Western Regional IPM Grants
Research/Extension Accomplishments Report

INSTRUCTIONS: PLEASE PROVIDE ONLY THE ESSENTIAL COMPONENTS OF ACCOMPLISHMENT WHICH ARE:

1. A CLEAR IDENTIFICATION OF THE PROBLEM/ISSUE ADDRESSED BY THE RESEARCH/EXTENSION.
2. A CONCISE EXPLANATION OF HOW THE RESEARCH/EXTENSION ACHIEVEMENT CONTRIBUTED TO THE SOLUTION OF THE PROBLEM/ISSUE BEING RESEARCHED.
3. THE IDENTIFICATION OF OTHER BENEFITS RESULTING FROM THE RESEARCH/EXTENSION, EVEN IF UNPLANNED.
4. PLEASE ATTACH A SUMMARY OF THE PAST YEARS PROGRESS, ONE PAGE MINIMUM.

CONTACT: _Virginia Stockwell________________

PROJECT NUMBER:___2006-03857________________

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PROJECT TITLE: Integration of a modified strain of BlightBan A506 with conventional fire blight management_____________________

PRINCIPAL INVESTIGATOR: _Virginia Stockwell_______________

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CO-PIs or TEAM MEMBERS: __Ken Johnson and Joyce Loper__________________

THE PROBLEM, ISSUE, OR REASON FOR CONDUCTING THE RESEARCH/EXTENSION:

Fire blight, caused by the bacterium *Erwinia amylovora* is the most serious bacterial disease of pear and apple. The bacterial pathogen infects trees through flowers and causes a progressive blight of branches. Fire blight epidemics regularly cause losses totaling tens of millions of dollars. Disease control focuses on preventing the pathogen from growing on and infecting flowers. Growers use two antibiotics, streptomycin (Agri-mycin 17) and the less effective compound oxytetracycline (Mycoshield), for disease control. Antibiotics generally are sprayed an average of 2 to 4 times per year to trees in bloom for disease control. The antibiotic streptomycin provided excellent control until streptomycin-resistant pathogens became prevalent in the western states.

Biological control agents are now available for disease control. We study two biocontrol agents BlightBan A506 (*Pseudomonas fluorescens* A506) and BlightBan C9-1 (*Pantoea agglomerans* C9-1). Biological control agents colonize flowers and are moderately effective in suppressing pathogen growth on flowers and subsequent infection.

We have steadily improved the effectiveness of BlightBan A506 and BlightBan C9-1. From W-IPM research, co-application of BlightBan A506 with the iron chelate FeEDDHA induces A506 to produce an antibiotic and improves disease control. We also generated a deletion mutant of A506 (A506 AprX-) that does not make protease and when used in combination with C9-1 improves disease control compared to single strain inoculants or combinations of the parental strain A506 with C9-1. Furthermore, in limited trials, we found that one application of A506 AprX- with C9-1 followed by one application of Mycoshield (oxytetracycline) improved fire blight control.

The research is intended to improve disease control further and provide growers an integrated disease control program composed of optimized mixtures of biocontrol agents (BlightBan C9-1 with A506 AprX-) with fewer antibiotic applications. The specific goals of this research are to determine 1) if A506 AprX- & C9-1 provides greater disease control compared to BlightBan A506 with C9-1, 2) if FeEDDHA added to biocontrol mixtures improves disease control and 3) if a single application of biocontrols and then Mycoshield consistently improves disease control compared to two sprays of biocontrols or Mycoshield alone.

THE SINGLE MOST IMPORTANT ACCOMPLISHMENT OR BENEFIT RESULTING FROM THIS RESEARCH/EXTENSION:

In anticipation of funding, we started this project in the spring of 2006. We obtained the following results during orchard trials in 2006 and 2007 (disease control results provided in Table 1).

- Confirmed that streptomycin provided better control of fire blight than oxytetracycline if the pathogen was sensitive to both antibiotics.
- The mixture of the biological control agents C9-1 with AprX- provided better control of fire blight than C9-1 with BlightBan A506 in four of five trials.
- The integrated strategy of applying biological control agents once followed by a single application of oxytetracycline provided better control than biological control agents alone. This control strategy often provided better control than the conventional method of spraying oxytetracycline twice.
- The addition of Sequestrene 138 (FeEDDHA) did not improve disease control in the integrated strategy.
- Biological control agents established on flowers survived during a subsequent spray of oxytetracycline, even though the biocontrol agents are sensitive to the antibiotic.
- The population size of the pathogen was lower on flowers treated with biological control agents and oxytetracycline compared to flowers treated with only biological control agents or two applications of oxytetracycline.

These results were obtained in field trials conducted in 2006 and 2007. We will repeat the experiments in 2008. The field trials were conducted in experimental pear and apple orchards located on the Oregon State University, Botany and Plant Pathology Field Laboratory near Corvallis, Oregon. Biological control agents *Pantoea agglomerans* C9-1 (BlightBan C9-1) and *Pseudomonas fluorescens* A506 (BlightBan A506) or the protease-deficient derivative AprX- were applied to pear and apple trees at 80% bloom. At full bloom, plots were inoculated with an antibiotic-sensitive strain of the target pathogen *Erwinia amylovora*, the causal agent of fire blight. After full bloom (36 hours after pathogen inoculation), selected trees were sprayed with Mycoshield. Control trees were sprayed with water or streptomycin at this time and at 80% bloom.

During bloom, flower samples were taken and populations of the fire blight pathogen and the biological control agents were monitored.

After bloom, each tree was inspected for fire blight infections. Fire blight is seen as a necrosis and blight (often with bacterial ooze) of blossom clusters generally about two weeks after inoculation. Infected clusters were counted and removed over a month after first symptoms were observed; the sum of the number of infections per tree were calculated and subjected to statistical analyses. Table 1 provides a summary of the disease control results.

Table 1. Relative control of fire blight in Oregon integrated disease control experiments

	Trial (cultivar and year)					
	Pear		Apple			
Treatment*	Bartlett 2006	Bartlett 2007	Golden Delicious 2006	Rome Beauty 2006	Golden Delicious 2007	Across trials
Inoculated water control	0 ab** (162) §	0 a (258)	0 a (1622)	0 a (230)	0 a (546)	0
Antibiotics (two applications):						
Mycoshield (oxytetracycline)	26 abc	70 c	32 ab	0 a	43 b	34
Agri-mycin 17 (streptomycin)	82 e	92 d	32 ab	75 c	77 d	72
Biological controls and integrated methods:						
C9-1 & A506	0 a	33 b	6 a	0 a	59 bc	20
C9-1 & A506 then Mycoshield	46 cd	80 c	49 c	6 a	65 c	49
C9-1 & A506 & Sequestrene 138 then Mycoshield	44 cd	81 c	31 ab	24 b	58 bc	48
C9-1 & AprX-	10 ab	51 b	33 ab	23 b	49 bc	33
C9-1 & AprX- then Mycoshield	57 de	81 c	45 bc	36 b	39 ab	52
C9-1 & AprX- & Sequestrene 138 then Mycoshield	31 bc	83 c	31 ab	3 a	69 c	43

* All trees in experimental orchards were inoculated during full bloom with 5×10^5 to 1×10^6 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain).

Biological control bacteria *Pantoea agglomerans* C9-1 and *Pseudomonas fluorescens* A506 were applied once as the commercial formulations called BlightBan at 5×10^7 CFU/ml for each strain. Resuspended freeze-dried cells of the protease-deficient mutant of A506 called Pf AprX- was substituted for A506 in mixtures and applied at 5×10^7 CFU/ml. Sequestrene 138

(3 mM FeEDDHA) was tank-mixed with biological control agents and applied once at 80% bloom. Water and antibiotics [Mycoshield (a.i. oxytetracycline, 200 ppm) and Agri-mycin 17 (a.i. streptomycin, 100 ppm)] were applied at 80% bloom and ca. 36 h after inoculation of trees in full bloom with the pathogen. For the integrated treatments, biological control agents were sprayed once at 80% bloom and Mycoshield was sprayed once after full bloom.

** Relative disease control presented as mean reduction in disease incidence. The incidence of disease on water-treated and inoculated trees was set at 100%. Disease control for treatments was calculated as percent decrease in disease incidence relative to water treatment. Values followed by the same letter within a column containing data from a single orchard trial are not significantly different according to Fischer's protected least significance difference at $P = 0.05$. Data were transformed arcsine (square root(x)) prior to analysis.

§ Numbers in parentheses are the average number of strikes (blossom clusters with symptoms of fire blight) on water-treated and inoculated trees.

ADDITIONAL BENEFITS, SUCH AS:

SOCIAL BENEFITS -

The methods developed in this proposal potentially will reduce the number of antibiotic sprays applied to pear and apple trees, while still providing disease control. Ninety percent of amount of antibiotics used to control diseases of plants are used against fire blight of pear and apple. Reduction in the number of antibiotic sprays for fire blight control will reduce exposure of the environment and orchard workers to antibiotics.

ECONOMIC BENEFITS -

Economically, the integrated strategy should cost growers similar amounts to conventional disease control methods consisting of multiple antibiotic sprays. The improved disease control with the integrated strategy would save growers costs associated with removal of diseased tissues by pruning.

ENVIRONMENTAL BENEFITS -

Reduction in the number of antibiotic sprays in orchards may reduce the selection pressure for antibiotic-resistant bacteria in orchards. This strategy also may reduce the probability of *Erwinia amylovora* becoming resistant to oxytetracycline. This compound is the only antibiotic that the growers can use and that the pathogen has not developed resistance. Loss of antibiotic sensitivity by the fire blight pathogen would be an additional burden for growers to manage this devastating disease of apple and pear.

OTHER -

PLEASE SUBMIT A HIGH RESOLUTION DIGITAL IMAGE REPRESENTATIVE OF YOUR RESEARCH/EXTENSION PROJECT THAT WE CAN USE TO HIGHLIGHT YOUR PROJECT!

<u>When you have completed this form, return to both:</u>

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