DESCRIPTION:	**Evaluation of New Bactericides for Control of Fireblight of Pears Caused by *Erwinia Amylovora***
PROJECT LEADER:	**James Adaskaveg, UC Riverside**
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Project Title:	Evaluation of new bactericides for control of fire blight of pears caused by *Erwinia amylovora*
Project Leader:	Dr. James E. Adaskaveg, Department of Plant Pathology, University of California, Riverside, CA 92521 (909) 787-7577
Cooperators:	Dr. H. Förster and D. Thompson

SUMMARY

1. Both DOW–01 (technical) and Starner were highly effective against *E. amylovora* in *in vitro* assays.
2. Starner was effective against fireblight in field studies during the low-disease-incidence-year 2001. Valent Biosciences asked us to submit the material into the IR-4 program. We complied, but then the company retracted the material for development until more efficacy data are obtained nationwide.
3. DOW-01 and DOW 117-2 were effective as a blossom and foliar spray treatment but not effective as a trunk and branch injection treatment in reducing foliar fire blight symptoms in one field trial in an experimental orchard at UC Davis. No data were obtained from the commercial orchard due to low disease incidence. Foliar treatments with Starner or DOW-01 reduced disease in field studies, but low levels of russeting were observed on fruit but not leaves as compared to the check.
4. New copper formulations were effective in reducing fireblight in field trails in an experimental orchard. These need to be tested in a commercial orchard with large fruit samples.
5. None of the other treatments evaluated, including terramycin (Mycoshield), gave satisfactory disease control when applied as an injection treatment. Cankers of treated trees still contained viable inoculum in two field trials with no significant reduction in the recovery of the pathogen after treatment application as compared to isolations made before application or compared to the check.

INTRODUCTION

Fire blight, caused by the bacterium *Erwinia amylovora,* is a very destructive disease of pome fruit trees worldwide. Of all the pomaceous crops, the disease is most severe on pears. The name 'fire blight' is descriptive of the most characteristic symptom of the disease, a blackening of twigs, flowers, and foliage as though they have been swept by fire. 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 blossom, the bacteria spread into the peduncle and spur. 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. If uncontrolled, eventually 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. Copper treatments are widely used only during dormant and bloom periods because phytotoxic effects commonly occur on fruit as russeting. Streptomycin, an antibiotic for 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 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 have to be developed to combat this destructive disease. These materials should be locally systemic and not phytotoxic and should target multiple sites of action within the bacterial pathogen or have a mode of action different from currently used bactericides. Materials with different modes of action could then be incorporated into a resistance management program. Systemic formulations could be used in trunk and branch injections to eliminate the primary inoculum of the pathogen, while protective treatments could be used topically to prevent spread and infection of secondary inoculum. During the past years we have identified a broad spectrum biocide (DOW-01 – Dow Chemicals) that is registered as a water treatment, and that is highly toxic at low concentrations in *in vitro* assays against bacterial plant pathogens (e.g. species of *Xanthomonas*), is very active against bacterial spot of peppers in greenhouse experiments, and has significantly reduced walnut blight incidence in field trials. Another compound evaluated by us recently is Starner, an antibiotic that also showed promising results in walnut blight and fire blight control in preliminary field trials. Thus, we conducted field and laboratory experiments for the evaluation of new potential fire blight control chemicals.

OBJECTIVES

I. Evaluate the toxicity of alternative, non-copper based chemicals to *E. amylovora*: DOW-01, Starner, and compare efficacy to copper and streptomycin.

A) Laboratory in vitro tests to evaluate the bactericidal activity: filter paper disk assay and direct contact assay.

B) Field studies using highly water-soluble formulations in trunk and branch injections on ornamental pear trees and in commercial pear orchards with a high natural fire blight incidence. Applications during tree dormancy and before bloom.

C) Field studies using less water-soluble formulations in protective spray treatments. Weekly applications will be made starting at bloom.

D) Field studies with mixtures of antibiotics and biocides. Weekly applications will be made starting at bloom.

MATERIALS AND METHODS

Laboratory in vitro tests to evaluate the bactericidal activity of new chemicals. In a direct contact assay, bacterial suspensions (10^7 cfu/ml of an *E. amylovora* isolate from pear) were incubated with selected concentrations of DOW-01 technical (5, 20, 100 ppm final concentration). After selected times (0.5, 1, 5 min) the bacterial-test substance mixture was diluted 1:1000 with sterile water and the resulting suspension was plated out onto nutrient agar using a spiral plater. Numbers of bacterial colonies on the agar plates were counted after 2-3 days of incubation. Colony numbers of the chemical treatments were compared to those of the water control. The toxicity of Starner was evaluated in amended agar media assays. For this, Starner was incorporated into nutrient agar at concentrations of 1 or 10 ppm. Diluted bacterial suspensions were spread onto the plates and colonies were counted after 2 days.

Field studies using bactericides and antibiotics in trunk and branch injections. In a small-scale field trial at UC Davis, water-soluble formulations of DOW-01 (117-2, EC; 5,000 ppm each), the antibiotic Starner (70-90 g/L), or terramycin (60-80 g/L) were injected into trunks and large branches of 6-year-old pear trees in the spring at bloom time (March 6, 2001). All trees had severe fire blight symptoms with extensive cankers. Injections were done using a high-pressure injector, and 2 injections of 50 ml each were made for each tree. Similar injections were done in a 20-year-old commercial pear orchard. There were three single-tree replications for each treatment. Isolations from cankers were made before and after application and were compared to the non-treated check. In the commercial orchard, cankers on each tree were evaluated after approximately two weeks; whereas in the experimental orchard, three to four branches with cankers on each tree were evaluated after approximately three weeks. Trees in the experimental orchard were also evaluated for twig strikes on May 31, 2001. Data for chemical control were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.12.

Field studies using bactericides and antibiotics as protective spray treatments. In field studies, the relative efficacy of protective treatments including experimental antibiotics and bactericidal treatments were compared to terramycin and copper-containing compounds. The following treatments were applied at the UC Davis orchard: Starner (0.33 lb a.i. or 0.5 lb a.i.), DOW-01-EC (500 or 1000 ppm), DOW 117-2 (1000 ppm), Mycoshield (200 ppm ai), Cuprofix D (10 lb a.i.), Cuprofix MZ (7.5 lb a.i.), Bioacumen (5%), Vacciplant (400 ml), and Vacciplant + Kocide 101 (400 ml + 8 lb). Treatments were applied using an air-blast backback sprayer at 100 gal/A on March 22, March 26, March 30, April 10, and May 4 2001 based on a temperature-threshold model. Disease incidence was evaluated on May 23 as the number of fire blight strikes on each of four single-tree replications. Data were analyzed using analysis of variance and LSD mean separation procedures of SAS 6.12.

Another trial was established in a commercial orchard in Marysville where fire blight caused crop losses in 2000. In this trial applications were done according to the Degree-Hour-Chart for fire blight risk on 6 single-tree replications for each treatment. Application dates were: March 21 (50-80% bloom), March 24 (full bloom), March 28 (full bloom/petal fall), April 2, April 10, April 18, April 25, and May 4. The same treatments were applied as in the Davis orchard with the exception of DOW 117-2, Bioacumen, and Vacciplant.

RESULTS

Laboratory* in vitro *tests to evaluate the bactericidal activity of new chemicals. In direct contact assays exposing *E. amylovora* to solutions of technical grade of DOW-01 for selected times, bacteria did not survive after a 5-min-exposure to 5 ppm DOW-01 or a 30-sec-exposure to 20 ppm of the chemical. Survival after a 30-sec-exposure or a 1-min-exposure in 5 ppm was 31.5% or 9.6%, respectively, as compared to the water control. In contrast, in similar experiments done with the walnut blight pathogen *Xanthomonas campestris* pv. *juglandis* higher concentrations of the chemical or longer exposure times were required to inhibit bacterial survival. With this pathogen, no bacteria survived after a 5-min-exposure at 20 ppm. Thus, DOW-01 is more active *in vitro* against the fire blight pathogen *E. amylovora* than it is to the walnut blight pathogen *X. campestris* pv. *juglandis.*

Two fire blight isolates from pear were tested in amended agar media assays using Starner. On nutrient agar that was amended with 1 ppm of the antibiotic no bacterial colonies were observed (100% inhibition), whereas 2.4×10^5 colonies were counted on the control plates. As with DOW-01, the fire blight pathogen was more sensitive to the chemical than the walnut blight pathogen. With *X. campestris* pv. *juglandis* there was a 50.6% inhibition at 1 ppm Starner and a 100% inhibition at 10 ppm.

Field studies using bactericides and antibiotics in trunk and branch injections. None of the injection treatments in the UC Davis or the commercial orchard eradicated the disease and *E. amylovora* still could be isolated from cankers after injection treatment. No significant reduction in the recovery of the pathogen from cankers was measured between before and after application samplings for each treatment or between the treatments and the check. In the experimental orchard, recovery ranged from 17% to 66% between the treatments. In the commercial orchard, recovery in re-isolations ranged from 50% to 100% for before and after application. The number of fireblight blossom/shoot infections (e.g., strikes) was also evaluated for the injected trees. For injection studies, there was no difference between any of the treatments as compared to the check based on the number of fire blight strikes per tree (Table 1). The 1000-ppm concentration of DOW-117-2 and Mycoshield slightly reduced the number of strikes from 2.3 per tree in the control to 1.7 in the treated trees but again, there was no significant difference from that of the check treatment. Concentrations of DOW 117-2 between 5,000 and 20,000 ppm increased the number of strikes as compared to the control. It may be, however, that some of the strikes at these high chemical concentrations actually were caused by phytotoxic effects of DOW-01. In the commercial orchard, the incidence of blight was very low and no disease incidence data were obtained.

Table 1. Injections of pear trees at UCD for fire blight control 2001

Treatment	Rate	Number of strikes/tree	LSD
Control	---	2.3	ab
DOW 117-2	20,000 ppm	5.0	a
DOW 117-2	10,000 ppm	4.7	a
DOW 117-2	5000 ppm	4.0	ab
DOW 117-2	1000 ppm	1.7	b
DOW 117-2	500 ppm	4.0	ab
DOW 117-2	250 ppm	3.0	ab
DOW-01 EC	5000 ppm	2.7	ab
Starner	70g/L	3.0	ab
Mycoshield	60g/L	1.7	b

*- Two 50-ml-injections for each chemical were done on each tree on March 6, 2001.The number of fire blight strikes were counted on each tree on May 31, 2001. Values followed by the same number are not significantly different based on an analysis of variance and LSD mean separation procedures of SAS version 6.12.

Field studies using bactericides and antibiotics as protective spray treatments. Five protective spray treatments with biocides and antibiotics were applied at the UC Davis orchard from the end of March to early May based on a temperature-threshold model. All treatments significantly reduced the incidence of fire blight based on the number of strikes per tree (Table 2). Furthermore, there was no significant difference between the treatments that ranged from antibiotics, biocides, to copper- and copper/silver based treatments. Thus, the spray treatments were more effective than the injection treatments. Because the number of fruit in this experimental orchard was very low, no evaluations for phytotoxicity or russeting effects on fruit caused by the treatments were made. No phytotoxicity was observed on leaves for any of the treatments.

In the commercial orchard disease incidence was very low and no data could be obtained from the treatments. As in the experimental orchard, treatments were applied based on a temperature-threshold model. Eight applications of each treatment were made from late March through early May. Phytotoxicity evaluations on pear fruit were made at harvest. Most treatments caused russeting with the exception of Mycoshield (Table 3). As compared to the check, no difference in russeting was observed between the high rate of Starner and low rate of the DOW-01 materials. Of the fruit showing russeting in the DOW-01 treatment, russeting often developed as a small circular or ring-like symptom (1 per fruit). The high rate of DOW-01 had 10%, the low rate 2.2%, and the check had 0% of the fruit showing these symptoms. The materials with copper caused the highest russeting.

Table 2. Efficacy of new bactericides and biological treatments for management of Fireblight on Bartlett pears in Solano Co.

Treatment	Rate/A	Disease Incidence (%)	LSD
Control		7.1	a
Starner	0.33 lb ai	1.8	b
Starner	0.5 lb ai	1.0	b
DOW-01 EC	500 ppm	2.3	b
DOW-01 EC	1000 ppm	2.7	b
DOW-117-2	1000 ppm	2.0	b
Cuprofix D	20/10 lbs	1.5	b
Cuprofix MZ	15/7.5 lbs	0.7	b
Mycoshield	200 ppm	0.9	b
Bioacumen	5%	2.0	b
Vacciplant	400 ml	3.7	b
Vacciplant + Kocide 101	400 ml + 8 lbs	0.7	b

* - Treatments were applied on: 3-22, 3-26, 3-30, 4-10, 5-4-01.

** - Disease was evaluated on 5-23-01. Disease Incidence is the average number of Fireblight strikes counted on each of four single-tree replications. No phytotoxicity was observed on leaves in any treatment (No fruit was available).

Table 3. Phytotoxicity of new bactericides for management of fireblight on harvested Bartlett pears in Yuba Co.

Treatment	Rate/A	Russetting Incidence (%)	LSD
Control	----	8.2	d
Starner	0.33 lb ai	26.9	b
Starner	0.5 lb ai	13.5	cd
DOW-01 EC	500 ppm	14.4	cd
DOW-01 EC	1000 ppm	21.2	bc
Cuprofix D	20/10 lbs	39.9	a
Cuprofix MZ	15/7.5 lbs	31.0	ab
Mycoshield	200 ppm	2.9	e

* - Application dates were: March 21 (50-80% bloom), March 24 (full bloom), March 28 (full bloom/petal fall), April 2, April 10, April 18, April 25, and May 4.

** - Russetting is expressed as the incidence of russetting per box of fruit (avg. 90 fruit/box) and there were 6 boxes (6 single tree replications) per treatment.