CONTINUING PROJECT REPORT **YEAR**: 1 of 2

WTFRC Project Number:

Project Title: Non-antibiotic fire blight control that minimizes fruit russet risk

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Budget: **Year 1: $25,000** **Year 2: $25,750**
Annually**:** FRA 3.5 mo plus fringe, 2K M&S, 1K local travel & plot fee, 3% inflation

Other funding sources

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Notes: Objectives 1 and 2 of this proposal are matching objectives for the above NIFA OREI project
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Amt. awarded: $495K to Johnson, Elkins, Granatstein and Smith 10/14 - 9/17
Notes: Objectives 1 and 2 of this proposal are related to objectives for the above NIFA ORG project

WTFRC Collaborative expenses: None

Budget
Organization Name: OSU Agric. Res. Foundation **Contract Administrator:** Kelvin Koong
Telephone: (541) 737-4066 **Email address:** .j.koong@oregonstate.edu

Item	**2014-15**	**2015-16**	
Salaries Faculty Res. Assist.	14,000	14420	
Benefits OPE 58%	8,120	8364	
Wages undergrads	900	927	
Benefits OPE 12%	108	111	
Equipment			
Supplies	1,000	1030	
Local Travel	372	383	
Miscellaneous			
Plot Fees	500	515	
Total	**$25,000**	**$25,750**	

Footnotes: Annually**:** FRA 3.5 mo plus fringe, 90 hr undergrad labor, 2K M&S, 1K local travel & plot fee, 3% inflation.

OBJECTIVES

1) Develop non-antibiotic fire blight control programs that minimize fruit russet risk.

1a. Understand the specific risks to fruit from biological material, Blossom Protect (*Aureobasidium pullulans*).

2) **Continued evaluation of alternative, organic-approved materials for fire blight suppression.**

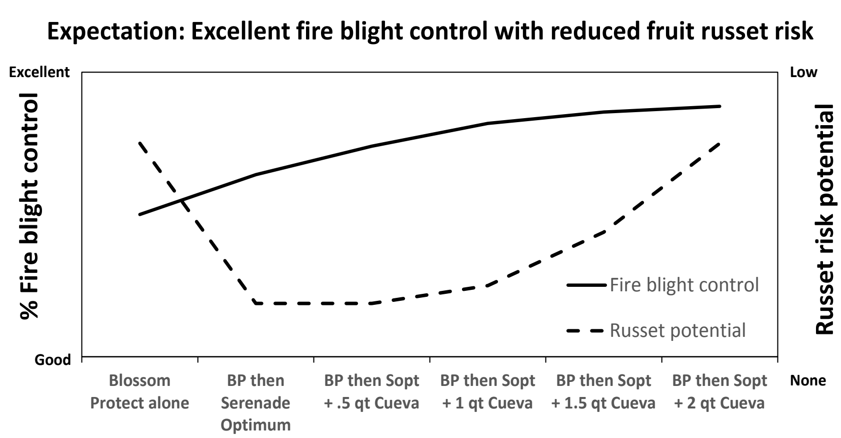
SIGNIFICANT FINDINGS

- Blossom Protect applied once at 70% bloom provided outstanding fire blight control in a pear trial with light disease pressure.
- Under severe disease pressure, fire blight control in Gala apple with Blossom Protect was enhanced by two oversprays Cueva soluble copper between full bloom and petal fall.
- After full bloom, *Aureobasidium pullulans* was detected on nearly 100% of flowers sampled from trees treated with Blossom Protect, and on most flowers (> 90%) sampled from non-treated trees.
- Blossom Protect induced russetting on Bartlett pear fruit in a wet climate (Corvallis) but not in semi-arid Lakeport, CA.
- Cueva induced russetting on Bartlett pear fruit in a wet climate (Corvallis), on Comice pear fruit in a semi-arid climate (Medford), but not on Bartlett pear fruit in a semi-arid climate (Lakeport).
- Molecular methods to identify Blossom Protect strains of *Aureobasidium pullulans* were verified and used to confirm that *A. pullulans*-induced damage to cherry and apple fruit from orchards not treated with Blossom Protect was not caused by Blossom Protect strains of *A. pullulans*.
- Several additional materials - Oxidate, Taegro, R42014 and Previsto – show potential to contribute to non-antibiotic fire blight control programs in certified organic orchards.

METHODS

Objective 1 hypothesis: We know from prior research that integrated programs of Blossom Protect followed by Serenade Optimum and/or a soluble copper (e.g., Cueva) will provide good to excellent fire blight control. We also know that there is a fruit russetting risk with both Blossom Protect and with soluble coppers. These russetting risks are poorly understood, and therefore, we want to more clearly define the risk when these materials are used in integrated blight control programs (hypothesized russeting risk is shown in **Fig. 1**).

Fig. 1. Hypothesized outcome of integrated, non-antibiotic fire blight control programs.



Experimental design. Objectives were addressed in experimental orchards located at Oregon State University field stations in Corvallis and Medford, and an organic pear orchard in Lake County, CA. Experiments were arranged in a randomized complete block design with 4 replications. Treatments were applied to trees during early morning (dates and bloom stages provided in results). Treatment suspensions and pathogen inoculum were sprayed to near runoff with backpack sprayers or with a motorized 25-gallon tank sprayer equipped with a hand wand.

Microbial colonization and disease assessment. Microbial populations were measured by washing flowers sampled from the experimental trees followed by dilution plating the wash onto a semi-selective culture medium to enumerate microbial populations. Fire blight was measured by counting the number of blighted flower clusters (strikes) on each tree during weekly inspections in May and early June. Microbial populations on flowers (log-transformed), total number of blighted flower clusters per tree, and disease incidence (diseased clusters divided by total clusters (based on prebloom counts)) were subjected to analysis of variance.

Fruit russet evaluation. Prior to harvest, 30 to 50 fruit were sampled from each replicate tree. For each fruit, percent surface russetting was graded using a modified Horsfall-Barratt rating system.

Molecular identification of Blossom Protect strains of Aureobasidium pullulans. The fire blight biocontrol product, Blossom Protect, consists of strains CF10 and CF40 of *A. pullulans* mixed in a 50:50 ratio. In recent situations of fruit rot of cherry (R. Kim, postharvest cherry lots, Yakima 2012) and fruit russet of apple (J. Pscheidt, Braeburn apple orchard, Corvallis 2014), *A. pullulans* was implicated as the cause of the fruit damage. This led us to investigate published molecular PCR protocols for specific identification of the Blossom Protect strains of *A. pullulans*.

RESULTS & DISCUSSION

Obj. 1. Non-antibiotic fire blight control programs that minimize fruit russet risk.

Fire blight control. *Bartlett pear trial.* Integrated programs of the non-antibiotic materials depicted in Figure 1 were evaluated for fire blight control in a 54-yr-old ‘Bartlett’ pear orchard near Corvallis, OR. Trees used in the study averaged 597 flower clusters per tree. Fire blight risk as determined by the heat unit risk model, COUGARBLIGHT, was moderate to high during the primary bloom period. Disease intensity was low with fire blight infections on water-treated trees averaging 12 strikes per tree (**Table 1**). Compared to the water-treated control, each of the treatments reduced significantly ($P < 0.05$) total strikes per tree and incidence of disease; the exception treatment was Luna Sensation, which performed similar to the water treated control (and was included as a control to suppress non-target floral colonization by *A. pullulans*). Antibiotic standards and all program combinations that began with one treatment of Blossom Protect provided a very high level of control including Blossom Protect by itself. Serenade Optimum by itself provided an intermediate level of control.

Gala apple trial. Integrated programs of the non-antibiotic materials depicted in Figure 1 were evaluated for fire blight control in a 15-yr-old ‘Gala’ orchard Corvallis, OR. Trees used in the study averaged 572 flower clusters per tree. Fire blight risk as determined by the heat unit risk model, COUGARBLIGHT, was low to moderate during the bloom period. Perhaps owing to a high dose of pathogen inoculum, disease intensity was very high with fire blight infections on water-treated trees averaging 389 strikes per tree (**Table 2**). Compared to the water-treated control, each of the treatments reduced significantly ($P < 0.05$) incidence of disease; the exception treatment was Luna Sensation, which performed similar to the water treated control. Based on ANOVA of total strikes per tree, Blossom Protect followed by Cueva twice at 3 quarts provided improved control compared to Blossom alone (64% control versus 34% control, respectively).

Discussion of fire blight control. With respect to fire blight severity, our 2014 trials yielded contrasting data with light disease pressure in Bartlett pear (water control averaged 12 strikes/tree) and severe disease pressure in gala apple (water control averaged 389 strikes/tree). In Bartlett pear, the 70% bloom treatment of Blossom Protect accounted for nearly all of the observed fire blight suppression. In apple, the high disease pressure resulted in Blossom protect alone providing an intermediate level of control in spite of nearly all flowers being colonized by *A. pullulans*. In apple, following Blossom Protect with Cueva (3qts/100 gallon) resulted in a level of control comparable to streptomycin.

Table 1. Bartlett pear, non-antibiotic fire blight trial, Corvallis, 2014.

Treatment	Rate per 100 gallons water	Date treatment applied* 7 Apr 80% bloom	10 Apr Full bloom	14 Apr Petal Fall	Number of blighted clusters per tree**		Percent blighted floral clusters***	
Water		---[§]	**X**	**X**	**11.8**	**a**[#]	**1.7**	**a**[#]
FireWall 100 ppm	8 oz.	---	X	---	1.3	cd	0.2	cd
FireLine 200 ppm	16 oz.	---	X	X	1.0	cd	0.2	cd
Serenade Optimum	20 oz.	---	X	X	6.0	ab	1.0	ab
Blossom Protect	21.4 oz.	X	---	---	0.3	d	0.1	d
plus Buffer Protect	150 oz.	X	---	---				
Blossom Protect	21.4 oz.	X	---	---	1.8	cd	0.3	cd
plus citric acid	150 oz.	X	---	---				
Blossom Protect	21.4 oz.	X	---	---	1.0	cd	0.1	cd
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	2.5	bc	0.4	bc
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
plus Cueva (one pint)	16 fl. oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	1.8	cd	0.3	cd
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
plus Cueva (one quart)	32 fl. oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	1.8	cd	0.3	cd
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
plus Cueva (1.5 quarts)	48 fl. oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	1.5	cd	0.2	cd
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
plus Cueva (two quarts)	64 fl. oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	2.8	bc	0.4	bc
plus Buffer Protect	150 oz.	X	---	---				
then Cueva (3 quarts)	96 fl. oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	1.0	cd	0.2	cd
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
plus Actigard	2 oz.	---	X	X				
Luna Sensation	4 oz.	---	X	X	10.8	a	1.7	a

*** Trees inoculated with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) on an evening 1 to 2 days before the full bloom treatment applications; total inoculum concentration was 1 x 10^6 CFU/ml. ** Transformed log(*x* + 1) prior to analysis of variance; non-transformed means are shown. *** Transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown. [§] X indicates material was sprayed on that specific date; --- indicates material was not applied on that specific date. [#] Means within a column followed by the same letter are not significantly different according to Fischer's protected least significance difference at *P* = 0.05.**

Table 2. Gala apple, non-antibiotic fire blight trial, Corvallis, 2014.

Treatment	Rate per 100 gallons water	Date treatment applied* 13 Apr 80% bloom	15 Apr Full bloom	19 Apr Petal Fall	Number of blighted clusters per tree**		Percent blighted floral clusters***	
Water		---[§]	**X**	**X**	**389**	**a**[#]	**69.8**	**a**[#]
FireWall 100 ppm	8 oz.	---	X	---	129	c	25.2	b
FireLine 200 ppm	16 oz.	---	X	X	245	b	43.8	b
Serenade Optimum	20 oz.	---	X	X	215	bc	32.6	b
Blossom Protect	21.4 oz.	X	---	---	258	b	41.5	b
plus Buffer Protect	150 oz.	X	---	---				
Blossom Protect	21.4 oz.	X	---	---	269	ab	44.5	b
plus citric acid	150 oz.	X	---	---				
Blossom Protect	21.4 oz.	X	---	---	213	bc	37.5	b
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	252	b	45.0	b
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
plus Cueva (one pint)	16 fl. oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	205	bc	34.8	b
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
plus Cueva (one quart)	32 fl. oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	192	bc	33.1	b
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
plus Cueva (1.5 quarts)	48 fl. oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	203	bc	37.8	b
plus Buffer Protect	150 oz.	X	---	---				
then Serenade Optimum	20 oz.	---	X	X				
plus Cueva (two quarts)	64 fl. oz.	---	X	X				
Blossom Protect	21.4 oz.	X	---	---	142	c	28.1	b
plus Buffer Protect	150 oz.	X	---	---				
then Cueva (3 quarts)	96 fl. oz.	---	X	X				
Luna Sensation	4 oz.	---	X	X	406	a	71.4	a

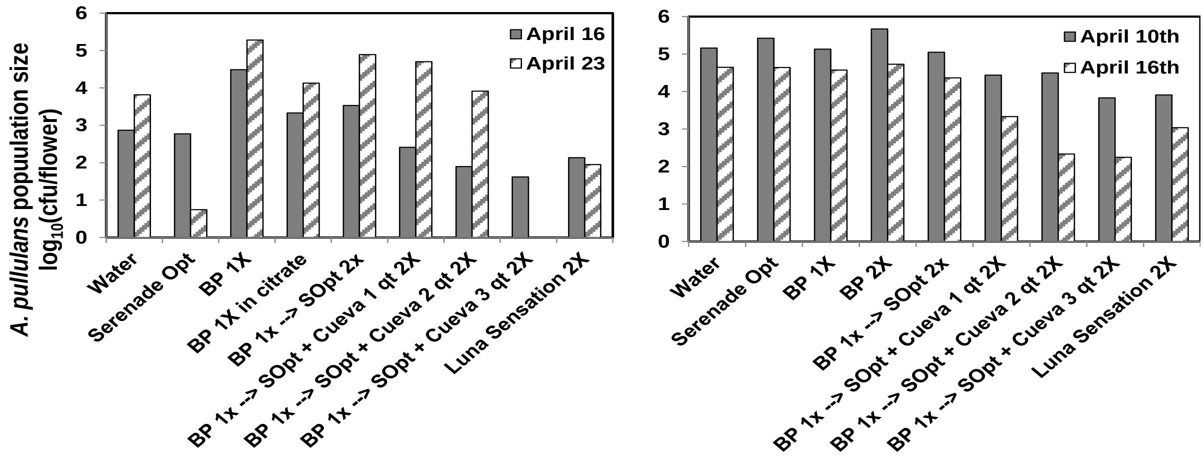
See table 1 for footnotes.

Yeast populations on flowers oversprayed with Serenade Optimum and Cueva copper.

In spray trials with Serenade Optimum and Cueva soluble copper, Blossom Protect was applied once at 70% bloom; populations of the Blossom Protect organism, *A. pullulans,* were measured on two sampling dates between full bloom and petal fall. Trials included those inoculated with the pathogen (**Tables 1 & 2**) and russet evaluation trials in southern Oregon (Comice pear, Medford) and northern California (Bartlett pear). Over all trials, *A. pullulans* was detected on nearly every flower (> 99%) from trees treated with Blossom Protect, and was detected on most flowers (> 90%) sampled from trees not treated with this material. In some trials (**Fig. 2A**), the measured population sizes of *A. pullulans* on non-treated trees was smaller than the population size of this organism on trees treated with Blossom Protect only. In contrast, in other trials (**Fig. 2B** and Gala apple, Corvallis (not shown)), the measured population size of *A. pullulans* on flowers from non-treated trees was statistically similar to the population size of this organism on trees treated with Blossom Protect only. Oversprays of Serenade Optimum after Blossom Protect did not significantly ($P > 0.05$) suppress *A. pullulans* populations compared to the population size of this organism on trees treated with Blossom Protect only. In contrast, mixing Serenade Optimum with 2 or 3 quarts of Cueva significantly suppressed *A. pullulans* populations ($P \leq 0.05$) compared to Blossom Protect only (i.e., Cueva copper caused a 10- to 1000-fold reduction in yeast population size).

Fig. 2. Population size on *Aureobasidium pullulans* (Blossom Protect organism) on pome fruit flower treated with integrated non-antibiotic fire blight control programs.

A. Bartlett pear, Corvallis **B. Comice pear, Medford**

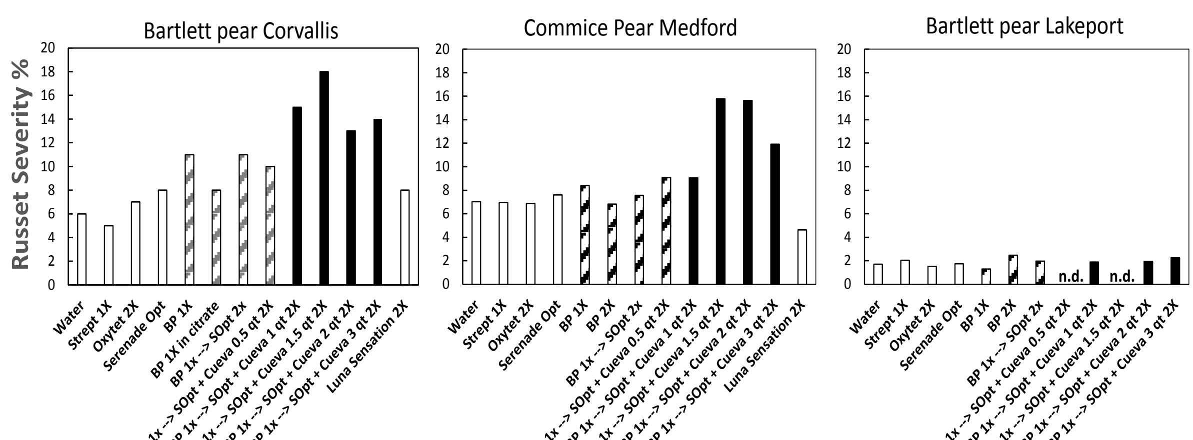


Fruit russeting associated with Blossom Protect, Serenade Opt and Cueva copper programs.

Russetting data was collected from all pear trials: Bartlett-Corvallis, Bartlett-Lakeport, Comice-Medford. The Gala apple trial had too much fire blight and apple scab to provide useful fruit russetting data. Within location, the Corvallis location showed significantly ($P \leq 0.05$) elevated

Fig. 3. Fruit russet severity on pear fruit flower treated with integrated non-antibiotic fire blight control programs. Open bar: low russet potential, hatched bar: Blossom Protect, solid bar: BP then Cueva.

A. **B** **C.**



russeting in Blossom Protect treatments (either alone or with Serenade Optimum) compared to treatments that received water only (Fig. 3A). Also in Corvallis, following Blossom Protect with Cueva treatments resulted in a significant ($P \leq 0.05$) enhancement in russetting severity compared to Blossom Protect by itself. Similary, at Medford, Cueva treatments after Blossom Protect significantly enhanced russetting of Comice pear compared to Blossom Protect alone, but severity of russetting on the Blossom Protect only treatments was not different than the water-treated control. At Lakeport, mean russetting severity was low (< 2% severity) and not effected by any of the treatments.

Discussion of A. pullulans populations and fruit russetting potential. Pears were chosen for the trials because they are more susceptible to russetting than apple, with Comice pear being exceptionally susceptible compared to the moderately susceptible, Bartlett pear. In addition, the trial locations represented two types of spring climate: semi-arid (Medford & Lakeport) and wet (Corvallis). Russetting was apparently influenced by climate with Bartlett pear in Corvallis showing a higher mean severity than the drier locations. Within drier climates, russetting was apparently influenced by cultivar with Comice pear in Medford showing a higher mean severity than Bartlett pear in Lakeport. In the semi-arid climates, Blossom Protect showed a little potential to enhance russet. In contrast, Cueva showed more potential to induce russetting; although based on the Lakeport data, this material appears relatively safe on tolerant cultivars as long as conditions remain dry during the period of high susceptibility (petal fall to plus 3 wk). In 2015, we intend to focus russet evaluation trials on apple.

In the upcoming 2015 season, implementation of non-antibiotic fire blight control is required for certified organic pome fruit. Based on the data above (and previous results), we have been communicating the following recommendations:

- Early bloom apple and pear Blossom Protect:
    - One full, or two half apps, or two full apps if blight in orchard last year
    - In apple, Blossom Protect immediately after 2nd lime sulfur thinning treatment
    - In smooth-skinned pears in wetter areas, russet risk might be unacceptably high
    - Bloomtime Biological is an alternative, fruit-safe biological material
- Full bloom to petal fall, depending on cultivar russet risk/CougarBlight model risk:
    - Serenade Optimum every 2 to 5 days (most fruit safe)
    - Improved control: Mix Serenade Opt with Cueva (2 to 3 qts/A)
    - Cueva every 3 to 6 days (3 to 4 qts/A) (good blight control but least fruit safe)

Obj. 1a. Understand the specific risks to fruit from the biological material, Blossom Protect: Molecular identification of *Aureobasidium pullulans* strains.

DNA extracted from CF10 and CF40 (positive controls) yielded their respective PCR products (Table 3). PACE- and BRAE-isolates (Table 3), which were recovered from cherry and apple fruit, respectively, all had pink coloration and filamentous edges consistent with *A. pullulans*. Amplification of DNA extractions using the general *A. pullulans* primers (CF40 ITS) resulted in positive amplification of all isolates and positive controls with the exceptions of isolate PACE-A758 and the no-DNA control. Amplification of isolate DNA with the specific CF10 and CF40 primer sets resulted in no PCR products for any of the PACE- or BRAE-isolates.

Table 3.

		PCR Primer set		
Isolate designator:	**Source:**	**CF40 ITS**	**SCAR6**	**SCH3RAPD**
CF10	Blossom protect	100 bp	307 bp	-
CF40	Blossom protect	100 bp	-	962 bp
PACE-A625, -A626, -A705, -A721, -A723, -A724, -A725, -A728, -A731, -A732, -A733, -A735, , -A759, -A760, -A762	cherry fruit, Yakima	100 bp	-	-
PACE-A758	cherry fruit	-	-	-
BRAE-1, -2, -3, -4, -5, -6, -7, -8, -9, -10	apple fruit, Corvallis	100 bp	-	-

Discussion. Specific PCR primers for the amplification of *A. pullulans* strains CF40 and CF10 (Blossom Protect) were used to successfully detect these strains from the product package and from treated pear and apple flowers (data not shown). We used these tools to investigate strain identity of *A. pullulans* isolates from cherries in Washington and Braeburn apple in Oregon; i.e., fruit damaged by *A. pullulans* from orchards that were not treated directly with Blossom Protect. Based on the general and specific primer sets, isolates from damaged fruit were identified as *A. pullulans* but not the Blossom Protect strains of this organism. We are continuing investigation into other methods for specific identification of Blossom Protect strains of *A. pullulans*.

Obj. 2. Evaluation of alternative, organic-approved materials for fire blight suppression.

Gala apple trial. Non-antibiotic materials for fire blight control were evaluated in a 15-yr-old ‘Gala’ orchard near Corvallis, OR. Trees used in the study averaged 572 flower clusters per tree. Fire blight risk as determined by the heat unit risk model, COUGARBLIGHT, was low to moderate during the bloom period. Perhaps owing to a high dose of pathogen inoculum, disease intensity was very high with fire blight infections on water-treated trees averaging 389 strikes per tree. Compared to the water-treated control, most treatments reduced significantly (*P* < 0.05) incidence of disease; exceptions were Luna Sensation, OxiPhos, BmJ alone, BMJ and Double Nickel combination, Double Nickel alone, and Double Nickel and Cueva Combination. Intermediate levels of control (36 to 53%) were provided by FireLine, Serenade Optimum, Taegro, Oxidate, R42014, and Blossom Protect. The highest levels of control (60 to 64%) were provided by FireWall (streptomycin), the Blossom Protect and Cueva combination, and the mineral material, LMA.

Table 3. Gala apple, alternative materials fire blight trial, Corvallis, 2014.

Treatment	Rate per 100 gallons water	Date treatment applied* 02 Apr Pre-bloom	13 Apr 80% bloom	15 Apr Full bloom	19 Apr Petal Fall	Number of blighted clusters per tree**		Percent blighted floral clusters***	
Water		---	---[§]	**X**	**X**	**389**	**a[#]**	**69.8**	**a[#]**
Luna Sensation	4 fl. oz.	---	---	X	X	406	a	71.4	a
FireWall 100 ppm	8 oz.	---	---	X	---	129	e	25.2	e
FireLine 200 ppm	16 oz.	---	---	X	X	245	ab	43.8	bcde
Blossom Protect plus Buffer Protect	21.4 oz. 150 oz.	---	X X	--- ---	--- ---	258	ab	41.5	bcde
Blossom Protect plus Buffer Protect then Cueva (3 quarts)	21.4 oz. 150 oz. 96 fl. oz.	---	X X ---	--- --- X	--- --- X	142	de	28.1	de
Double Nickel then Cueva (4 quarts)	32 fl. oz. 128 fl. oz.	---	X ---	X ---	--- X	274	ab	47.3	abcde
BmJ	4.5 oz.	X	X	X	---	290	ab	52.2	abcd
BmJ alone/plus/ then Double Nickel	4.5 oz. 32 fl. oz.	X	X X	--- X	--- ---	290	ab	56.7	abc
Double Nickel	64 fl. oz.	---	---	X	X	261	ab	49.8	abcde
Serenade Optimum	20 oz.	---	---	X	X	215	bc	32.6	cde
Taegro	5.2 oz.	---	---	X	X	230	bc	44.9	bcde
OxiDate plus HoldIt sticker	128 fl. oz. 32 fl. oz.	---	--- ---	X X	X X	227	bcd	42.0	bcde
OxiPhos plus HoldIt sticker	160 fl. oz. 32 fl. oz.	---	X X	X X	X X	314	ab	63.3	ab
R42014	64 oz.	---	---	X	X	194	cde	34.0	cde
LMA 2%	16.6 lb.	---	---	X	X	121	e	24.9	e

See table 1 for footnotes.

Discussion. From the perspective of certified organic production, the materials Previsto (2014 data not shown), Oxidate, Taegro, and R42014 either have or are expected to be placed on the National Organic Program’s approved material list. We will re-evaluate most of these materials in 2015 within integrated programs with other materials (e.g., Blossom Protect). The mineral material, LMA, is being used for fire blight control in Europe, but it is not yet clear if this material will receive organic approval. In addition to the above materials, we used our Golden Delicious block in 2014 to evaluate a mix of experimental phage (bacterial viruses) from Brigham Young University. The mix contained phage that specifically infect the fire blight pathogen. The phage material did not provide significant fire blight control, but will be looked at again in 2015.