Annual Report - 2010

Prepared for the California Pear Board

Project Title:	Evaluation of new bactericides for control of fire blight of pears caused by *Erwinia amylovora*
Project Leaders:	Drs. J. E. Adaskaveg and D. Gubler, Department of Plant Pathology, University of California, Riverside CA 92521 and Davis, CA 95616, respectively.
Cooperators:	Dr. H. Förster, L. Wade (Arysta Life Science), D. Thompson, C. Ingels (UCCE, Sacramento Co.) and R. Elkins (UCCE, Lake Co.)

SUMMARY

1. Baseline sensitivity concentrations for kasugamycin were established for growth of 376 isolates of *E. amylovora* from California. The frequency histogram demonstrates the natural variation in sensitivity of a large pathogen population previously not exposed to the antibiotic and will serve as a baseline for sensitivity monitoring.
2. The in vitro activity of kasugamycin, streptomycin, and oxytetracycline was highly dependent on the agar medium used in the sensitivity assay. For kasugamycin and oxytetracycline, inhibition was greatest using NA, whereas for streptomycin it was greatest using either NA, Czapek agar, or KMB.
3. The activity of kasugamycin was also highly dependent on the pH of the medium and was significantly higher at pH 5.1 than pH 7.3.
4. Population studies of the pathogen in 2010 indicated an intermediate incidence of streptomycin resistance with results varying within and between locations. Resistance was found in 8 of 13 locations. Strains of *E. amylovora* were moderately resistant to streptomycin. No strains less sensitive to oxytetracycline or kasugamycin were obtained in the 2010 survey.
5. Several field trials were conducted on the management of fire blight. The following products were evaluated: the antibiotics kasugamycin (Kasumin), streptomycin, and oxytetracycline (Mycoshield), the compounds copper hydroxide (Kocide 3000), copper hydroxide/copper oxychloride (Badge), and mancozeb (Manzate F45), as well as the biocontrols *Streptomyces lydicus* (Actinovate) and *Aureobasidium pullulans* (Blossom Protect), and the natural products Cerebrocide, Proalexin, and Citrox.
    a. In a small-scale trial, treatments with kasugamycin, streptomycin, oxytetracycline, and kasugamycin/ mancozeb mixtures were highly effective in controlling the disease when applied before inoculation with a streptomycin-sensitive isolate of the pathogen. Kasugamycin treatments applied 16 h after inoculation significantly reduced disease incidence as compared to the untreated control and most of the other treatments. Oxytetracycline was the least effective of the three antibiotics as a post-infection treatment.
    b. 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 oxytetracyline. Kasugamycin was also very effective in mixtures with mancozeb and in mixture rotations of antibiotics with mancozeb.
    c. The copper compounds Badge and Kocide 3000, the natural products Cerebrocide, Proalexin, and Citrox, as well as the biocontrols Actinovate and Blossom Protect also significantly reduced the incidence of fire blight.
6. Kasugamycin (Kasumin) registration in the US is being pursued on pome fruit with federal registration expected in Jan. 2011.
7. Studies on the extent of *E. amylovora* colonization inside diseased twigs will be continued next season under more conducive conditions for disease.

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. 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 oxytetracycline (Mycoshield). 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 2010 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 of

copper hydroxide (Kocide 3000) and copper oxychloride (Badge) that use less copper (e.g., 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 mancozeb (e.g., Manzate, Penncozeb, etc.) by themselves and in mixtures with kasugamycin. Additive effects of bactericides mixed with EBDC have been reported for fire blight and other bacterial diseases. We are also evaluating single and mixture programs with copper, mancozeb, and antibiotics (i.e., streptomycin and oxytetracycline) 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 2010 studies we included the biocontrol Actinovate (*Streptomyces lydicus*), Blossom Protect, and the natural products Citrox, Proalexin, and Cerebrocide.

OBJECTIVES

1. Evaluate the efficacy of the antibiotic kasugamycin (Kasumin) as compared to streptomycin, oxytetracycline, or terramycin (Mycoshield) in cooperation with UCCE.
   a. Laboratory in vitro tests to evaluate the bactericidal activity with and without adjuvants: 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 adjuvants, fungicides (mancozeb, dodine), new formulations of copper (e.g., Kocide 3000, GWN4620), and the plant defense activator ProAlexin). Evaluate product rates, timings, and rotations.
   d. Evaluate the efficacy of integrated programs using copper, fungicides, antibiotics and biological controls (Actinovate).
2. Determine the distribution of streptomycin- or terramycin-sensitive and -resistant isolates of *E. amylovora* in pear orchards in California (continuation of 2006-09 surveys)
   a. Laboratory in vitro tests to evaluate the bactericidal activity with and without adjuvants in different amended agar media.
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), oxytetracycline (Sigma) and mancozeb 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 taken for two inhibitory concentrations as determined visually: 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.). Each isolate was evaluated in two independent experiments with two or four replications per experiment.

Eight randomly selected isolates were evaluated for their sensitivity against kasugamycin, streptomycin, and oxytetracycline using the same method as described above and using NA, King's medium B (KMB), Luria-Bertani agar (LBA), Czapek agar (Difco Laboratories), Kado medium 523, and a modified yeast-salts agar. Stock concentrations used in the spiral plating for streptomycin (streptomycin sulfate; Sigma Aldrich, St. Louis, MO), and oxytetracycline (oxytetracycline hydrochloride; Sigma-Aldrich) were 1,000 mg/L and 500 mg/L, respectively.

In additional studies, the effect of pH of the culture medium on the activity of kasugamycin was investigated. To obtain selected pH values, a previously published method was adopted. For this, 1.1 ml of a sterilized buffer (1.3 g citric acid, 1.9 g glycine, 1.9 g KH_2PO_4/50 ml of water) was added to 18.9 ml nutrient broth and the pH was adjusted to mean values of 5.1 (5.0 to 5.2) or 7.3 (7.1 to 7.5) using 1 N NaOH. Measurements of pH were done with a pH meter. The test was conducted in micro-titer plates (0.2 ml reaction volume per well) with a initial bacterial concentration of 10^7 cfu/ml. Plates were incubated on a shaker at 180 rpm at 25°C. After 12 h, bacterial growth was measured at 600 nm using a plate reader.

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 2010 from orchards in the main pear-growing areas in central and northern California (i.e., Lake, Sacramento, and Yuba-Sutter Co.). A total of 376 isolates of *E. amylovora* were obtained from pear orchards in the major growing areas in central and northern California between 2006 and 2009. 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 sections (ca. 1 mm x 2 mm x 5 mm), incubated in 1 ml of sterile water for 15 to 30 min to allow bacteria to stream out of the tissue, and the aqueous suspension was 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 small-scale field trials on Asian pear (cvs. Shinko and 20th Century) at the University of California, Davis, the pre- and post-infection activity of kasugamycin in controlling fire blight was evaluated. Treatments (see Results) were applied to run-off to branches with open flowers using a household hand sprayer on 15 and 22 March 2010 on the two cultivars, respectively. To evaluate pre-infection activity, treatments were applied, allowed to air-dry for1 or 2 h, and then flowers were inoculated with *E. amylovora*. To evaluate post-infection activity, flowers were inoculated and treated after 16 h. Treatments included a water control and aqueous preparations of streptomycin at 100 mg/L, oxytetracycline at 200 mg/L, kasugamycin at 100 mg/L, and a mixtures of kasugamycin at 100 mg/L with mancozeb (Manzate F45) at 5,400 mg/L or other antibiotics. Inoculations were done with a strain of *E. amylovora* (10^6 cfu/ml in sterile distilled water), isolated from diseased flowers in a commercial Bartlett pear orchard in Marysville, CA, and determined to be sensitive to all compounds evaluated. Inoculations were applied to run-off using a hand sprayer in late afternoon and inoculated branches were enclosed in white plastic bags for 16 to 18 h to maintain leaf wetness and reflect sunlight to prevent over-heating inside the bags. Disease was evaluated after 10 to 14 days as incidence (the number of diseased flowers per 20 to 50 flowers used for each replication expressed as a percentage). All treatments were applied to each of four single-tree replications.

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), full bloom/petal fall (April 7) or petal fall (April 15) using a back-pack air-blast sprayer at 100 gal/A. The number of blossom and shoot infections per tree was evaluated on April 22 and 29, 2010, 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. Due to the low incidence of fire blight this year in selected field locations this objective was not conducted. We plan to continue this objective in the 2011 season.

RESULTS AND DISCUSSION

Laboratory studies on inhibition of *E. amylovora* by kasugamycin, streptomycin, oxytetracycline, and mancozeb. The inhibition of bacterial growth by a chemical compound is typically described as the minimum inhibitory concentration (MIC) or the lowest concentration of an antimicrobial that prevents growth of the organism. We could not easily obtain these concentrations for kasugamycin, streptomycin, and oxytetracycline because there was generally a very gradual reduction in growth with increasing concentrations of the antimicrobials. This made it difficult to assess where growth was completely prevented. Thus, we chose to quantify in vitro inhibitory activity as both LIC and MIC values (defined here as the minimum inhibitory

concentration to inhibit growth by 95%). Using these two values, shifts in sensitivities among strains in sample populations can be detected.

Values for the lowest concentration (LIC) of kasugamycin where a reduction in growth on NA was observed among 376 isolates of *E. amylovora* ranged from 3.5 to 18.3 mg/L with a mean value of 8.7 mg/L (Fig. 1A). Values for the antibiotic where $\geq$95% of growth was inhibited (MIC) ranged from 6.9 to 46.7 mg/L with a mean value of 18.5 mg/L (Fig. 1B). Thirty-five percent of the total number of isolates were resistant to streptomycin at 6 to >50 mg/L. The frequency histogram in Figure 1 demonstrates the natural variation in sensitivity of a large population of the pathogen previously not exposed to the antibiotic and can function as a baseline for future comparative studies. Mancozeb was also inhibitory to *E. amylovora* with average (range) LIC and MIC values of 3.75 mg/L (2.94 to 4.66 mg/L) and 5.27 mg/L (4.11 to 6.59 mg/L), respectively.

The *p* values for the regression models of LIC values of kasugamycin plotted against those for oxytetracycline or streptomycin were highly significant ($P < 0.0001$). Still, correlation coefficients were low for both regressions ($r^2 = 0.07$ or $r^2 = 0.05$, respectively) (Fig. 2A,B). Using a large collection of strains of *E. amylovora* with a wide range of sensitivities against streptomycin or oxytetracycline, the regression analyses resulted in highly significant models. Low correlation coefficients, however, were found between sensitivity to kasugamycin and that to either of the other two antibiotics. Thus, cross resistance was not observed within the sample population although two of the antibiotics (i.e., kasugamycin and streptomycin) are aminoglycoside antibiotics. This reflects the unique modes of action of each of the antibiotics. The sensitivity of strains to streptomycin appeared to be stepwise with low and high levels of resistance measured within the sample populations. Streptomycin resistance levels ranged from 6 to >50 mg/L. This possibly reflects the presence of different resistance genes and mechanisms as has been described previously for *E. amylovora*.

When kasugamycin, streptomycin, and oxytetracycline were tested for their inhibitory effect against growth of eight randomly selected isolates of *E. amylovora* using six different growth media. For the combined data set, significant ($P < 0.0001$) differences were observed among the media. Inhibition by each of the three compounds was lowest (LIC values were highest) using YSA, but growth responses for the remaining media were different for kasugamycin, streptomycin, and oxytetracycline (Table 1). For kasugamycin and oxytetracycline, inhibition was greatest using NA, whereas for streptomycin it was greatest using either NA, Czapek agar, or KMB. Among the media, there were 6.5-fold differences in inhibition for kasugamycin, 4.2-fold differences for streptomycin, and 20-fold differences for oxytetracycline. Although in field trials the efficacy of kasugamycin against fire blight was similar to streptomycin and oxytetracycline (equal amounts of active ingredient for kasugamycin and streptomycin and double the amount of active ingredient for oxytetracycline), concentrations required to inhibit bacterial growth in vitro were higher for kasugamycin than for the other two antibiotics.

The in vitro inhibition by kasugamycin was also highly dependent on the pH of the culture medium. Growth of *E. amylovora* using nutrient broth not amended with kasugamycin and adjusted to pH 5.1 was significantly ($P < 0.0001$) increased by 20.6% or 28.2% compared to growth using non-adjusted nutrient broth or broth adjusted to pH 7.3, respectively. Relative inhibition of growth in the presence of kasugamycin was significantly ($P < 0.0001$) higher at pH 5.1 than at pH 7.3 (Table 2). Thus, in comparison to the non-amended control, relative growth in the presence of 5 mg/L kasugamycin was 78.9% at pH 7.3 and 13.8% at pH 5.1. In the presence of 10 mg/L kasugamycin growth was 64.9% at pH 7.3 and 3.9% at pH 5.1. This pH effect was not tested for streptomycin and oxytetracycline. The effect of pH on the inhibition by kasugamycin may be important in preparing spray tank mixtures with water of different sources and pH. Some pesticides can degrade quickly in alkaline solutions and thus, additional studies are needed to evaluate pH of spray solutions and their effect on the activity of kasugamycin in managing fire blight. Additionally, these results indicate that the in vitro inhibitory concentration ranges for kasugamycin that we present in this study may not reflect the inhibitory activity of the compound in and on the host plant where nutritional and other chemical conditions are complex. Thus, considering the high efficacy of the compound in our field studies, it is possible that the activity of kasugamycin may be enhanced *in planta* due to pH effects alone. The pH of pear flowers and leaves was measured and determined to be between 5 and 6.

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 a plasmid that is ubiquitously found in this bacterium. A total of 66 isolates from 13 California pear orchard locations (2 to 7 isolates per location) were subsequently tested for their sensitivity against the

antibiotics streptomycin, oxytetracycline, and kasugamycin. For oxytetracycline, none of the collected strains showed reduced sensitivity and all isolates were considered sensitive to the antibiotic. For kasugamycin, there was again a wide sensitivity range among isolates, but all isolates were determined to be sensitive to kasugamycin (Table 3). For streptomycin, a wide range of streptomycin resistance was observed among the strains between locations and within each site sampled (Table 3). Annual fluctuations of the incidence of streptomycin resistance are shown in Table 4. 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), 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 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. As indicated last year, this information emphasizes the need for registration of new bactericides. The apparent difference in competitiveness between streptomycin-resistant and highly resistant isolates needs to be further investigated.

Field studies using pre-infection or post-infection treatments in controlling fire blight during the growing season. For the two experiments conducted on Asian pear cvs. Shinko and 20th Century, when flowers were first treated and then inoculated after 1 to 2 h, all treatments including kasugamycin, kasugamycin-mancozeb, as well as the registered streptomycin and oxytetracycline reduced the incidence of fire blight compared to the control (Fig. 3A). The incidence of diseased flowers was significantly (P <0.001) reduced from 40.8% in the untreated control to between 5.4% (kasugamycin) and 11% (streptomycin). When flowers were first inoculated and then treated after 16 h, all treatments significantly reduced the incidence compared to the control (Fig. 3B). Kasugamycin was significantly (P <0.001) more effective than oxytetracycline or kasugamycin-mancozeb. Streptomycin showed an intermediate efficacy.

In an air-blast spray field trial on Bartlett pear where 4 applications of each treatment were made, all treatments significantly reduced the incidence of blossom blight and shoot infections from that of the control and the efficacy of most treatments could not be statistically separated (Fig. 4). Treatments with kasugamycin (Kasumin 2L), kasugamycin-oxytetracycline (Mycoshield), or rotations of each antibiotic (kasugamycin, streptomycin, or oxytetracycline) with mancozeb (Manzate F45) resulted in the lowest incidence of shoot infections. Streptomycin and oxytetracycline treatments were intermediate in their performance. The copper compounds Badge and Kocide 3000 also performed well with no phytotoxicity observed. The biocontrols Actinovate and Blossom Protect, as well as the natural products Cerebrocide (+biozyme), Citrox, and Proalexin also significantly reduced the incidence of fire blight under low disease pressure (Fig. 4).

In summary, our field trials in 2010 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 four consecutive applications at 100 ppm. Arysta LifeScience 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. The registration package was submitted to the US-EPA in January 2010 and federal registration of kasugamycin for management of fire blight is expected in Jan. 2011. The registration of kasugamycin in California may take an additional year. Still, the registration of kasugamycin will be timely. 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. The effect of water pH will also need to be studied based on this year's results. Thus, identification of integrated fire blight programs with copper, fungicides, and antibiotics as well as optimum application conditions (e.g., water pH) is successfully being pursued for the California pome fruit industries.

Table 1. In vitro inhibitory effects (lowest concentration where a reduction of growth is observed -LIC) for kasugamycin, streptomycin, and oxytetracycline against *E. amylovora* using selected agar media.

	Kasugamycin		Streptomycin		Oxytetracycline	
Agar medium[a]	LIC (mg/L)[b]	LSD[c]	LIC (mg/L)	LSD	LIC (mg/L)	LSD
NA	6.93	e	0.301	cd	0.106	e
Czapek	12.25	d	0.225	d	0.722	bc
KMB	20.63	c	0.288	cd	0.668	c
523	28.54	b	0.357	c	0.822	b
LBA	43.09	a	0.791	b	0.359	d
YSA	45.58	a	1.260	a	2.156	a

[a] - NA = nutrient agar, KMB = King's medium B, LBA = Luria-Bertani agar, 523 = Kado medium 523, YSA = Yeast-salts agar.

[b] - Inhibitory concentrations were determined using the spiral gradient dilution method. Values are the average of 8 isolates of *E. amylovora* from 2 repeated experiments.

[c] - For each column, values followed by different letters indicate significant differences according to an analysis of variance and least significant mean separation procedures ($P<0.05$).

Table 2. In vitro inhibitory effect of kasugamycin on growth of *Erwinia amylovora* at selected pH values.

Kasugamycin	Growth (%)[a]			
(mg/L)	pH 5.1[b]	LSD[c]	pH 7.3	LSD
0	100.0	Aa	100.0	Aa
5	13.8	Bb	78.9	Ba
10	3.9	Cb	64.9	Ca

[a] - Growth in microtiter plates was measured as optical density. For each pH treatment, growth in the presence of kasugamycin was expressed as percent growth compared to the non-amended control. Values are the average of three repeated experiments.

[b] - The pH of nutrient broth was adjusted using a citrate-glycine-potassium phosphate buffer.

[c] - Values followed by different letters (capitalized letters for each column and non-capitalized letters for each row) indicate significant differences according to an analysis of variance and least significant mean separation procedures ($P<0.05$).

Table 3. Distribution of isolates of *Erwinia amylovora* sensitive or less sensitive to streptomycin or oxytetetracycline in a survey of 13 California pear orchards in 2010

No.	County	No. isolates	Incidence Steptomycin Resistance (%)*	Incidence Oxyteracycline Resistance (%)*	Incidence Kasugamycin Resistance (%)*
1	Placer	5	0	0	0
2	Placer	2	0	0	0
3	Placer	3	0	0	0
4	Sacramento	6	100	0	0
5	Sacramento	7	85.7	0	0
6	Sacramento	5	100	0	0
7	Sacramento	5	20	0	0
8	Sacramento	6	33.3	0	0
9	Sacramento	6	16.6	0	0
10	Sacramento	9	44.4	0	0
11	Sacramento	4	0	0	0
12	Sacramento	3	33.3	0	0
13	Sacramento	5	0	0	0
Total		66			

* - Inhibitory concentrations were determined on nutrient agar using the SGD method. Minimum inhibitory concentrations (LIC) of isolates sensitive to streptomycin were 0.157-0.481 ppm; whereas LIC of isolates resistant to streptomycin were 7.45-15.72 ppm.

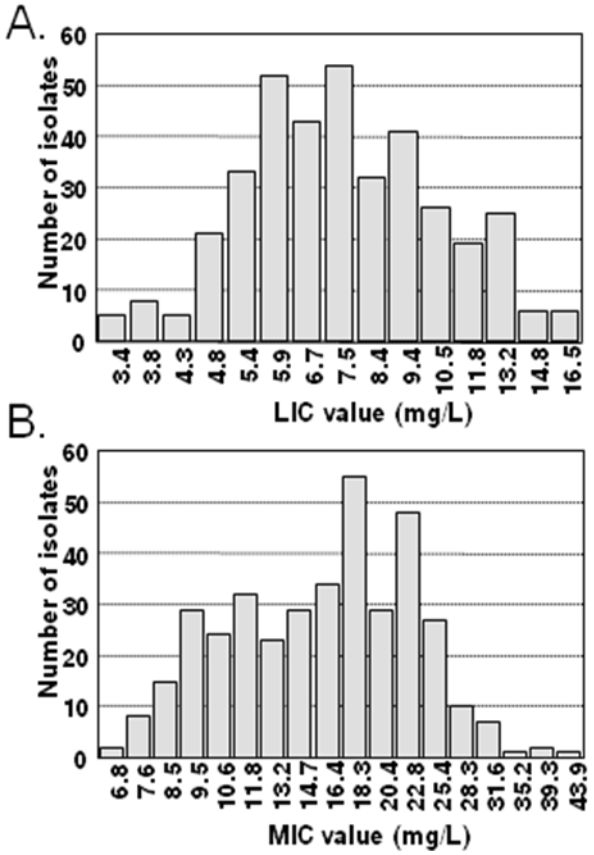
** - Minimum inhibitory concentrations (LIC) of isolates for oxytetracycline were 0.097-0.168 ppm; whereas those for kasugamycin were 4.29-9.15 ppm.

Table 4. Incidence of streptomycin resistance among isolates of *Erwinia amylovora* in surveys of California pear orchards 2006-2010*

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
2010	39.4	61.5

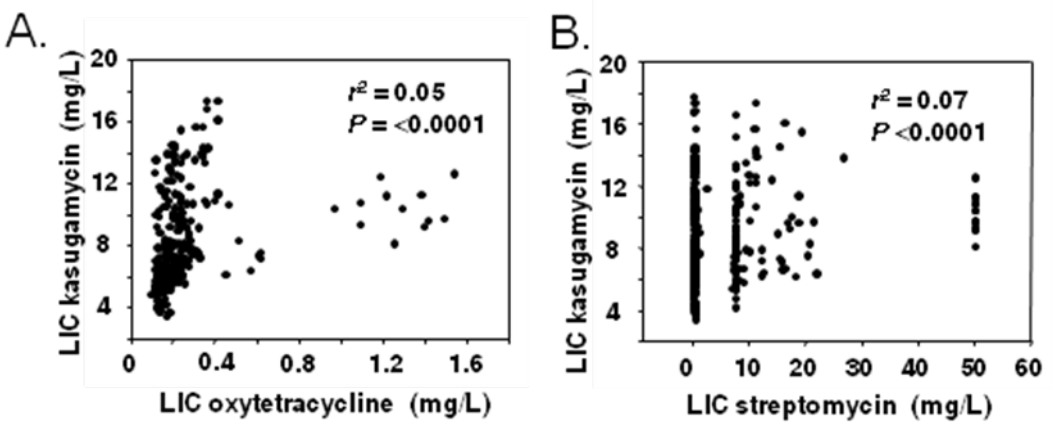
* - Inhibitory concentrations were determined on nutrient agar using the SGD method.

Fig. 1. In vitro sensitivity to kasugamycin of 376 isolates of *Erwinia amylovora* collected throughout California pear growing regions.



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 the minimal concentration that inhibits growth by ≥95% (MIC) were determined using the spiral gradient dilution method. Bar height indicates the number of isolates within each bin and bin width was based on Scott's method using $\log_{10}$ transformed values

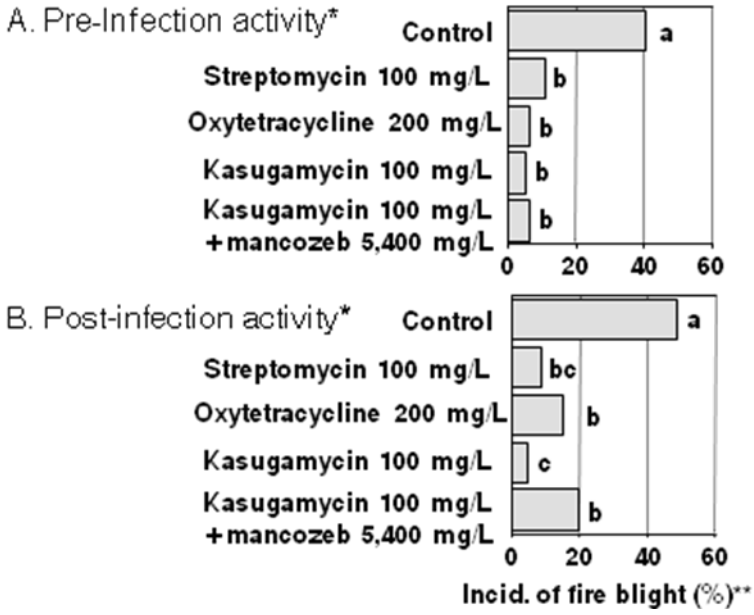
Fig. 2. Scatter plots of lowest inhibitory concentrations of kasugamycin for growth of *Erwinia amylovora* plotted against those for streptomycin and oxytetracycline.



*- For streptomycin, 376 isolates were evaluated whereas for oxytetracycline 257 isolates were evaluated.

**- The coefficients of determination (r^2) and *P* values for the linear regressions are indicated.

Fig. 3. Evaluation of bactericides for control of fire blight of cvs. 20th Century and Shinko Asian pear in field trials.

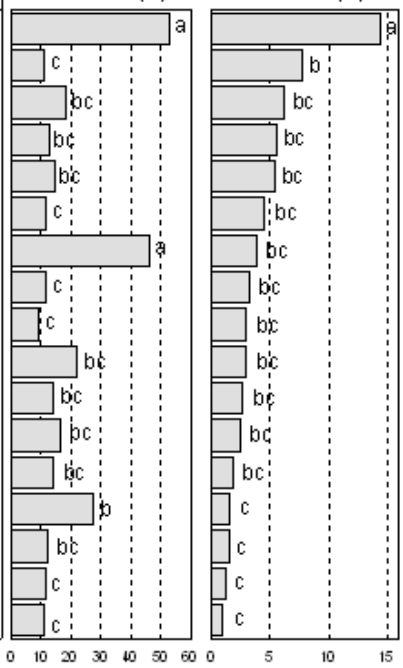


*- Treatments were applied to run-off using a hand sprayer either 1 or 2 h before (i.e., pre-infection activity) or 16 h after (i.e., post-infection activity) inoculation with *Erwinia amylovora* (1 x 10^6 cfu/ml).

**- Disease was evaluated as the incidence of blighted flowers per 20 to 50 flowers for each of the four replications after 10 to 14 days. Data were combined for the two cultivars after testing for homogeneity of variance.

Fig. 4. Evaluation of new bactericides for fireblight management on Bartlett pears in a field trial in Live Oak CA - 2010

Treatment*	80% bloom (3-23)	Full Bloom (3-31)	FB/PF (4-7)	Petal Fall (4/15)
Control	---	---	---	---
Kasumin + Streptomycin (100+100 ppm)	@	@	@	@
Kasumin 10L (100 ppm)	@	@	@	@
Citrox BC (1%)	@	@	@	@
Kocide 3000 (8 oz)	@	@	@	@
Manzate F45 (150 fl oz)	@	@	@	@
ProAlexin (1%)	@	@	@	@
Blossom Protect + Buffer (21.5 oz+9.3 lb)	@	@	@	@
Mycoshield (200 ppm)	@	@	@	@
Badge 2.27SC (14.5 fl oz)	@	@	@	@
Kasumin 10L + Manzate F45 (100 ppm+75 fl oz)	@	@	@	@
Actinovate + NuFilm P (12 oz+8 fl oz)	@	@	@	@
Streptomycin (100 ppm)	@	@	@	@
Cerebrocide + Biozyme (67 fl oz+32 fl oz)	@	@	@	@
Kasumin 2L (100 ppm)	@	@	@	@
Kasumin 10L, Strep., Myco + Manzate F45**	@	@	@	@
Kasumin + Mycoshield (100+200 ppm)	@	@	@	@



* - Treatments were applied using an air-blast sprayer at 100 gal/A. Disease was evaluated on 4-22 (blossom infections) and 4-29-10 (shoot infections for each of the 6-7 single-tree replications.

** - Rotation treatment of Kasumin 10L (100 ppm), Streptomycin (100 ppm), or Mycoshield (200 ppm) with each mixed with Manzate F45 applied in sequence.