Project Title: **Evaluation of new bactericides for control of fire blight of pears caused by *Erwinia amylovora***

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SUMMARY

1. For a third year, blossom and foliar spray treatments with the DOW bactericide effectively reduced the incidence of fireblight in two field studies. Efficacy was similar to the industry standard terramycin. Three formulations of the DOW bactericide that were evaluated in one of the plots were similarly effective.
2. The acidified hydrogen peroxide Zerotol also significantly reduced the incidence of fireblight with a similar efficacy as terramycin.
3. Phytotoxicity as measured by a general fruit russeting was not significantly different between the control and the terramycin and DOW biocide treatments, but was increased by Zerotol. The DOW biocide and Zerotol treatments also caused another type of fruit symptoms with circular russeting lesions.

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. 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

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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 in California, and that is highly toxic at low concentrations in *in vitro* assays against bacterial plant pathogens (e.g. species of *Xanthomonas* and *Erwinia*), is very active against bacterial spot of peppers in greenhouse experiments, and has significantly reduced walnut blight incidence in field trials. In the first two years of our fire blight trials (2001 and 2002) we found that formulations of this compound significantly reduced the incidence of the disease, similar to terramycin (Mycoshield). Thus, the DOW bactericide was evaluated again in 2003. Other compounds evaluated previously include the antibiotic Starner and the activated host resistance compound Vacciplant. Although very effective, Starner was not included into our trials in 2003 because the registration of this antibiotic is not being supported by the registrant or by the US-EPA. The efficacy for Vacciplant was inconsistent in our experiments. Because acidified hydrogen peroxide compounds (e.g. Zerotol) have shown efficacy against other bacterial diseases (e.g., walnut blight), Zerotol was included into our 2003 fireblight trials. Thus, in 2003 we conducted additional field experiments for the evaluation of new potential fire blight control chemicals.

OBJECTIVES

I. Evaluate the toxicity of new agriculturally registered acidified peroxide formulations (e.g., Zerotol, Oxidate) and alternative, non-copper based chemicals to *E. amylovora*: formulations of the DOW material (DBNPA), silver-based compounds (e.g., Axenohl), and the SAR compound Vacciplant as compared to terramycin.
   A) Laboratory in vitro tests to evaluate the bactericidal activity: Direct contact assays, filter disk assays, amended agar assays.
   B) Field studies with protective spray treatments. Applications will be made based on existing Fireblight forecasting models starting at bloom.

II. Measure residues of the DOW material on fruit over time (Tentative objective based on EPA requirements).
   A) Develop in collaboration with DOW Chemical Company a residue procedure on pear fruit for the DOW compound.
   B) Evaluate residues of the DOW material on pear fruit at selected time intervals after application.

MATERIALS AND METHODS

Field studies using protective treatments during the growing season. In small-scale field studies, the relative efficacy of protective treatments including bactericidal treatments (e.g., the DOW compound DB 918-48C), and a new acidified, hydrogen peroxide material (e.g., Zerotol) was compared to terramycin (e.g., Mycoshield). Concentrations that were evaluated were based on laboratory tests and current product registration rates for other uses. An adjuvant Bond (2 pt/A) was used with the DB 918-48C treatments. Trials were established at UC Davis and in a commercial orchard in Marysville where fire blight caused crop losses in 2000-2001. In the Marysville plot, treatments were applied on 3/24, 3/31, 4/7, 4/11, 4/18, 4/30, 5/7, 5/14, and 5/21/03. In the Davis plot, treatments were applied on 3/29, 4/11, 4/18, and 4/25. In both plots treatments were applied using an air-blast sprayer at 100 gal/A. using an air-blast sprayer at 100 gal/A. Tree growth and incidence of new blight infections on blossoms and leaves in addition to potential phytotoxic effects of the treatments will be evaluated. For this, infected shoots were removed periodically during the spring season. The number of fire blight strikes was counted on each of four single-tree replications and disease incidence

determined for each tree and data was accumulated throughout the season. Disease was evaluated May 12 and May 27, 2003 in the Marysville plot and on May 13, 2003 in the Davis plot. Phytotoxicity evaluations on pear fruit were made at harvest in the first orchard. For this 100 fruit from each tree were randomly evaluated for the incidence of russeting and for fruit phytotoxicity symptoms as described in last years report. Treatments will be replicated four times on different trees. Data for chemical control will be analyzed using analysis of variance and LSD mean separation procedures of SAS 6.12.

RESULTS AND DISCUSSION

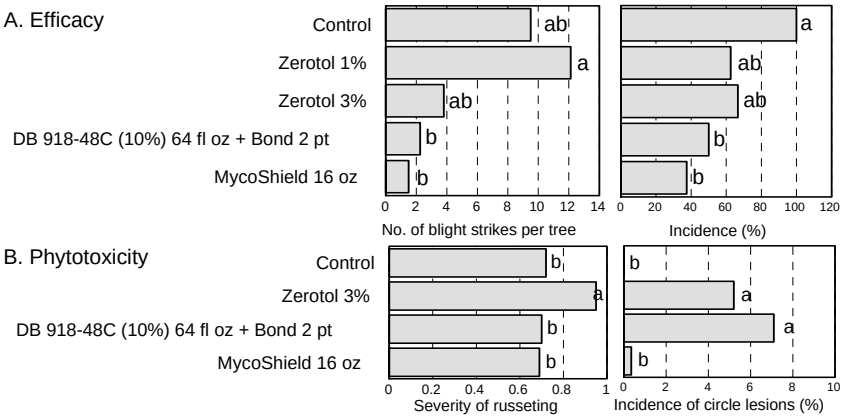
Two field trials on Bartlett pear were conducted using bactericides and antibiotics as protective spray treatments, one in a commercial orchard in Marysville and one in an experimental orchard in Davis, CA. Nine treatments of Zerotol, the DOW bactericide, or MycoShield between the end of March to May were applied in the Marysville orchard based on a temperature-threshold model. In the Davis orchard four treatments were applied based on forecasted rains. The Marysville orchard was evaluated twice (May 12 and May 27) and significant differences were observed in the total number of fire blight strikes per tree between most of the treatments and the control (Fig. 1A). The most effective treatments were Mycoshield and the DB 918-48C treatments that significantly reduced the incidence and average number of strikes per tree from that of the untreated control. Furthermore for these treatments, ratings of phytotoxicity in the form of fruit russeting were not significantly different from the control (Fig. 1B). The Zerotol 1% treatment had the most variable performance with a similar average number of strikes and incidence of disease per tree as compared to the untreated control (Fig. 1A). The Zerotol 3% treatment was also similar but a trend for a lower number of strikes per tree was observed. The rate selected for this product was based on registered rates for other crops. A higher range of rates needs to be evaluated, however, a significantly higher level of fruit russeting occurred in our ratings of treated fruit (Fig. 1B). Additionally both the Zerotol 3% and the DB 918-48C treatments caused a higher level of phytotoxicity ‘circles’ on the lower side of the fruit that developed in the form of faint pigmented circles or rings approximately 10-15 mm in diameter. These symptoms were consistently observed in the last two years DB 918-48C was evaluated. In the second orchard, all of the treatments significantly reduced the incidence of disease as compared to the control (Fig. 2). Disease incidence was 48.5% in the control and 17 to 26% in the treatments. There was no difference in efficacy for the three formulations of the DOW bactericide and no difference between the two rates of the DB 918-48C formulation. No evaluations for russeting effects on fruit were made in the second orchard because the number of fruit in this experimental orchard was very low. No phytotoxicity was observed on leaves for any of the treatments.

The DOW DB 918-48C bactericide was effective against fire blight and performed similarly to Mycoshield but neither could prevent the disease from developing. In both treatments, new infections continued to develop during the season, although applications were applied whenever the disease prediction model indicated a high-risk level of accumulated degree-hours. Thus, the DOW material does not eradicate the disease and is probably not very persistent. The non-persistence of the chemical, however, can also be considered a positive characteristic. The chemical quickly dissipates, potentially leaving no residues in the developing pear fruit. This aspect of the bactericide possibly could facilitate its registration by EPA if pursued by the manufacturer. At this time, however, animal feeding, environmental metabolite, and residue studies will need to be conducted to fully characterize the DOW biocide before any food use registration is permitted. As indicated in the introduction, the active ingredient of DB 918-48C is registered with the US-EPA in California as a water treatment. The additional studies, however, are required for use on an agricultural crop. At this time, DOW Chemical is not proceeding with the registration because of the estimated costs ($2-4 million) and time required (5-7 years) to complete the required studies.

Thus, in our trials during the past three years, materials were identified that reduced the incidence of fireblight similar to the standard terramycin. In summary, the antibiotic Starner will no longer be evaluated because it is known to develop resistance in pathogen populations and the class of antibiotics that the bactericide belongs to is not being developed in agriculture because of their importance in medicine. The new

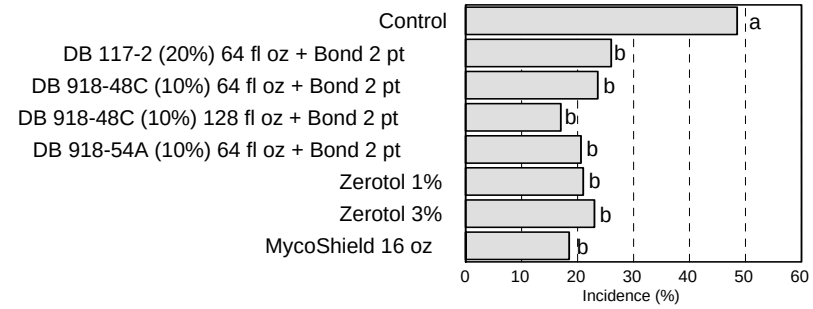
copper and silver formulations Bioacumen and Axenohl, respectively, that were evaluated in 2001 and 2002 were effective, but EPA may not accept silver-based materials for registration due to environmental persistence and mammalian toxicity problems. The new copper-formulation Bioacumen showed no clear advantage from other copper products with similar or higher costs and russeting symptoms associated with Bartlett fruit development. Efficacy of the systemic acquired resistance material Vacciplant was also variable but this compound may still be effectively used in a program with terramycin or streptomycin. Lastly, Zerotol and the DOW material were effective, but with some minor phytotoxicity associated with Bartlett fruit. The DOW material will not be evaluated further for the reasons discussed above. Thus, Zerotol is the only material that should continued to be evaluated of the materials evaluated to date. Still, the importance of finding an alternative material to antibiotics for fireblight control is imperative. Therefore, to develop an integrated disease management program, new materials have to be introduced and used in conjunction with existing compounds.

Fig. 1. Evaluation of new bactericides for fireblight management on Bartlett pear
Field trial in Marysville, CA - 2003



Treatments were applied on: 3/24, 3/31, 4/7, 4/11, 4/18, 4/30, 5/7, 5/14, and 5/21/03 using an air-blast sprayer at 100 gal/A. Disease was evaluated May 12 and May 27, 2003. The number of fire blight strikes were counted on each of four single-tree replications and disease incidence determined for each tree.

Fig. 2. Evaluation of new bactericides for fireblight management on Bartlett pear
Field trial in Davis, CA - 2003



Treatments were applied on: 3/29, 4/11, 4/18, and 4/25 using an air-blast sprayer at 100 gal/A. Disease was evaluated May 13, 2003. The number of fire blight strikes were counted on each of four single-tree replications and disease incidence was determined based on the total number of branches evaluated.