Annual Report - 2005

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. In two field trials, the efficacy of blossom and foliar spray treatments with the antibiotics kasugamycin (Kasumin) and terramycin, the fungicide famoxadone, and the biocontrol Bloomtime Bio (*Pantoea agglomerans*) were evaluated.
2. Because of the low and inconsistent incidence of fire blight in the spring of 2005, data were only obtained from one of the trials. In this trial, the biocontrol Bloomtime Bio was highly effective in reducing the incidence of fire blight.
3. The efficacy of kasugamycin was rate-dependent. Rates of 100 ppm were equally effective as Bloomtime Bio and no phytotoxicity was observed.
4. The registration of kasugamycin (Kasumin) is supported by its US registrant. The registration is also supported by EPA because this antibiotic is not used in medicine.

INTRODUCTION

Fire blight, caused by the bacterium *Erwinia amylovora,* is a very destructive disease of pome fruit trees, especially pears, worldwide. 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 very difficult to control. Even with an integrated program of chemical control combined with sanitation and orchard management this serious disease is almost impossible to eliminate with the current methods available. Thus, every effort should be made to keep the disease out of the orchard. If the disease is in its early stage and only a few twigs are blighted it often can be eliminated by pruning. 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. Streptomycin, an antibiotic for fire blight control, came into general commercial use during the late 1950s, followed by the less effective oxytetracycline (Terramycin). Because of the lack of alternative control materials, antibiotics are still being used commercially, although pathogen resistance against the antibiotic streptomycin is widespread and concerns are growing regarding using 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 have identified a broad-spectrum biocide from Dow Chemicals that is registered as a water treatment and the antibiotic Starner. Because of registration costs, however, the manufacturer of the biocide will not proceed with registration. The antibiotic Starner (Valent Biosciences) is not being developed for agricultural use because the class of antibiotics that Starner belongs to is important in medicine. The acidified hydrogen peroxide Zerotol showed efficacy in our 2003 trials, but its efficacy in 2004 was not satisfactory. The antibiotic kasugamycin (Kasumin), however, showed promising results in 2004. Members of the kasugamycin antibiotic class are not being 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.

In 2005 we conducted additional field experiments for the evaluation of new potential fire blight control chemicals. We evaluated the antibiotic kasugamycin and famoxodone, a fungicide that has been reported to have efficacy against bacterial diseases. Interestingly, this material has no in vitro toxicity and may induce host resistance within the plant. In addition we included the biological control Bloomtime Bio (*Pantoea agglomerans*). This biocontrol agent has been reported to active against *E. amylovora* in vitro and in vivo and its mode of action has been reported to be based on the production of antibiotics.

OBJECTIVES

1. Evaluate the toxicity of the antibiotic kasugamycin (Kasumin) as compared to terramycin (Mycoshield) in cooperation with UCCE.
   a. Laboratory in vitro tests to evaluate the bactericidal activity with and without adjuvants: Direct contact assays, filter disk assays, amended agar assays.
   b. Studies with protective spray treatments will be done in field trials. Adjuvants and product rates will be evaluated.

MATERIALS AND METHODS

***Laboratory studies on the toxicity of bactericides and on the interaction of* P. agglomerans *with* E. amylovora.** Antibiotics (e.g., kasugamycin, streptomycin) were evaluated for their toxicity using the spiral gradient dilution method. For this, a bactericidal concentration gradient was established in agar media in Petri dishes. After inoculation of the media with two isolates of the test pathogen and incubation for 2-3 days at 20 C, minimum inhibitory concentrations were obtained using a computer program. For evaluation of the activity of the biocontrol, a suspension of *P. agglomerans* was spiral-plated onto agar media and after 4 h, plates were inoculated with two isolates of *E. amylovora*. Plates were incubated at 15 or 20C.

***Field studies using protective treatments during the growing season*.** In small-scale field studies, the relative efficacy of the protective treatments kasugamycin (an antibiotic), famoxadone (two formulations), and the biological control Bloomtime Bio was compared to terramycin (e.g., Mycoshield). Concentrations that were evaluated were based on current product registration rates for other uses. Trials were established at UC Davis in an Asian pear orchard and in a commercial Bartlett orchard in Marysville where fire blight caused crop losses in previous years. In the Marysville plot, treatments were applied on 3/10, 3/17, 3/23, 3/30, 4/4, and 4/25/05 and disease was evaluated on May 5, 2005. In the Davis plot, treatments were applied on 3/10, 3/18, 3/26, 4/1, and 4/9/05 and disease was evaluated May 10, 2005. In both plots, Bloomtime Bio was applied three times at the low rate and twice at the high rate starting with the first application timing. All treatments were applied using an air-blast sprayer at 100 gal/A. Incidence of new blight infections on

blossoms and leaves in addition to potential phytotoxic effects of the treatments on foliage and fruit were evaluated. Data for were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.12.

RESULTS AND DISCUSSION

Laboratory studies on the toxicity of bactericides and on the interaction of *P. agglomerans* with *E. amylovora*. The quantitative activity of the antibiotics kasugamycin and streptomycin against *E. amylovora* was found to be dependent on the culture medium used. The minimum inhibitory concentration for kasugamycin was 4-19 ppm on potato dextrose agar (a high-nutrition growth medium), 5.1-5.7 ppm on nutrient agar (a moderate-nutrition growth medium) and 1.8-2.1 ppm on casitone agar (a low-nutrition growth medium). Minimum inhibitory concentrations for streptomycin were 7.3-9.5 ppm on potato dextrose agar and 1.4-1.8 ppm on nutrient agar (casitone agar was not evaluated with this antibiotic). Thus, kasugamycin is active against the fire blight pathogen at similar concentrations as streptomycin and this warranted its evaluation in field trials. The biocontrol agent in Bloomtime Bio, *P. agglomerans*, was not inhibited by streptomycin at concentrations of $\geq$50 ppm, whereas for kasugamycin the minimal inhibitory concentration was 15 ppm and no growth occurred at 50 ppm. This indicates that this biocontrol could be well incorporated into a spray program that includes streptomycin, but use of kasugamycin may affect the results of a program that uses Bloomtime Bio. Additional studies are required to evaluate these fire blight control materials in an integrated program using different products.

In interaction studies of the biocontrol agent *P. agglomerans* with *E. amylovora*, a range of concentrations of the biocontrol agent ($5 \times 10^5 - 1 \times 10^8$) was evaluated in a spiral gradient dilution assay. At both temperatures evaluated (15 and 20 C) no inhibition of growth of *E. amylovora* was observed on the high-nutrition potato dextrose agar medium. It was observed, however, that the growth rate of the biocontrol agent was much higher than that of *E. amylovora* and the final amount of bacterial growth produced on the substrate was much greater than that of the pathogen. Previously, others (Vanneste et al., 1992; Wright et al., 2001) have reported that the mode of action of *P. agglomerans* was based in part on the production of several antibiotics. The antibiotic action was only observed in a minimal growth medium that was deficient of histidine. Based on studies using mutants of *P. agglomerans*, it was concluded that other mechanisms might also be involved in the biological control action. Our studies indicate that *P. agglomerans* is highly competitive in colonizing food substrates and thus, another mode of action may be site exclusion where the biocontrol agent quickly colonizes food substrates that are no longer available to the fire blight pathogen.

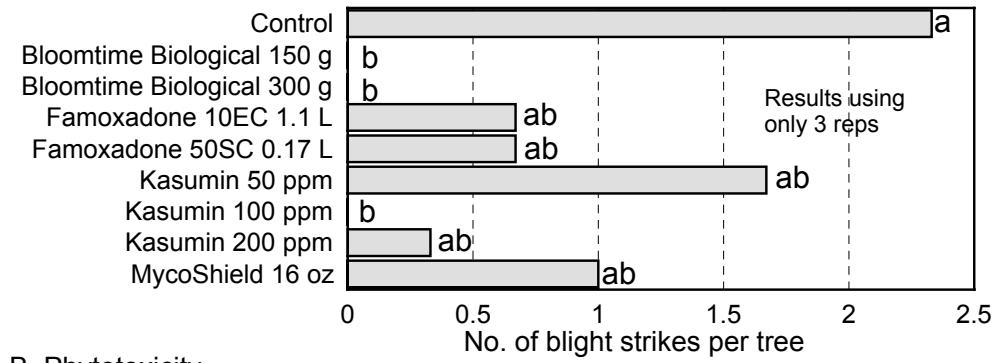
***Field studies using protective treatments during the growing season*.** Two field trials were conducted using bactericides and antibiotics as protective spray treatments, one in a commercial orchard in Marysville and one in an experimental orchard in Davis, CA. In both plots the natural incidence of disease was low in the spring of 2005. At the Marysville trial, an average of only 2.3 strikes were found on the untreated control trees at evaluation time on May 5 (Fig. 1A). All treatments reduced the incidence of disease numerically. Bloomtime Bio and the intermediate rate of kasugamycin (100 ppm) had a significant effect and no disease was observed in these latter treatments. Overall, famoxodone and streptomycin were the least effective materials tested. Phytotoxicity was observed after treatment with one of the famoxodone formulations and with the high rate (200 ppm) of Kasumin, but not with the intermediate and low rates of this antibiotic (Fig. 1B). In the last two years, Kasumin at 100 ppm has had an efficacy similar to MycoShield.

The incidence of fire blight in the Asian pear orchard at UC Davis was highly variable and no significant differences were found among the treatments. Still, the data presented in Fig. 2 show a trend for reduction in the natural incidence of disease using the high rate of Bloomtime Biological, the 100-ppm rate of Kasumin, and the 113-g (0.25 lb/A) rate of streptomycin in an orchard with no streptomycin resistance.

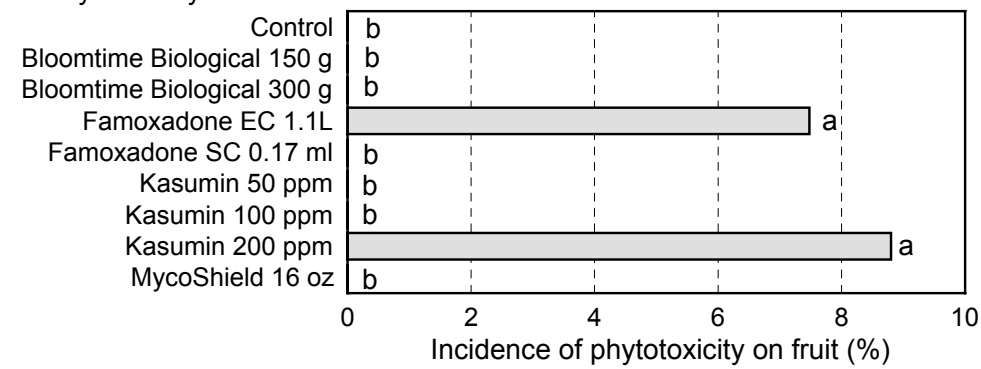
Arysta Life Sciences Corp. is the company that is supporting registration of kasugamycin for agricultural use in the United States. The antibiotic is not being used in medicine and is registered in other countries for agricultural use. Recently, the US EPA approved an Arysta Life Sciences request for an import tolerance for agricultural crops. Since September 2005, the US-EPA has approved 2006 IR-4 studies on pear for establishing a tolerance of the antibiotic for use in the United States for managing fire blight.

Fig. 1. Evaluation of new bactericides for fireblight management on Bartlett pear
Field trial in Marysville, CA - 2005

A. Disease incidence

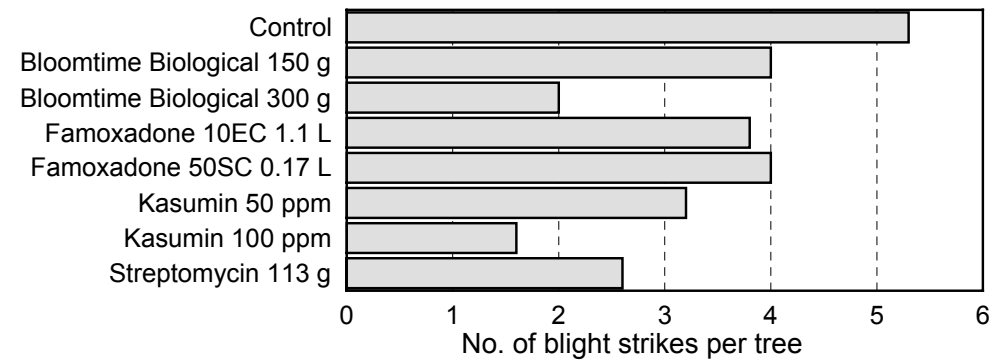


B. Phytotoxicity



Treatments were applied on: 3/11, 3/17, 3/23, 3/30, 4/14, and 4/25/05 using an air-blast sprayer at 100 gal/A. Disease was evaluated on May 5, 2005. Bloomtime Bio was applied three times at the low rate and twice at the high rate starting with the first application timing. The number of fire blight strikes was counted on each of four single-tree replications. Phytotoxicity on fruit was evaluated at harvest in July. Phytotoxicity was evident as circular lesions. In the Kasumin 200 ppm treatment phytotoxicity was also observed on some leaves but no evaluation was done.

Fig. 2. Evaluation of new bactericides for fireblight management on Asian pears
Field trial in Davis, CA - 2005



Treatments were applied on: 3/10, 3/18, 3/26, 4/1, and 4/9/05 using an air-blast sprayer at 100 gal/A. Disease was evaluated on May 10, 2005. Bloomtime Bio was applied three times at the low rate and twice at the high rate starting with the first application timing. The number of fire blight strikes was counted on each of four single-tree replications. Data presented are the average of 4 replications.