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

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ABSTRACT

1. Antibiotic resistance

- a. **Surveys** of *Erwinia amylovora* in California pear orchards were continued, but only two samples were obtained from 2 orchards in Sacramento Co. All 19 strains were sensitive to kasugamycin and oxytetracycline. One strain was moderately resistant to streptomycin, whereas the other ones were sensitive.

2. Efficacy studies with new bactericides for management of fire blight.

- a. In **laboratory studies with ornamental pear flowers**, differences in EPL formulations were detected. Mixtures of EPL with Seican or Cinnerate were more effective than EPL alone and sometimes statistically similar to Kasumin.
- b. **Field trials** were conducted under high natural disease pressure in a commercial Bartlett pear orchard and in inoculation studies with Comice and apple pear at UC Davis.
 - In the Bartlett study, all treatments were effective in reducing blight from the control. The most effective one, NUP 17010 (oxytetracycline)+Syllit, was significantly different from most other treatments (i.e., Cinnerate, Blossom Protect, QAM, JAX (A+B)+QAM, VirusShield, Kasumin 8L (organic formulation), JAX (A+B) high rate, RAA-A, and Curezin+NuFilm P). JAX (A+B) at the medium rate, Kasumin 8L or 2L mixed with Vacciplant, Instill+Problad, and Curezin+LI700 were statistically intermediately effective. Kasumin use is restricted to a maximum of four applications per season (no more than two consecutive applications) and is recommended in mixture with another bactericide to help minimize the risk for resistance development.
 - On Comice pear, JAX (A+B), Instill+Problad, FireLine, and Kasumin 8L+Vacciplant significantly reduced fire blight from the control. FireLine and Kasumin had numerically the lowest levels of disease, whereas JAX (A+B) and Instill+Problad were statistically intermediate.
 - On Shinko apple-pear, Thyme Guard, Cinnerate, and RAA were not significantly different from the untreated control. YSY+activator, JAX (A+B), and Blossom Protect formed intermediate statistical groups, whereas Kasumin 8L+Vacciplant and FireWall were the most effective and were significantly different from all other treatments.

INTRODUCTION

Fire blight caused by the bacterium *Erwinia amylovora* is the most destructive disease of pome fruit trees worldwide, especially pears. In California, prolonged rat-tail bloom contributes to a long infection period and the difficulty of management. Few effective treatments are available. An integrated program with sanitation practices and applications of chemical and biological controls is the best approach. If the disease occurs at low incidence, it may be eliminated by pruning. Regular aggressive pruning out of diseased tissue is essential for keeping inoculum levels low.

Current chemical control programs for fire blight are mostly based on protective treatments with antibiotics or copper. On Bartlett pears, copper treatments traditionally have been used only during the dormant and bloom periods because they can cause fruit russeting. Some newer formulations of copper, however, are labeled at lower metallic copper equivalent (MCE) and can be used after bloom without causing russeting. At low disease pressure, copper compounds can provide satisfactory disease control, and they can be an effective rotational or mix partner. At high disease pressure, however, copper applications generally fail to control fire blight at satisfactory levels. Therefore, copper is ranked as '+/++' in our UCIPM ratings indicating inconsistent performance depending on environmental conditions. In addition, we reported reduced copper sensitivity in strains of *E. amylovora* from pear that can also explain the moderate and inconsistent performance of copper. Lack of systemic action and low registered rates are other factors contributing to low efficacy. However, copper in the new product Instill (soluble chelated copper sulfate pentahydrate) is thought to have some systemic properties.

Treatments with the antibiotics streptomycin (STR) and oxytetracycline (OXY) have been employed for many years to manage fire blight. Continued use for many seasons and lack of effective alternative control materials resulted in resistance to develop against STR at many locations in California, mostly in Sacramento Co. Strains with moderate plasmid-based resistance and strains with chromosome-based high resistance have been identified. The incidence of STR resistance has been fluctuating widely among years, and this has been attributed to disease pressure and the intensity of STR use. Strains of *E. amylovora* with reduced sensitivity to OXY were found several times during our surveys. Starting in 2018, however, we detected strains with high resistance to this antibiotic for the first time at several locations in Sacramento Co. These strains were also highly resistant to STR, they were similarly virulent as sensitive strains, and they competed well in co-inoculation studies with sensitive strains. This is a serious concern, but it is not known if these resistant strains will persist in the field in the absence of selection pressure. We molecularly characterized this new resistance phenotype and demonstrated that tet, strA, and strB resistance genes were acquired by the novel conjugative IncX plasmid pX11-7. These results were published (Phytopathology 113:2165-2173). Resistance to kasugamycin that became available in California in 2018, however, has never been detected in our surveys.

Surveys on antibiotic resistance were continued in 2024 but samples were only obtained from two orchards in Sacramento Co. Concerns have been expressed by regulatory agencies regarding the use of antibiotics in agriculture, but kasugamycin is not used in

human or animal medicine and has a different mode of action from STR or OXY (no cross-resistance). In 2020, after 7 years of environmental resistance monitoring with no detected shifts in sensitivity among non-target bacteria, the EPA has suspended this requirement for the kasugamycin registration.

Table 1. Bactericides evaluated in studies on fire blight 2024

Category	FRAC Code	Active ingredient	Trade name/Code
Antibiotics	24	kasugamycin	Kasumin 2L, 8L
	41	oxytetracycline	FireLine 45, Mycoshield NUP-17010
	25	streptomycin	FireWall
Natural products	BM 01	<i>Acacia</i> sp. extract	QAM
	BM 01	bacterial metabolite	RAA-A
	BM 01	cinnamaldehyde	Seican
	BM 01	cinnamaldehyde + EPL	JAX (A+B)
	BM 01	cinnamon oil	Cinnerate
	BM 01	<i>Lupinus alba</i> extract	ProBlad
	BM 01	laminarin	Vacciplant
	BM 01	thyme oil	Thyme Guard
Biocontrols	BM 02	<i>Aureobasidium pullulans</i>	Blossom Protect
	BM 02	<i>Papiliotrema terrestris</i> PT22AV	YSY
Food preservative	---	ε-poly-L-lysine, EPL	food additive
Other antimicrobials	M01	copper-octanoate	Cueva
	M01	Soluble chelated copper sulfate pentahydrate	Instill
	U12	dodine	Syllit
	P07/33 + M01	phosphite + copper + zinc	Curezoin
	---	experimental	PST
	---	oxidizer	VirusShield

With the current emphasis on identifying alternative bactericides, we continued our evaluations of exempt-from-tolerance and potential organic compounds in 2024 (Table 1). These included the food preservative ε-poly-L-lysine (EPL) that was formulated as a mixture with cinnamaldehyde (JAX (A+B)) by formulation chemists of Summit Agro, a potential registrant. Other compounds evaluated were extracts from an *Acacia* sp. (QAM) or *Lupinus alba* (ProBlad), cinnamon (Cinnerate) and thyme (Thyme Guard) oils, cinnamaldehyde (Seican), a bacterial metabolite (RAA-A), and a new biocontrol (YSY). All these potentially could qualify as biopesticides with the EPA and ultimately as organic compounds that could be OMRI certified. Therefore, we continue to try to improve their efficacy by using selected additives. Our goal is to develop effective rotational programs for organic farming practices with the use of copper, biologicals, and innovative strategies such as registering food preservatives and OMRI-approved natural products (including kasugamycin and EPL). We also will work on conventional programs with the use of antibiotics alone or in mixtures with copper, biologicals, or natural products during bloom or as cover sprays during early fruit development.

OBJECTIVES

1. Continue resistance surveys for streptomycin, oxytetracycline, and kasugamycin in *E. amylovora* populations from pear orchards in California.
 - a) Collaborate with farm advisors, PCAs, and growers
2. Evaluate and optimize the performance of antibiotics, new formulations of copper, natural products, biocontrols, and GRAS food additives in combination with adjuvants and low concentrations of copper. Treatments identified as effective in small-scale laboratory studies will be included in field studies.
 - a) Kasumin and new formulations of oxytetracycline in combination with exempt-from-tolerance antimicrobials including copper sulfate or dodine (Syllit).
 - b) Low-copper concentration products such as copper sulfate pentahydrate (MasterCop) and copper octanoate (Cueva) alone and in mixtures.
 - c) New formulations of ϵ -poly-L-lysine and nisin (KFD 623, and KFD 622, respectively) in combination with capric/caprylic acids (Dart), essential oils (Cinnerate), or cinnamaldehyde (JAX), KFD.
 - d) The FDA GRAS plant extracts from *Lupinus albus* (ProBlad Verde), cinnamaldehyde (Seican), cinnamon oil (Cinnerate), thyme oil (ThymeGuard), a soap bark tree extract (BTS), and an *Acacia* sp. extract (QAM). Some of these are already OMRI-certified.
 - e) New biocontrols derived from yeasts (YSY) or bacteria and bacterial fermentation products (such as the metabolite RAA).

MATERIALS AND METHODS

Isolation and culturing of *E. amylovora*. Samples with fire blight symptoms were obtained in early summer of 2024 from two pear orchards in Sacramento Co. Infected fruit, peduncles, and twigs were cut into small sections and incubated in 1 ml of sterile water for 15 to 30 min to allow bacteria to diffuse out of the tissue. Suspensions were streaked onto yeast extract-dextrose-CaCO₃ agar, and single colonies of *E. amylovora* were transferred. A total of 19 strains were obtained and evaluated for their sensitivity to antibiotics.

Laboratory studies on the toxicity of bactericides against *E. amylovora*. Using the spiral gradient endpoint method, radial concentration gradients of STR, OXY, or kasugamycin were established in nutrient agar by spirally plating stock concentrations of each antimicrobial using a spiral plater. After radially streaking out suspensions of the test bacteria (10 μ l of 10⁸ cfu/ml as determined by measuring optical density at 600 nm) along the concentration gradient, plates were incubated for 2 days at 25°C. Measurements were taken visually for the minimal concentration that inhibited growth by >95% (MIC). The actual antibiotic concentrations were obtained by entering the radial distances of inhibition (measured from the center of the plate) into the Spiral Gradient Endpoint computer program.

Evaluation of new bactericide treatments. Preliminary early-season studies focusing on EPL and EPL + cinnamaldehyde nisin were conducted in the laboratory on flowering ornamental pear (*Pyrus calleryana*). Twigs were placed into 100-ml Erlenmeyer flasks

containing water with 20 ppm gibberellic acid (to delay senescence), treated using an air-nozzle hand sprayer, allowed to air-dry, and were spray-inoculated with *E. amylovora* (5x10⁶ cfu/ml). The incidence of fire blight was determined after 5 days based on the number of blackened flowers of the total number of flowers.

Comparative field studies were conducted on cv. Bartlett pear in a commercial orchard and on cvs. Shinko apple-pear and Comice pear at UC Davis. Three or four applications were done with timings based on temperature, rainfall, and host development. The relative efficacy of protective treatments was evaluated including new formulations of copper (e.g., Instill, Cueva) and zinc (Curezin), food additives (poly-L-lysine - EPL), biocontrols (e.g., Blossom Protect, YSY), natural products (e.g., Cinnerate, Seican, ProBlad, PST, QAM, RAA, Vacciplant), and other products (e.g., VirusShield) as compared to antibiotics (kasugamycin – Kasumin 2L or 8L, streptomycin, and oxytetracycline). These products were evaluated alone or in selected mixtures (e.g., Seican+EPL – JAX (A+B), JAX (A+B)+QAM, Kasumin+Vacciplant, Instill+ ProBlad) to develop integrated programs for resistance management. Incidence of blight infections on flowers and shoots in addition to potential phytotoxic effects of the treatments (e.g., fruit russeting) were evaluated. Treatments were replicated on four trees. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 9.4.

RESULTS AND DISCUSSION

Survey of antibiotic sensitivity in *E. amylovora* strains from pear in California in 2024. Due to lack of sample submissions by farm advisors and PCAs, only 19 strains of *E. amylovora* were obtained from two locations in Sacramento. All were sensitive to OXY and kasugamycin. All were sensitive to STR except for one strain that was moderately resistant (Table 2). Thus, only limited information is available on antibiotic resistance in the fire blight pathogen in 2024. Our previous detections of STR and OXY resistance and the need to protect the efficacy of kasugamycin stress the high importance of resistance management.

Table 2. Sensitivity of *E. amylovora* strains from pear orchards in California to streptomycin, oxytetracycline, and kasugamycin in 2024

County	Orchard No.	No. isolates	In vitro sensitivity (MIC)		
			Streptomycin	Oxytetracycline	Kasugamycin
Sacramento	1	9	1 MR 8 S	S	S
Sacramento	2	10	S	S	S
total	2	19			

Sensitivity to the three antibiotics was determined using the spiral gradient endpoint method.

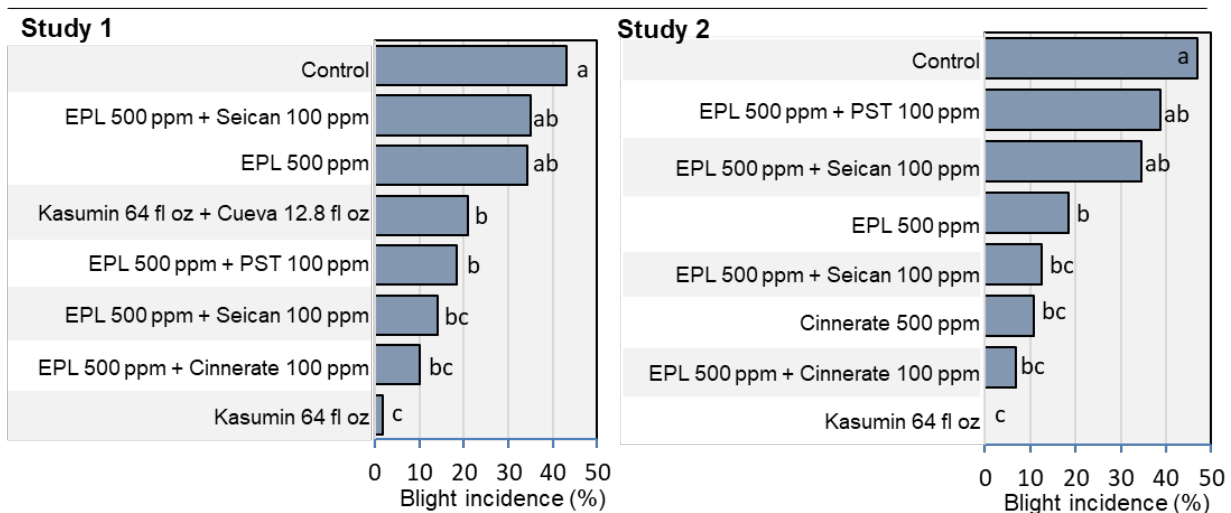
MIC rating: S = 0.6 - 1.4 ppm

MR = 20 - 35 ppm

Antibiotics should only be used in mixtures or rotations, the number of applications of each per season should be limited to two if possible, new alternatives (e.g., early-season copper, biologicals) should be used, and new options should be developed.

Laboratory studies with ornamental pear flowers to evaluate new bactericide treatments. In two early-season laboratory studies with ornamental pear flowers, two formulations of EPL were compared by themselves and in mixtures with other bactericides. EPL-1 generally was significantly less effective than EPL-2 in reducing incidence of fire blight, indicating that sources of EPL need to be carefully selected in the planned design of field formulations. As previously shown, mixtures of EPL with Seican or Cinnerate were more effective than EPL alone, and mixtures including EPL-2 were statistically similar to Kasumin (Fig. 1, Studies 1 and 2). These promising results were used to design the treatment lists for our field trials later in the spring.

Fig. 1. Efficacy of bactericides for management of fire blight of ornamental pear flowers in laboratory studies, 2024

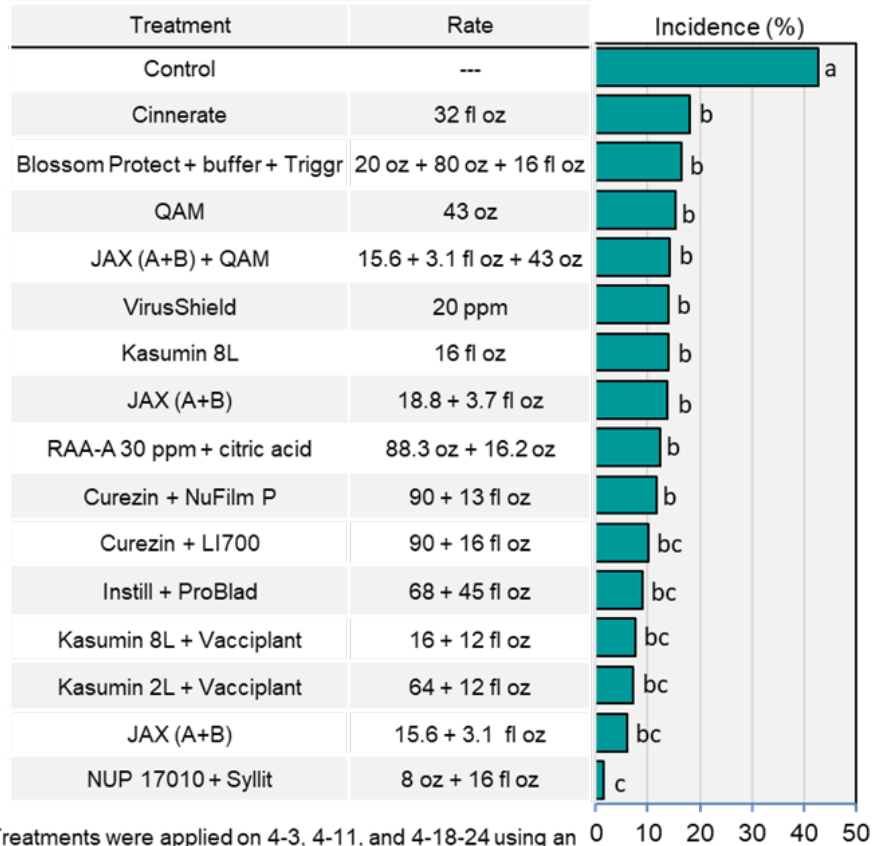


Flowering twigs of ornamental pear (*Pyrus calleryana*) were placed into 100-ml Erlenmeyer flasks containing water with 20 ppm gibberellic acid. Flowers were treated using a hand sprayer and were spray-inoculated with *E. amylovora*. Twigs were covered with plastic bags, and the incidence of fire blight was determined after 5 days based on the number of blackened flowers of the total number of flowers evaluated

Field studies to evaluate new bactericide treatments. In all studies, treatments with the antibiotics streptomycin, oxytetracycline, or kasugamycin numerically resulted in the lowest incidence of blight, but several alternative treatments showed promising results with efficacy statistically similar to these antibiotics.

In a trial in a commercial Bartlett orchard, all treatments effectively reduced fire blight from the control where an average of 42.8% of flower clusters was affected at the evaluation date (Fig. 2). The most effective treatment, NUP 17010+Syllit, reduced disease incidence to 1.4% and was significantly different from most other treatments (i.e., Cinnerate, Blossom Protect, QAM, JAX (A+B)+QAM, VirusShield, Kasumin 8L (an organic formulation), JAX (A+B) high rate, RAA-A, and Curezin+NuFilm P) and the untreated control. JAX (A+B) at the medium rate, Kasumin 8L or 2L mixed with Vacciplant, Instill+ProBlad, and Curezin+LI700 were statistically intermediately effective, and these treatments and the new formulation oxytetracycline and the organic kasugamycin mixed with Vacciplant (laminarin) were identified as the most effective in reducing the disease to <10% (Fig. 2).

Fig. 2. Efficacy of bactericides for management of fire blight of cv. Bartlett pears, Live Oak, 2024



On Comice pear where trees were inoculated with *E. amylovora*, Thyme Guard was not significantly different from the untreated control (Fig. 3). The other treatments including JAX (A+B), Instill+Problad, FireLine, and Kasumin 8L+Vacciplant significantly reduced fire blight from the control. FireLine and Kasumin had numerically the lowest levels of disease, whereas JAX (A+B) and Instill+Problad were statistically intermediate.

On Shinko apple-pear, trees were inoculated twice at full bloom. Thyme Guard, Cinnerate, and RAA were not significantly different from the untreated control (Fig. 4). YSY+activator, JAX (A+B), and Blossom Protect formed intermediate statistical groups, whereas Kasumin 8L+Vacciplant and FireWall were the most effective and were significantly different from all other treatments and from the untreated control.

Conclusions. The organic kasugamycin formulation Kasumin 8L, JAX (A+B), Curezin with either NuFilm P or LI-700, RAA-A, and VirusShield were identified as new treatments with commercially acceptable efficacy in reducing fire blight in our 2024 trials. Overall, Kasumin 8L, the natural products Cinnerate, RAA-A, and JAX (A+B) were consistently effective on all pear cultivars. JAX (A+B) is a mixture of EPL and cinnamaldehyde (Seican; an organically approved commercial product) that the registrant is interested in registering as an organic mixture bactericide. Blossom Protect+Buffer continued to perform well with mostly

Fig. 3. Efficacy of new bactericides for management of fire blight of cv. Comice pear after inoculation in a field study at UC Davis 2024

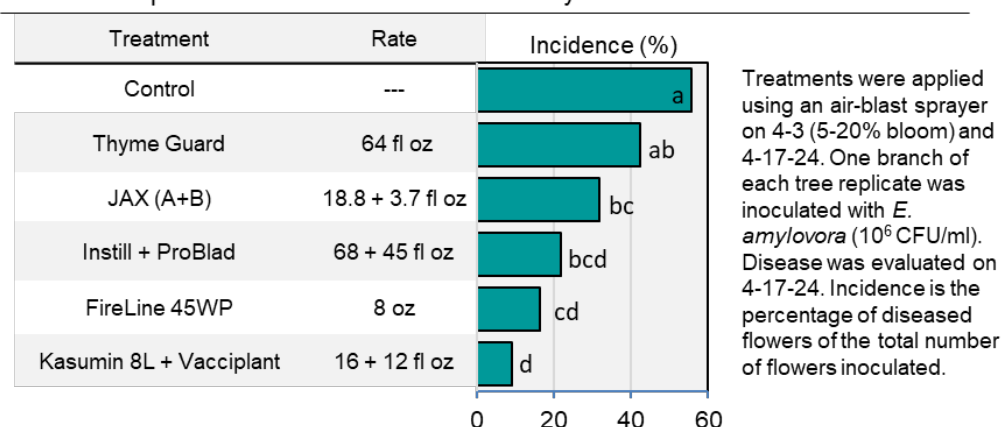
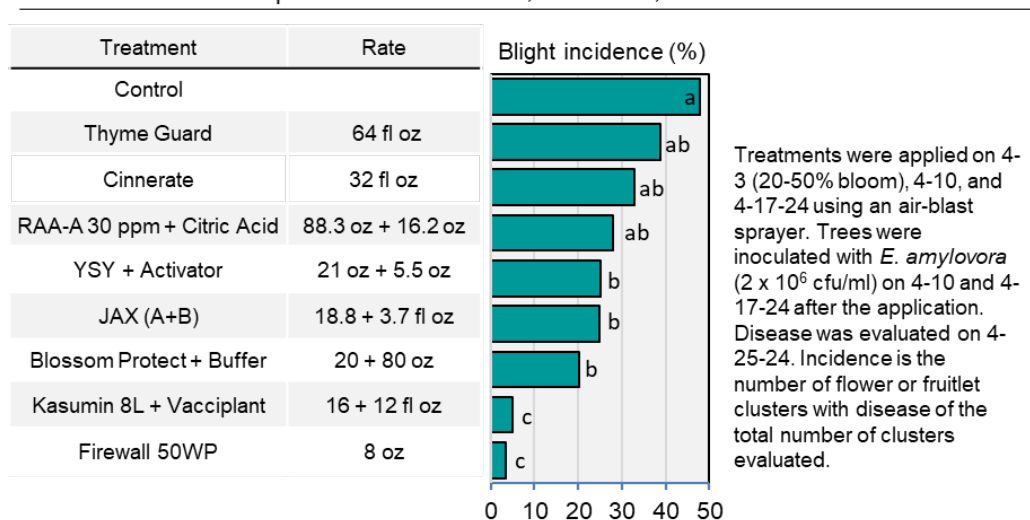


Fig. 4. Efficacy of bactericides for management of fire blight of cv. Shinko apple pears after inoculation, UC Davis, 2024



intermediate efficacy. New alternative treatments like JAX, Cinnerate, and RAA products need to be pursued with registrants in collaboration with us to continue evaluation of their efficacy and consistency as compared to conventional antibiotics with ultimate registrations in the United States as organically approved bactericides.

The most consistently effective treatments in our trials were the two Kasumin products (one conventional and one potentially organic) mixed with Vacciplant, as well as Fireline and the new 45% NUP oxytetracycline formulation. Thus, the three registered antibiotics streptomycin, oxytetracycline, and kasugamycin were again highly and consistently effective. Efforts are underway to obtain an organic formulation of Kasumin 8L and to register it in the United States in cooperation with UPL.