrose-$CaCO_3$ agar (YDC). Single colonies were transferred and the identity of the isolates as *E. amylovora* was verified by colony morphology and by PCR using primers specific for the ubiquitous *E. amylovora* plasmid pEA29 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.

Field studies using protective treatments during the growing season. In two field studies in a commercial Bartlett orchard in Live Oak, four applications of selected treatments (see Results) were done at 80% bloom (Apr 1), full bloom (Apr 6), full bloom/petal fall (April 13) or petal fall (April 21) using a back-pack air-blast sprayer at 100 gal/A. One branch of each tree was spray-inoculated with *E. amylovora* on Apr 14. Evaluation of inoculated branches was done on Apr 21 and evaluation of natural incidence of fire blight was done on Apr 27. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.1.

Characterization of streptomycin-resistant strains using molecular approaches. The genomic region of the *rps*L gene from highly and moderately resistant as well as from sensitive isolates of *E. amylovora* was amplified and sequences were compared (Chiou and Jones, Phytopathology 85:324-328, 1995). The presence of *strA-strB* and of transposon Tn*5393* was evaluated using published primers (McGhee et al., Phytopathology 101:182-191, 2011). For representative isolates containing *strA-strB* and Tn*5393,* amplifications were done using primers derived from the transposon and from sequences of selected plasmids. A positive amplification would thus indicate the location of the resistance genes on a particular plasmid. Additional sequence analysis was done to confirm the results. This work was done in collaboration with Dr. G. Sundin at Michigan State University.

RESULTS AND DISCUSSION

Survey of antibiotic sensitivity among* E. amylovora *strains collected in California. Isolates of *E. amylovora* were confirmed for species identity by PCR amplification of a 1-kb DNA fragment using specific primers for plasmid pEa29 that is ubiquitously found in this bacterium. A total of only 47 isolates from 10 pear orchard locations in Sacramento Co. (2 to 8 isolates per location) were obtained and were subsequently tested for their sensitivity against antibiotics. For oxytetracycline and kasugamycin, none of the collected strains showed reduced sensitivity and all isolates were considered sensitive (Table 1). For streptomycin, moderate resistance (LIC values 13.9 to 24.3 ppm, MIC values 20.8 to 37.6 ppm) was found at 5 locations with an incidence of between 42.8% and 75% (Table 1). Thus, the occurrence of streptomycin resistance was moderate again in 2011. This is in agreement with our conclusion that in low-disease years when fewer antibiotic applications are made and selection pressure on the pathogen population is lower, wild-type sensitive isolates will gradually replace the resistant population that appears to be less fit as compared to sensitive isolates. This information is very useful for the implementation of resistance management strategies. It implies that at locations with resistance against streptomycin, the incidence can possibly be reduced if more rotational treatments are available, making this important management tool more effective again. This information emphasizes the need for registration of new bactericides.

Laboratory studies on the interaction between kasugamycin and captan, mancozeb, or dodine. Our results from a microtiter plate growth assay demonstrate the in vitro inhibition of growth of *E. amylovora* by the fungicides captan, mancozeb, and dodine and support our previous findings that these compounds can reduce the incidence of fire blight in the field in comparison to the control. As clearly seen in Table 2 in the interaction between captan and kasugamycin and between mancozeb and kasugamycin, the inhibitory action of the compounds is additive and not synergistic (Table 2). Concentrations of each of these chemicals were used that only partially inhibited growth of *E. amylovora*, so the additive effect could be more clearly seen.

***Field studies using protective treatments during the growing season*.** In the first trial on Bartlett pear, a wide range of efficacy was obtained using 16 treatments (Fig. 1). Among the new treatments, kasugamycin continued to be highly effective in reducing the incidence of fire blight after inoculation and the natural incidence resulting in a numerically lower disease incidence than for streptomycin or oxytetracyline. The new 8L formulation was similarly effective as the previous 2L formulation. The biological treatments Cerebrocide, Blossom Protect, Actinovate, and Ph-D also significantly reduced the incidence of disease after inoculation, but Cerebrocide and Blossom Protect did not reduce the natural incidence of fire blight. Citrox/Proalexin significantly reduced the natural incidence but not the incidence of disease after inoculation. Among the mixture treatments, Kasumin + streptomycin, Actinovate, Quintec, or Prophyt and Cerebrocide + Ph-D showed high efficacy after inoculation, and Kasumin + streptomycin and Cerebrocide + Ph-D were also very effective in reducing the natural incidence of blight.

The reduced-copper product Badge X2 also significantly reduced the incidence of blight after inoculation, and numerically reduced the natural incidence. No phytotoxicity (russeting) on the developing Bartlett fruit was observed after four applications. Thus, new copper products can be an effective integral part of a fire blight management program as rotational treatments especially when disease pressure is low to moderate. Registrants, however, are reluctant to change their labels that currently indicate that a risk of russeting exists if copper is applied to developing fruit. Use of reduced MCE copper formulations in mixtures with antibiotics is a use strategy that needs continued evaluation.

In the second field trial, all treatments significantly reduced the natural incidence of blight and all except Actinovate reduced the incidence after inoculation (Fig. 2). Kasumin again performed very well with an efficacy similar to Mycoshield or Kasumin + streptomycin. Actinovate was the least effective, followed by Ph-D, Blossom Protect, and then the antibiotic treatments.

In summary, our field trials in 2011 again indicate that kasugamycin is a highly effective treatment against fire blight of pear that can be used in resistance management programs with rotations and possibly mixtures. No phytotoxicity was observed after four consecutive applications at 100 ppm. Registration of the product for California is expected for 2012. Mixture partners for kasugamycin and the registered antibiotics need continued evaluation to maximize the efficacy of treatments and as part of a resistance management program. Overall the natural products/fermentation products and biocontrols used in our field studies showed varying degrees of efficacy that ranged from very good to not effective. Efficacy in the two trials was not always consistent (Actinovate and Blossom Protect). Still, they warrant further testing and they potentially can be used as valuable rotation products for the antibiotics when disease pressure is low to moderate.

Characterization of streptomycin-resistant strains using molecular approaches. *Isolates highly resistant to streptomycin.* After sequencing the ribosomal protein S12 (*rps*L) gene from three highly resistant (MIC>50 ppm), two moderately resistant, and two sensitive isolates, sequences for codon 43 were AGA, AAA, and AAA, respectively. Thus, the amino acid change in the highly resistant isolates was from lysine to arginine as has been described for highly resistant isolates from other locations (Chio and Jones, 1995). Other mutations at this site previously reported (i.e., changes to asparagin or threonine) were not detected. This mechanism of resistance was described to be the primary mechanism for isolates from the western United States in the 1990s. In our surveys over the last years, however, highly resistant isolates of *E. amylovora* were only detected at one location and thus, these isolates have been replaced by strains with different resistance mechanisms.

Isolates with moderate resistance to streptomycin. StrA and *strB* genes, as well as transposon Tn*5393* sequences were amplified from representative isolates with moderate resistance but not from sensitive or highly resistant isolates (Fig. 3). Amplifications for plasmid pEa8.7 that was described for a few isolates from central California in 1997 were negative. Additionally, *strA-strB* genes could not be located on plasmid pEa34 that often harbors the resistance gene in isolates in Michigan. This indicated that a different mechanism of resistance is present in the current California population of the pathogen with moderate resistance. Sequence analysis of DNA regions flanking the transposon indicated that it was located on plasmid pEU30. This plasmid was first described from isolates from the western United States in 2004, but is not known to date to carry resistance genes. PCR amplifications confirmed the association of *strA-strB* with pEa30 in all evaluated moderately resistant isolates that were collected between 2006 and 2011 from various locations in California. Further characterization of the genetic base of resistance of these isolates is ongoing, but data indicate that the

pathogen is highly adaptive in changing its defense strategies against streptomycin and will continue to challenge the use of this antibiotic. A diagram summarizing current knowledge on mechanisms of streptomycin resistance in *E. amylovora* is presented in Fig. 4.

Table 1. Incidence of resistance against streptomycin, oxytetetracycline, or kasugamycin in isolates of *Erwinia amylovora* collected in surveys of 13 California pear orchards in 2011

Orchard No.	County	No. isolates	Incidence Steptomycin Resistance (%)*	Incidence Oxytetracycline Resistance (%)**	Incidence Kasugamycin Resistance (%)**
1	Sacramento	7	42.8	0	0
2	Sacramento	7	71.4	0	0
3	Sacramento	6	0	0	0
4	Sacramento	3	66.7	0	0
5	Sacramento	3	0	0	0
6	Sacramento	3	33.3	0	0
7	Sacramento	4	75	0	0
8	Sacramento	2	0	0	0
9	Sacramento	4	0	0	0
10	Sacramento	8	0	0	0

* - Inhibitory concentrations were determined on nutrient agar using the SGD method. Minimum inhibitory concentrations (MIC, >95% inhibition) of isolates sensitive to streptomycin were 0.465-1.2 ppm; whereas LIC of isolates resistant to streptomycin were 20.8-37.6 ppm.

**- MICs of isolates for oxytetracycline were 0.27-0.42 ppm; whereas those for kasugamycin were 7.1-15.2 ppm.

Table 2. Evaluation of the additive activity of kasugamycin and captan, mancozeb, or dodine in inhibiting growth of *Erwinia amylovora*

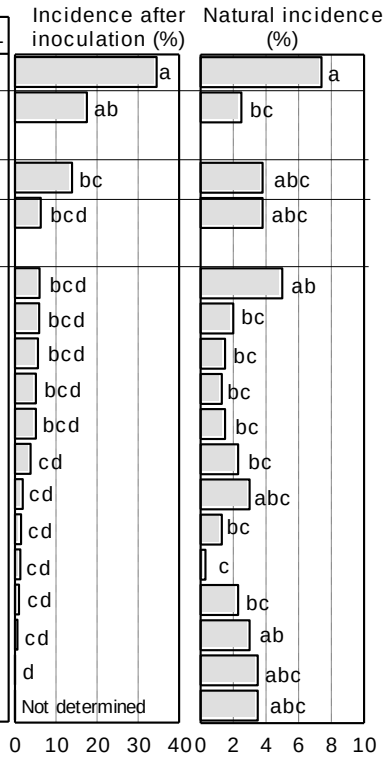
Treatment*	% inhibition of growth**
Kasugamycin 1 ppm	43.8
Captan 5 ppm	53.3
Kasugamycin 1 ppm + Captan 5 ppm	89.7
Mancozeb 10 ppm	37.3
Kasugamycin 1 ppm + Mancozeb 10 ppm	78.9
Dodine 0.5 ppm	99.2
Kasugamycin 1 ppm + dodine 0.5 ppm	100

*- *E. amylovora* was grown in microtiter plates in nutrient broth without or with the addition of test substances. Growth was measured by optical density readings at 600 nm.

** - % inhibition as compared to the non-amended control

Fig. 1. Evaluation of new bactericides for fireblight management on Bartlett pears in a field trial in Live Oak CA - 2011

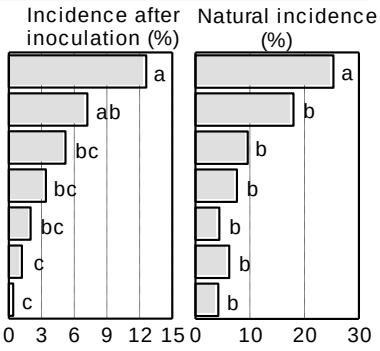
No.	Treatment	4-1	4-6	4-13	4-21
1	Control	---	---	---	
2	Citrox BC 133 ml + Proalexin 133 ml	@	---	@	@
	Biozyme	---	@	---	---
3	Blossom Protect 21.5 oz + Buffer 9.35 lb	@	@	@	@
4	Blossom Protect 21.5 oz + Buffer 9.35 lb	@	@	@	@
	Citrox BC 133 ml + Proalexin 133 ml	@	@	@	@
5	Cerebrocide 64 fl oz	@	@	@	@
6	Actinovate 12 oz + Tactic 8 fl oz	@	@	@	@
7	Kasumin 8L 100 ppm + Actinovate 12 oz	@	@	@	@
8	Cerebrocide 64 fl oz + Ph-D org. 6.2 oz	@	@	@	@
9	Kasumin 8L 100 ppm + Streptomycin 100 ppm	@	@	@	@
10	Fireline 200 ppm	@	@	@	@
11	Badge X2 8 oz	@	@	@	@
12	Firewall 100 ppm	@	@	@	@
13	Kasumin 8L 100 ppm	@	@	@	@
14	Kasumin 2L 100 ppm	@	@	@	@
15	Kasumin 8L 100 ppm + Quintec 6 fl oz	@	@	@	@
16	Kasumin 8L 100 ppm + Prophyt 4 pt + Tactic	@	@	@	@
17	Quintec 6 fl oz	@	@	@	@



Treatments were applied using an air-blast sprayer at 100 gal/A (80% bloom was on 4-1, full bloom was on 4-6, and full bloom/petal fall was on 4-13-11). Treatments 2 and 4 were rotations. Branches with blossoms were inoculated with *E. amylovora* on 4-14-11. Evaluation of inoculated branches was done on 4-21-11 and evaluation of natural incidence of fire blight was done on 4-27-11.

Fig. 2. Evaluation of new biologicals and antibiotics for fireblight management on Bartlett pears in a field trial in Live Oak CA - 2011

No.	Treatment	4-1	4-6	4-13	4-21
1	Control	---	---	---	---
2	Actinovate 12 oz + Tactic 8 fl oz	@	@	@	@
3	Ph-D org. 6.2 oz	@	@	@	@
4	Blossom Protect 21.5 oz + Buffer 9.35 lb	@	@	@	@
5	Kasumin 8L 100 ppm	@	@	@	@
6	Mycoshield 200 ppm	@	@	@	@
7	Kasumin 8L 100 ppm + Streptomycin 100 ppm	@	@	@	@



Treatments were applied using an air-blast sprayer at 100 gal/A (80% bloom was on 4-1, full bloom was on 4-6, and full bloom/petal fall was on 4-13-11). Branches with blossoms were inoculated with *E. amylovora* on 4-14-11. Evaluation of inoculated branches was done on 4-21-11 and evalaution of natural incidence of fire blight was done on 4-27-11.

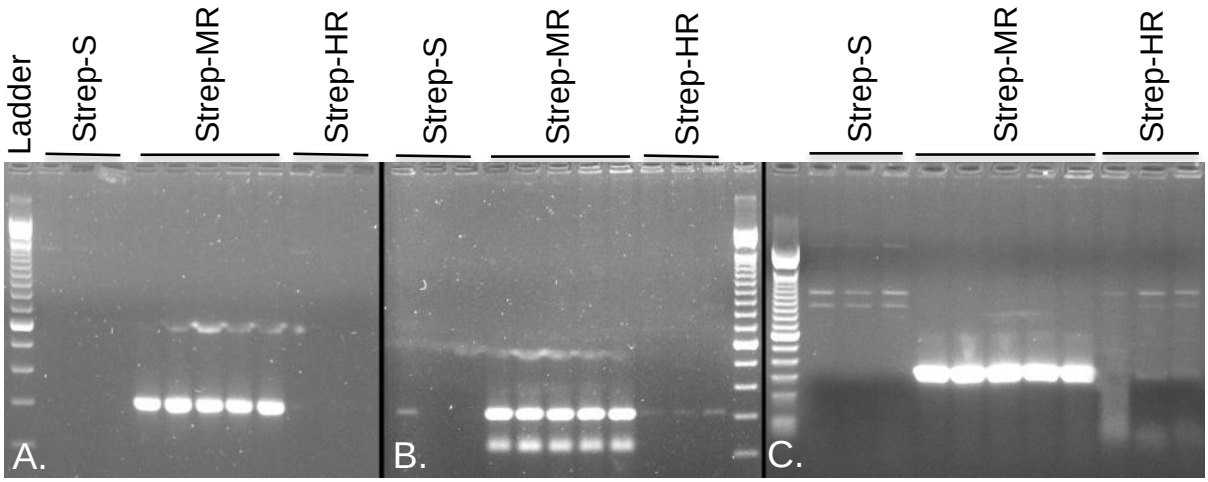


Fig. 3. PCR amplification of streptomycin resistance genes A) *StrA* and B) *StrB,* as well as C) transposon Tn*5393* in isolates of *Erwinia amylovora* sensitive (Strep-S), moderately resistant (Strep-MR), or highly resistant (Strep-HR) to streptomycin.

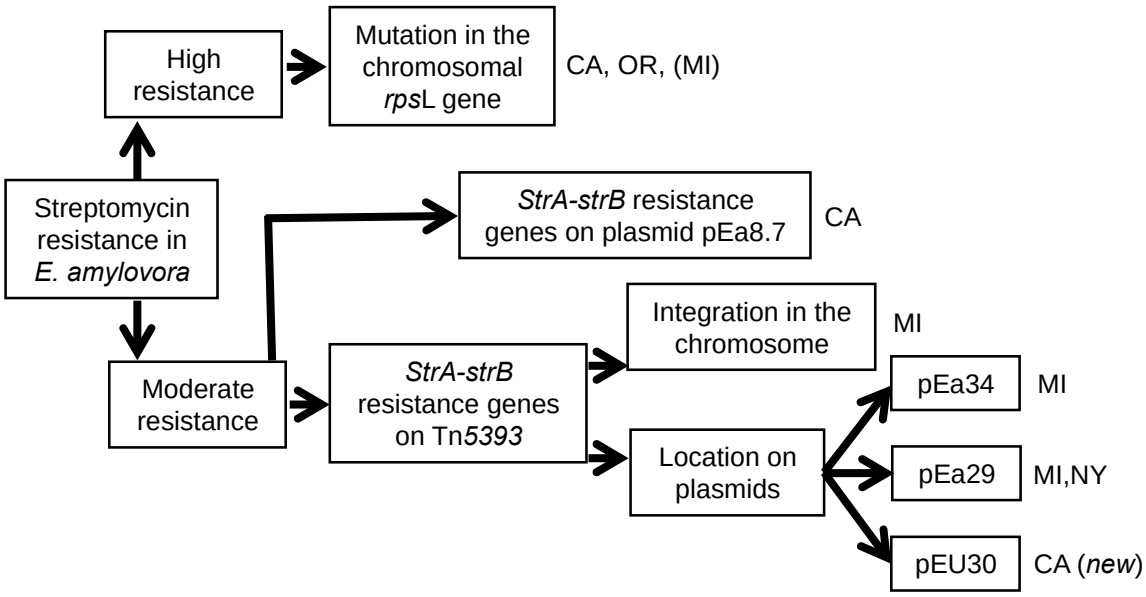


Fig. 4. Genetic mechanisms of streptomycin resistance in *Erwinia amylovora.* US States abbreviations indicate where each mechanism has been reported. Tn*5393* is a transposon.