FINAL PROJECT REPORT
WTFRC Project Number:

Project Title: Integrated management of fire blight of pear and apple

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Budget History:

Item	**Year 1:** 2004	**Year 2:** 2005	**Year 3:** 2006
Salaries	9,000	4,000	4,200
Benefits	4,680	2,080	2,730
Wages			
Benefits			
Equipment			
Supplies	1000	300	300
Travel	450	300	300
Miscellaneous	1000	1000	1000
Total	16130	7680	8530

Project Title: Integrated management of fire blight of pear and apple
PIs: Ken Johnson & Virginia Stockwell, Oregon State University

Objective:
Field-test an optimized biopesticide strategy in combination with oxytetracycline.

Significant findings:

- Temperature conditions in western Oregon during spring 2006 were favorable for fire blight development. Consequently, as measured against what is typical in commercial pear and apple orchards, the amount of disease that developed in the experimental orchards was extreme. Thus, even antibiotic standards performed below their longer term averages.
- Nonetheless, our experiments concerned with fire blight control continued to show excellent results with what we term an 'integrated strategy', which is one biopesticide treatment followed by one oxytetracycline treatment.
- In Bartlett pear, all treatments that involved a biopesticide applied once near full bloom followed by a single application of Mycoshield provided a significant level of disease control. In Golden Delicious apple, disease intensity was extremely high with two of the integrated treatments providing significant control of fire blight compared to water treated controls; antibiotic standards did not. In Rome Beauty apple, disease intensity was heavy; the mixture of A506 AprX- and BlightBan C9-1 followed by Mycoshield also provided a substantial level of disease control.
- *Pantoea agglomerans* strain C9-1 was registered by US EPA on April 10, 2006 as BlightBan C9-1. NuFarm Americas intends to market BlightBan C9-1 in combination with *Pseudomonas fluorescens* strain A506 AprX- , which is a mutant selection of the active bacterium in BlightBan A506 (also a registered product). Use of A506 AprX- still requires EPA approval.

Background

Fire blight, caused by the bacterium, *Erwinia amylovora,* is a serious disease of pear and apple. The pathogen overwinters in cankers and moves to flowers as temperatures warm in spring. On flowers, the pathogen grows rapidly to attain an infective population size. Diseased flowers become necrotic; the pathogen then invades shoots and can progressively kill larger branches. Once infected, pruning is the only management option to reduce disease. Consequently, control focuses on spraying antibiotics and/or biopesticides onto flowers to prevent initial infections.

Antibiotics were first registered for fire blight suppression in the 1950s. Streptomycin kills cells of the pathogen and provides a ~80% reduction in disease if the pathogen population is sensitive to this antibiotic. In contrast, oxytetracycline is bacteriostatic and is less effective (~40% control). Streptomycin-resistant populations of the pathogen are widespread in the western U.S., and thus, oxytetracycline is used widely for fire blight control.

Two biopesticides are currently registered for fire blight suppression. Serenade (AgraQuest) is an air-dried fermentation culture of *Bacillus subtilis*. BlightBan A506 (NuFarm Americas) is a freeze-dried culture of *Pseudomonas fluorescens* strain A506. These products have provided a ~25% reduction of disease in small-scale, pathogen-inoculated trials, but have been somewhat more effective in orchards when combined within a conventional antibiotic program.

Numerous experiments have shown that strains of *Pantoea agglomerans* are the most effective biopesticides for fire blight suppression. NuFarm Americas has recently completed registration of *P. agglomerans* strain C9-1 under the trade name BlightBan C9-1. They intend to market C9-1 a combination product with a mutant selection of the active bacterium in BlightBan A506 (strain A506 AprX -).

Recently, our experiments concerned with fire blight control have focused on what we term an 'integrated strategy', which is one biopesticide treatment followed by one oxytetracycline treatment. To date, the integrated strategy has resulted in greater disease control than either biopesticides or oxytetracycline applied alone. Over a longer time period, we also have improved the effectiveness of the biopesticides BlightBan A506 and BlightBan C9-1. One improvement requires co-application of BlightBan A506 with the iron chelate, FeEDDHA. This non-phytotoxic chelate induces A506 to produce a potent antibiotic that inhibits E. amylovora (the antibiotic is not produced when iron is absent). A506 plus FeEDDHA already has some adoption among Oregon pear growers. The other improvement involves a derived knockout mutant of A506 that is deficient in an extracellular protease; this strain is called A506 AprX -. When A506 AprX - and C9-1 are applied as a combination, deletion of A506's ability to make the extracellular protease lengthens the half-life of the antibiotics produced by C9-1. In our earlier trials, the combination of A506 AprX - with C9-1 has been the most effective biopesticide treatment. Moreover, the effectiveness of one application of the combination of A506 AprX - with C9-1 followed by one application of oxytetracycline (i.e., the integrated strategy) has provided consistent control, which in many trials has approached equaled the control obtained from two applications of streptomycin.

MATERIALS AND METHODS

Materials tested. The commercial formulation of the biological agent, *Pantoea agglomerans* C9-1S (BlightBan C9-1, NuFarm) was evaluated for disease control in mixture with *Pseudomonas fluorescens* strain A506 (BlightBan A506, NuFarm). The iron chelate Sequestrene 138 (6% FeEDDHA, Becker Underwood, Ames, IA) was combined some of the bacterial treatments. We included treatments consisting of an extracellular protease-deficient deletion mutant of *P. fluorescens* strain A506 called A506 AprX - in mixture with BlightBan C9-1. We also included several treatments where biological control applications were followed by a single application of Mycoshield. Disease control efficacy by an avirulent *hrpL* mutant of Ea153 alone and in mixture with A506 AprX - and BlightBan C9-1 also was assessed. The *hrpL* mutant and A506 AprX - were cultured and freeze-dried in the Johnson laboratory for use in field trials. Additional treatments included Physpé (plant defense elicitor extracted from brown algae, Goëmar, Saint-Malo, France), and famoxate and Tanos (chemical agents manufactured by DuPont, Wilmington, DE). The chemical agents Agri-mycin 17 (streptomycin sulfate 17% a.i, NuFarm Americas, Burr Ridge, IL) and Mycoshield (oxytetracycline calcium complex, 17% a.i, NuFarm) were included as standard controls.

Experimental protocol. Biological agents, antibiotics and experimental chemical materials were evaluated for control of fire blight in a 46-yr-old 'Bartlett' pear orchard, a 26-yr-old 'Gold Delicious' apple orchard, and a 48-year-old 'Rome Beauty' apple orchard. All orchards were spaced 20' x 20' and located at the OSU BPP Field Laboratory near Corvallis.. The experiments were arranged in a randomized, complete block design with 4 replications and 12 to 17 treatments applied to single tree plots. Blossom cluster density on individual trees was estimated prior to bloom; cluster counts and tree location in the orchard were considered in assignment of trees to blocks in the plot design.

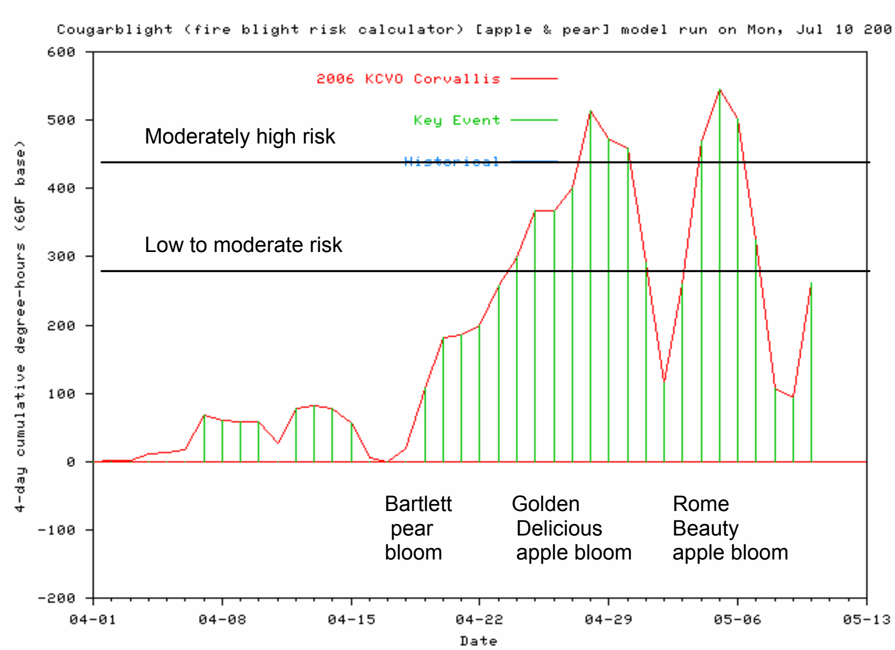
Treatments were applied during early morning on the following phenological stages (dates are in the data tables): green tip (Physpé only), popcorn; Physpé only, 30-40% bloom (Physpé, and some biocontrol bacteria treatments), 80- 90% bloom (all treatments) and full bloom (antibiotics and chemical agents). Treatment suspensions (except second application of Mycoshield were sprayed to near runoff with backpack sprayers equipped with hand wands; because of the large number of trees that received the second application of Mycoshield, this treatment was applied with a motorized, 25 gal tank sprayer equipped with a hand wand (~0.75 gal/tree). The same motorized tank sprayer was used to fog a suspension of freeze-dried cells of *Erwinia amylovora* strain 153N (streptomycin- and oxytetracycline-sensitive pathogen strain), which was prepared at 5 x 10^6 (pear) and at 1 x 10^6 (apple) CFU per ml. The pathogen inoculation occurred 2 days after the 80-90% bloom treatments.

Incidence of fire blight was determined by counting blighted blossom clusters (i.e., strikes) on each tree during weekly inspections from 4 to 25 May. Blighted blossom clusters were removed immediately after counting. Total number of blighted blossom clusters per tree ($\log_{10}$-transformed) and disease incidence (total diseased clusters/total number of clusters per tree, arcsine-square root transformed) were subjected to analysis of variance.

RESULTS

Weather conditions during bloom. Temperature conditions in western Oregon during spring 2006 were favorable for fire blight development. The COUGARBLIGHT disease risk model indicated a building risk period during bloom of Bartlett pear, and high risk periods during bloom of Golden Delicious and Rome Beauty apple (Fig. 1).

Fig. 1. Fire blight risk as estimated by COUGARBLIGHT, Corvallis. Oregon, 2006.



Bartlett pear. Trees used in the study averaged 1037 blossom clusters per tree. Symptoms of fire blight were observed first on 30 April. Disease intensity was moderate with symptoms of fire blight developing on 16% of inoculated blossom clusters treated with water only **(Table 1; at end of report).** Because of a cold front and associated rainfall that occurred at full bloom (1.2 inches of precipitation from April 14 to 16), the concentration of pathogen inoculum applied to the plot area on 13 April prior was five times greater than we have typically used for similar field experiments. Agri-mycin 17 provided a high degree of fire blight control. As measured by either the mean number or mean incidence of infected blossom clusters per tree, all treatments that involved a biological applied once near full bloom followed by a single application of Mycoshield provided a significant level of disease control. The mixture of A506 AprX - and BlightBan C9-1 followed by Mycoshield was the only treatment that was similar statistically to the result obtained with Agri-mycin 17.

Golden Delicious apple. Trees used in the study were moderately sized with an average of 1340 blossom clusters per tree. In the evening of 3 May, all trees were fogged with water to compensate for extremely dry conditions during bloom. Symptoms of fire blight were observed first on 3 May. Disease intensity was extremely high **(Table 2)**; symptoms of fire blight developed on ~ 100% of inoculated blossom clusters treated with water-only. Intense disease pressure was likely due to usually warm weather (up to 26°C) after inoculation of flowers with the pathogen. Under these conditions, the standard chemical treatments AgriMycin-17 and Mycoshield failed to significantly reduce the incidence of fire blight compared to water treated controls. Of all of treatments, only two integrated disease control methods provided significant control of fire blight compared to water-treated controls. The treatments consisting of 1) BlightBan C9-1 combined with A506 AprX - and FeEDDHA applied once near full bloom followed by a single application of Mycoshield or 2) BlightBan C9-1 combined with BlightBan A506 applied once near full bloom followed by a single application of Mycoshield provided a significant level of disease control by analysis of mean number of infected blossom clusters per tree and transformed disease incidence data.

Rome Beauty apple. Trees used in the study averaged 625 blossom clusters per tree. Symptoms of fire blight were observed first on 22 May. Disease intensity was heavy with symptoms of fire blight developing on 39% of inoculated blossom clusters treated with water only **(Table 3)**. Average daily temperatures were 68°F ranging from 58 to 91°F and two major rain events. Agri-mycin 17 provided a high degree of fire blight control. The mixture of A506 AprX - and BlightBan C9-1 followed by Mycoshield also provided a substantial level of disease control.

Overall performance of treatment groups. With treatments scaled relative to the amount of disease observed on the water treated control, Agri-mycin 17 (streptomycin) provided an average of 60% contro1 of fire blight caused by streptomycin-sensitive strain Ea153N (Fig. 2). Mycoshield averaged 20% control, and the average control of all treatments that involved **only** a biopesticide was 12%. The average control obtained from all treatments that followed an ‘integrated’ treatment regimen (a biopesticide follow by Mycoshield) was 37% (Fig. 2). The most efficacious biopesticide treatment, *P. agglomerans* C9-1 combined with *P. fluorescens* A506 AprX -, provided and average of 34% control (Fig. 3). One application of C9-1 plus A506 AprX -

Fig. 2

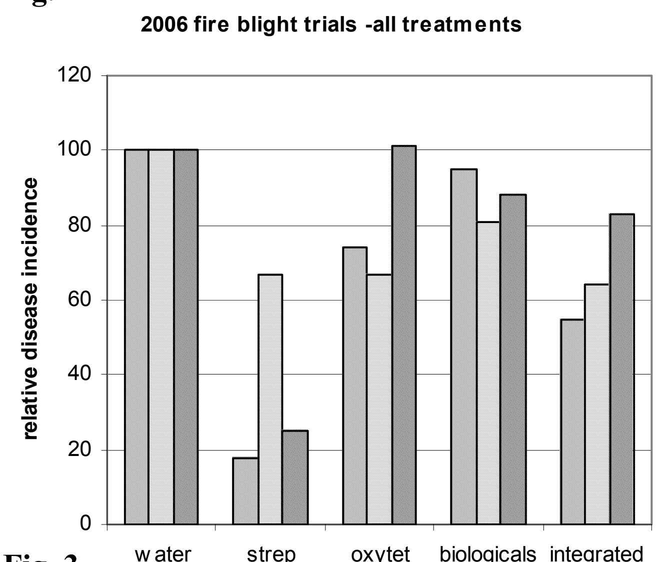
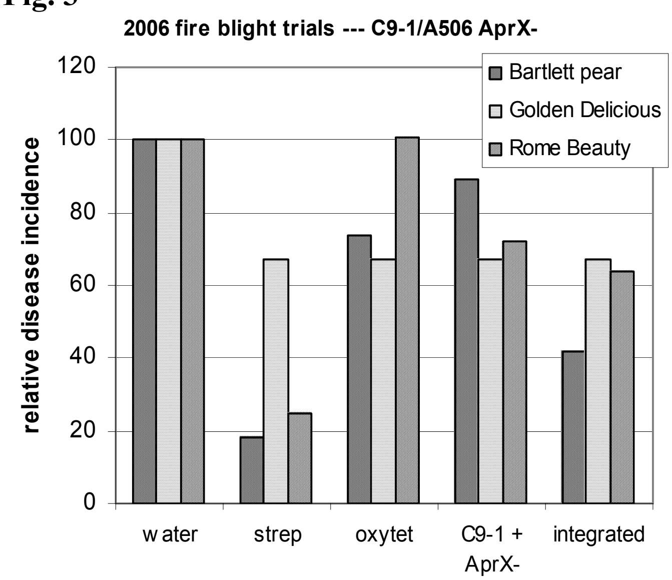


Fig. 3



followed by Mycoshield reduced the relative incidence of disease flower cluster by 44% (Fig. 3).

DISCUSSION

As measured against what is typical in commercial pear and apple orchards, the amount of disease that developed in the experimental orchards was extreme. Fire blight infection was promoted by favorable weather conditions in combination with inoculation of the pathogen onto the trees at full bloom. The high disease pressure is the principal reason the Mycoshield (oxytetracycline) performed below its longer term average (40 to 50% disease reduction) in all three trials. Nonetheless, as we have observed in previous seasons, the 'integrated strategy' resulted in greater disease control than either biopesticides or oxytetracycline applied alone. This strategy is has evolved from years of research trials (Fig. 4 next page) involving biopesticides, antibiotics and other chemical agents. A draw back of experimental trials is that the inoculation event introduces the pathogen to flowers all at once, whereas in a commercial orchard, pathogen populations build slowly over a period of several days. Consequently, we predict that integrated treatments will perform better in commercial orchards than we observe in our inoculated plots.

A fire blight forecasting model has been adapted to employ the integrated biopesticide and antibiotic strategy (illustrated in Fig. 5 next page; publication: Johnson, K. B., Stockwell, V. O. and Sawyer, T. L. 2004. Adaptation of fire blight forecasting to optimize the use of biological controls. Plant Disease 88:41-48)

In April 2006, BlightBan C9-1 was granted a registration and tolerance exemption by EPA (Fig. 6 next page). NuFarm has indicated to us that they will now pursue registration of A506 AprX- (a strain of Blight A506 that enhances effectiveness C9-1). A506 AprX- is a protease-deficient mutant of previously registered strain A506. Their intention is to market BlightBan C9-1 in combination with A506 AprX-.

Fig. 4. Summary of fire blight trials conducted at Oregon State University from 1991 to 2006. All treatments scaled relative to the amount of disease observed on the water treated control.

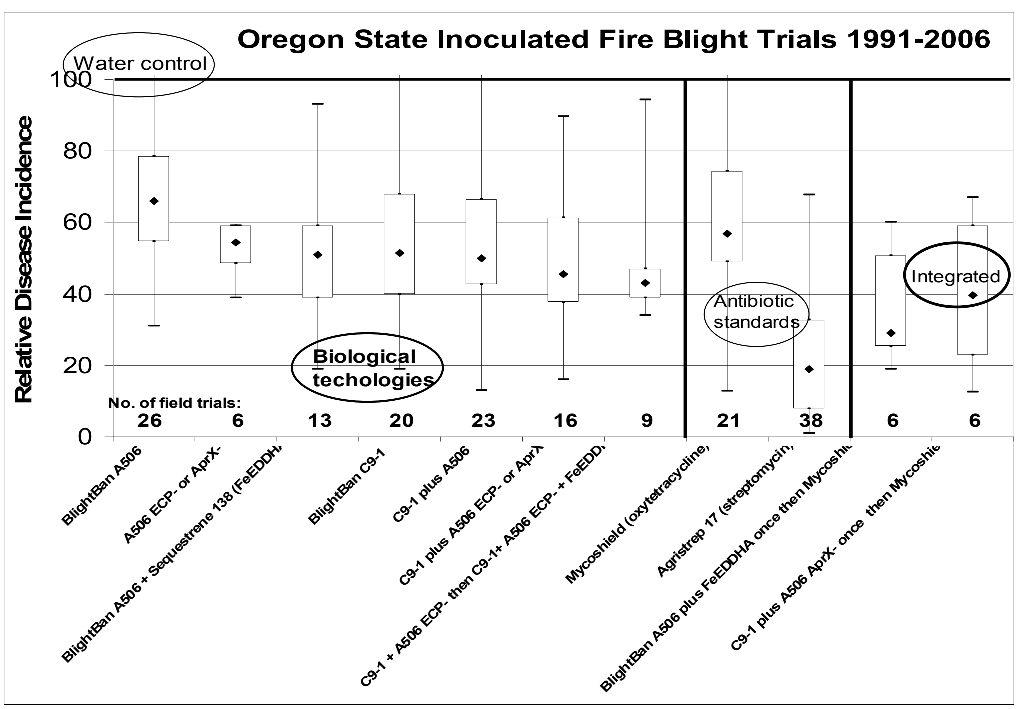


Fig. 5. Working model of the ‘integrated’ biopesticide/antibiotic strategy. Timing of fire blight treatments is based on bloom stage and a temperature-based disease risk assessment.

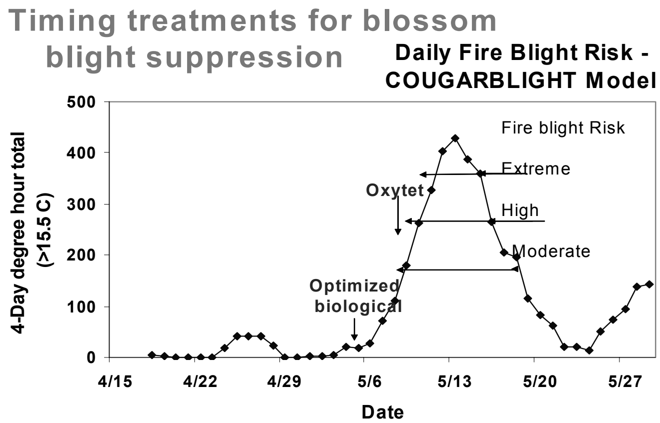


Fig. 6. EPA Registration of Blight Ban C9-1:

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division
(7511C)
1200 Pennsylvania Ave., NW
Washington, D.C. 20460

NOTICE OF PESTICIDE:
x Registration
Reregistration
(under FIFRA, as amended)

EPA Reg. Number: 71368-45
Date of Issuance: 4/10/06
Term of Issuance: Unconditional
Name of Pesticide Product: BlightBan C9-1®

Name and Address of Registrant (include ZIP Code):
Ted Head
Nufarm Agriculture Inc.
1333 Burr Ridge Parkway, Suite 125A
Burr Ridge, IL 60527

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Registration Division prior to use of the label in commerce. In any correspondence on this product always refer to the above EPA registration number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered under the Federal Insecticide, Fungicide and Rodenticide Act. Registration is in no way to be construed as an endorsement or recommendation of this product by the Agency. In order to protect health and the environment, the Administrator, on his motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under this Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

This registration does not eliminate the need for continual reassessment of the pesticide. If EPA determines at any time, that additional data are required to maintain in effect an existing registration, the Agency will require submission of such data under section 3(c)(2)(B) of FIFRA.

This product is registered in accordance with FIFRA section 3(c)(5) and is subject to the following conditions:

1) This registration is limited to the end-product, BlightBan C9-1®, which contains the active ingredient, *Pantoea agglomerans* strain C9-1.

2) Change “EPA Registration Number 71368-UL” to “EPA Registration Number 71368-45” on the final printed label.

3) The registrant must provide the Agency an upgraded analysis of samples.

Signature of Approving Official:
(Signature on the second page) JZH

Date: 4/10/06

EPA Form 8570-6

TABLE 1. Bartlett pear, Corvallis, Oregon, 2006 Fire blight trial

Treatment	Rate per 100 gallons water	Date treatment applied*: 10 April 30% bloom	12 April 90%-bloom	18 April full bloom	Mean number of blighted clusters per tree**		Mean percent of clusters blighted***	
BlightBan C9-1&	2.5 oz.	X	X[§]	---				
BlightBan A506	2.5 oz.	X	X	---	154	a	17.4	a
BlightBan C9-1 &		X	X	---				
A506 AprX- &	total 10^8	X	X	---				
hrpL mutant	CFU/ml	X	X	---	153	a	15.5	ab
Water control	**------**	**---**	**X**	**X**	**162**	**a**	**15.6**	**abc**
Tanos	12.0 fl oz	---	X	X	153	ab	15.3	abc
Avirulent *hprL* mutant of *E. amylovora*	10^8 CFU/ml.	X	X	---	145	abc	14.6	abcd
BlightBan C9-1 & A506 AprX-	total 10^8 CFU/ml.	X	X	---	138	abc	14.0	abcde
Physpé	9.7 fl. oz.	X	X	X	114	abc	13.4	abcdef
BlightBan C9-1 &	2.5 oz.	X	X	---				
BlightBan A506 &	2.5 oz.	X	X	---				
Sequestrene 138	16 oz.	X	X	---	108	abc	12.8	abcdef
Mycoshield	16 oz.	---	X	X	116	abc	11.6	abcdefg
Famoxate	11.4 fl oz.	---	X	X	114	abc	11.0	bcdefg
BlightBan C9-1&	2.5 oz.	---	X	---				
A506 AprX- &	2.5 oz.	---	X	---				
Sequestrene 138	16 oz	---	X	---				
then Mycoshield	16 oz.	---	---	X	96	bcd	10.7	cdefg
BlightBan C9-1	5 oz.	X	X	---	86	cd	8.9	defg
BlightBan C9-1&	2.5 oz.	---	X	---				
BlightBan A506 &	2.5 oz.	---	X	---				
Sequestrene 138	16 oz	---	X	---				
then Mycoshield	16 oz.	---	---	X	87	cd	8.8	efg
BlightBan C9-1	5 oz.	---	X	---				
then Mycoshield	16 oz.	---	---	X	90	cd	8.3	efg
BlightBan C9-1&	2.5 oz.	---	X	---				
BlightBan A506	2.5 oz.	---	X	---				
then Mycoshield	16 oz.	---	---	X	87	d	8.5	fg
BlightBan C9-1 &	total 10^8	---	X	---				
A506 AprX-	CFU/m.	---	X	---				
then Mycoshield	16 oz.	---	---	X	76	d	6.7	gh
Agri-mycin 17	8 oz	---	X	X	30	e	2.8	h

TABLE 2. Golden Delicious Apple, Corvallis, Oregon, 2006 Fire blight trial

Treatment	Rate per 100 gallons water	Date treatment applied* 22 April 30% bloom	 24 April 80%- bloom	 27 April full bloom	Mean number of blighted clusters per tree**		Mean percent of clusters blighted***	
Water control	**------**	**---**	**X[§]**	**X**	**1622**	**a**	**121**	**a**
BlightBan C9-1 & BlightBan A506 & Sequestrene 138	2.5 oz. 2.5 oz. 16 oz.	X X X	X X X	--- --- ---	1518	ab	114	a
Avirulent *hpr*L- *E. amylovora*	total 10^8 CFU/ml	X	X	---	1445	abc	105	ab
Famoxate	11.4 fl oz.	---	X	X	1408	abc	103	abc
Tanos	12.0 fl oz	---	X	X	1376	abc	102	abc
BlightBan C9-1 & A506 AprX- & *hrp*L- mutant	 total 10^8 CFU/ml	X X X	X X X	--- --- ---	1363	abc	101	abc
BlightBan C9-1 then Mycoshield	5 oz. 16 oz.	--- ---	X ---	--- X	1352	abc	95	abc
Physpé	9.7 fl. oz.	X	X	X	1203	abc	90	abc
BlightBan C9-1 & A506 AprX-	total 10^8 CFU/ml	**X**	X	---	1147	abc	81	abc
Mycoshield	16 oz.	---	X	X	1126	abc	82	abc
BlightBan C9-1& BlightBan A506 & Sequestrene 138 then Mycoshield	2.5 oz. 2.5 oz. 16 oz 16 oz.	--- --- --- ---	X X X ---	--- --- --- X	1124	abc	83	abc
BlightBan C9-1	5 oz.	X	X	---	1103	abc	86	abc
BlightBan C9-1 & A506 AprX- then Mycoshield	total 10^8 CFU/ml. 16 oz.	--- --- ---	X X ---	--- --- X	1076	abc	81	abc
Agri-mycin 17	8 oz	---	X	X	1015	abc	82	abc
BlightBan C9-1& A506 AprX- & Sequestrene 138 then Mycoshield	2.5 oz. 2.5 oz. 16 oz 16 oz.	--- --- --- ---	X X X ---	--- --- --- X	901	bc	67	bc
BlightBan C9-1& BlightBan A506 then Mycoshield	2.5 oz. 2.5 oz. 16 oz.	--- --- ---	X X ---	--- --- X	837	c	62	c

TABLE 3. Rome Apple, Corvallis, Oregon, 2006 Fire blight trial

Treatment	Rate per 100 gallons water	Date treatment applied* 26 April 30% bloom	28 April 70%-bloom	1 May full bloom	Mean number of blighted clusters per tree**	Mean percent of clusters blighted***
Mycoshield	16 oz.	---§	X§	X	259 a	39.6 a
BlightBan C9-1&	2.5 oz.	X	X	---		
BlightBan A506	2.5 oz.	X	X	---	242 a	39.3 a
Water control	**------**	---	**X**	**X**	**230 a**	**39.1 ab**
BlightBan C9-1 &		X	X	---		
A506 AprX- &	total 10^8	X	X	---		
*hrp*L- mutant	CFU/ml	X	X	---	242 a	38.9 ab
BlightBan C9-1&	2.5 oz.	---	X	---		
A506 AprX- &	2.5 oz.	---	X	---		
Sequestrene 138	16 oz	---	X	---		
then Mycoshield	16 oz.	---	---	X	239 a	38.1 ab
BlightBan C9-1&	2.5 oz.	---	X	---		
BlightBan A506	2.5 oz.	---	X	---		
then Mycoshield	16 oz.	---	---	X	231 a	36.9 ab
BlightBan C9-1	2.5 oz.	---	X	---		
then Mycoshield	16 oz.	---	---	X	200 a	32.4 ab
BlightBan C9-1 &	total 10^8					
A506 AprX-	CFU/ml.	X	X	---	176 a	30.2 ab
Avirulent *hpr*L- mutant of *E. amylovora*	10^8 CFU/ml.	X	X	---	193 a	29.9 ab
BlightBan C9-1&	2.5 oz.	---	X	---		
BlightBan A506 &	2.5 oz.	---	X	---		
Sequestrene 138	16 oz	---	X	---		
then Mycoshield	16 oz.	---	---	X	186 a	29.7 ab
BlightBan C9-1 &	total 10^8	---	X	---		
A506 AprX-	CFU/m.	---	X	---		
then Mycoshield	16 oz.	---	---	X	156 a	25.2 b
Agri-mycin 17	8 oz	---	X	X	60 b	9.7 c

Key to tables:* Trees inoculated on 13 April (Bartlett pear), 25 April (Golden Delicious apple) or 29 April (Rome Beauty apple) with 1 x 10^6 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain).
**Means of the sum of strikes per tree followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$. Data were transformed log(x) prior to analysis
*** Mean disease incidence values followed by the same letter 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.
§ X indicates date material sprayed, --- indicates material not applied on that date.