Western Regional IPM Grants
Annual Progress Report Guidelines
Due October 15, 2008

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.**

PROJECT NUMBER: 2006-03857

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

LEAD PRINCIPAL INVESTIGATOR: Virginia Stockwell

INSTITUTIONAL ADDRESS: Dept. of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon

TELEPHONE NUMBER AND EMAIL ADDRESS:
541-738-4078; stockwev@science.oregonstate.edu

CO-PIs or OTHER KEY PERSONNEL, and their INSTITUTIONS or AFFILIATIONS:
Kenneth Johnson, Dept. Botany and Plant Pathology, OSU and Joyce Loper, USDA-ARS-Horticultural Crops Research Laboratory, Corvallis, Oregon

WHO MAY WE CONTACT FOR ADDITIONAL INFORMATION IF NOT THE LEAD PI?
NAME: Contact Lead PI

THE PROBLEM, ISSUE, OR REASON FOR PURSUING THIS RESEARCH/ EXTENSION PROJECT.

Fire blight, caused by the bacterium *Erwinia amylovora* is the most serious bacterial disease of pear and apple. The pathogen infects trees through flowers and causes a progressive killing of branches. Disease control focuses on preventing the pathogen from growing on and infecting flowers. Conventional and organic fruit 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. The development of resistance of the pathogen to streptomycin left growers vulnerable to epidemics of fire blight. Fire blight epidemics in the western states regularly cause losses totaling tens of millions of dollars.

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) [NuFarm Americas, Burr Ridge, IL]. Biological control agents colonize sites on floral tissues required for growth and infection by the pathogen.

Colonization of these sites by biocontrol agents suppresses pathogen growth and provides moderate disease control.

We have improved the effectiveness of biocontrol with BlightBan A506 and BlightBan C9-1. From WR-IPM research, co-application of BlightBan A506 with the iron chelate FeEDDHA induces A506 to produce an antibiotic and improves disease control by this strain. We also generated a deletion mutant of A506 (A506 AprX-) that does not make an extracellular 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.

Many pear and apple growers are using BlightBan A506, but want the flexibility to be able to use such products in conjunction with antibiotics for control of fire blight. The products BlightBan A506 and C9-1 are fully compatible with streptomycin and can even be tank-mixed with the antibiotic. Integration of biological control agents with the antibiotic oxytetracycline has been challenging because the biological control agents are sensitive to the compound and are killed when suspended in a solution of oxytetracycline. We needed to develop a method whereby growers could use biocontrol agents in conjunction with application of oxytetracycline for optimal disease control.

The research is intended to improve disease control and provide growers an integrated disease control program composed of optimized mixtures of biocontrol agents (BlightBan C9-1 with A506 AprX-) and 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 biocontrols or two sprays of Mycoshield alone.

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

- We developed an effective integrated disease control strategy where biological control agents are applied in mid- to near full-bloom and oversprayed with Mycoshield (oxytetracycline) after full bloom (disease control results provided in Table 1).
- Biological control agents established on flowers survived during a subsequent spray of oxytetracycline, even though the biocontrol agents are sensitive to the antibiotic in vitro (Figure 1).
- 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 (Figure 1).
- The mixture of the biological control agents C9-1 with AprX- provided better control of fire blight than C9-1 with BlightBan A506 in orchard trials (Table 1).
- The integrated strategy of applying biological control agents once followed by a single application of oxytetracycline provided better control than biological control agents alone (Table 1). This control strategy often provided better control than

the conventional method of spraying oxytetracycline twice.

- The addition of Sequestrene 138 (FeEDDHA) to biocontrol agents did not improve disease control in the integrated strategy when trees treated with biological control agents and the iron chelate were oversprayed with Mycoshield.
- We confirmed that streptomycin provided better control of fire blight than oxytetracycline if the pathogen was sensitive to both antibiotics (Table 1). The duration of suppressive activity of both antibiotics on flowers was similar (Figure 2). The superior disease control by streptomycin was likely due to the capacity of the antibiotic to kill bacterial cells, whereas oxytetracycline only inhibits multiplication of the pathogen.

These results were obtained in orchard trials conducted during the funding period 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 (200 ppm a.i. oxytetracycline). Control trees were sprayed with water, Mycoshield, or Agri-mycin 17 (100 ppm a.i. streptomycin) at this time after full bloom and also earlier 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.

BRIEFLY DESCRIBE ADDITIONAL BENEFITS, SUCH AS:

SOCIAL BENEFITS -

The integrated disease control strategy developed in this project will reduce the number of antibiotic sprays applied to pear and apple trees and provide excellent control of fire blight. 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. In the western states where streptomycin-resistant isolates of *Erwinia amylovora* are prevalent, growers use Mycoshield for disease control. The integrated strategy provided significantly better control of fire blight than two applications of Mycoshield alone. The improved disease control with the integrated strategy would save growers costs associated with removal of diseased branches 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 RESOLUSION DIGITAL IMAGE REPRESENTATIVE OF YOUR RESEARCH/EXTENSION PROJECT THAT WE CAN USE IN WESTERN IPM CENTER PUBLICATIONS WHICH MENTION YOUR PROJECT.

When you have completed this form, return to
Frank Zalom
Department of Entomology
One Shields Avenue
University of California
Davis, CA 95616
fgzalom@ucdavis.edu

and

Rick Melnicoe
Western IPM Center
Department of Environmental Toxicology
One Shields Avenue
University of California
Davis, CA 95616
rsmelnicoe@ucdavis.edu

THIS FORM WAS COMPLETED BY:

_Virginia Stockwell, Asst. Prof. of Research
(Name and Title)

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

	Trial (year and cultivar)											
	2005			2006			2007		2008			
Treatment[a]	Bartlett	Golden Delicious	Rome	Bartlett	Golden Delicious	Rome	Bartlett	Golden Delicious	Bartlett	Golden Delicious	Gala	Pooled
Water	0[b] A [66][c]	0 A [296]	0 A [19]	0 AB [162]	0 A [1622]	0 AB [230]	0 A [258]	0 A [546]	0 A [32}	0 A [1402]	0 A [248]	0 A
A506 & C9-1	NT[d]	NT	NT	5 A	6 A	-5 A	25 B	63 BC	NT	NT	NT	19 B
A506 & C9-1 then Mycoshield	NT	NT	NT	46 BC	48 C	0 A	83 D	73 BC	NT	NT	NT	50 CD
AprX$^-$&C9-1	54 B	64 B	40 B	15 AB	29 BC	23 A	48C	54BC	55 B	43 ABC	22 B	41 C
AprX$^-$&C9-1 then Mycoshield	86 C	83 C	64 BC	53 CD	34 BC	32 AB	81 D	53 B	78 B	52 C	32 BC	59 D
Mycoshield	84 C	55 B	49 BC	29 ABC	31 BC	-12 A	73 D	54 BC	62 B	22 AB	42 C	44 C
Agri-mycin 17	94 C	77 BC	83 C	81 D	37 BC	74 B	94 E	82 C	68 B	45 BC	72 D	73 E

[a] All trees in experimental orchards were inoculated during full bloom with 5 x 10^5 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain). Bacterial antagonists *Pantoea agglomerans* C9-1 and *Pseudomonas fluorescens* A506 were applied once as the commercial BlightBan formulations at 5 X 10^7 CFU/ml for each strain. Resuspended freeze-dried cells of the protease-deficient mutant of A506 called AprX$^-$ was substituted for A506 in mixtures and applied at 5 X 10^7 CFU/ml. Water and antibiotics [Mycoshield (oxytetracycline, 200 ppm) and Agri-mycin 17 (streptomycin, 100 ppm)] were applied at 80% bloom and ca. 36 h after inoculation with the pathogen. For the integrated treatments, biological control agents were sprayed once at 70% bloom (or in 2008 at 30% and 70% bloom) and Mycoshield was sprayed once after full bloom.

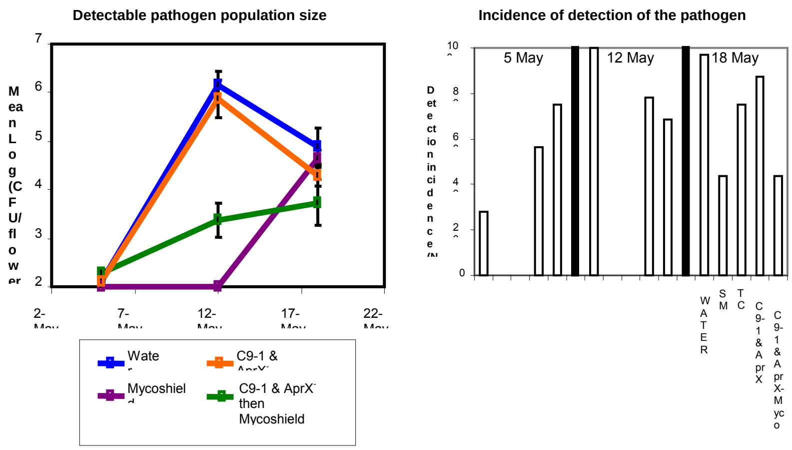
[b] Relative disease control presented as mean reduction in disease incidence. The incidence of disease on inoculated water-treated trees was set at 100%. Disease control for treatments was calculated as percent decrease in disease incidence relative to water treatment. Values followed by the same letter 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.

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

[d] NT indicates treatment not tested in that trial.

Figure 1. Colonization of flowers and persistence of biological control agents and the pathogen following Mycoshield oversprays.

Influence of treatments on colonization of Golden Delicious apple flowers in 2008 by *Erwinia amylovora*



Influence of Mycoshield on colonization of apple flowers by bacterial antagonists

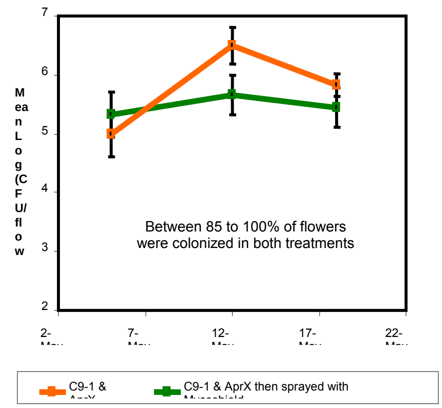
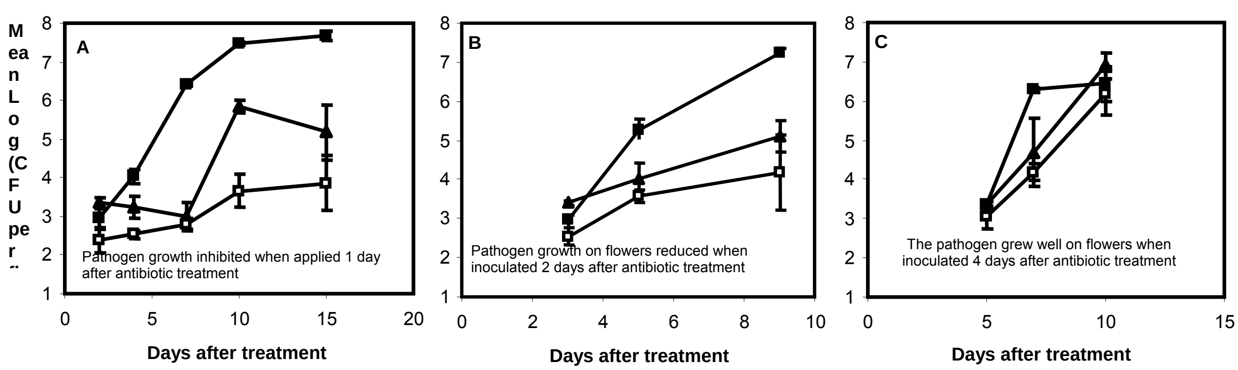


Figure 2. Persistence of antibiosis by streptomycin or oxytetracycline on flowers

Duration of suppression of *Erwinia amylovora* on antibiotic-treated



Mean population size (log_{10} CFU per flower) of *Erwinia amylovora* strain 153N on 'Bartlett' pear flowers sprayed with water (■), Agri-mycin 17 (□, 100 ppm streptomycin), or Mycoshield (▲, 200 ppm oxytetracycline) and then inoculated with the pathogen 1, 2, or 4 days later. A. Populations of Ea153N on flowers inoculated one day after water or antibiotic sprays. B. Populations on flowers inoculated two days after sprays. C. Populations on flowers inoculated four days after sprays. Each point represents the mean population size and vertical bars represent ± one standard error.