FINAL PROJECT REPORT **YEAR**: 2 of 2

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

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David Sugar, OSU, Medford, OR

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

Agency Name: USDA NIFA OREI
Amt. awarded: $476K to Johnson, Elkins, and Smith 10/11 - 9/14
Notes: Objectives 1 and 2 of this proposal are matching objectives for the above NIFA OREI project
Agency Name: USDA NIFA ORG
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:** Russ Karow
Telephone: (541) 737-4066 **Email address:** Russell.Karow@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.

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

SIGNIFICANT FINDINGS

- Blossom Protect applied once at 70% bloom continued to provide significant fire blight control in apple and pear.
- In 2015, integrated fire blight control programs that began with Blossom Protect and followed by Serenade Opti, Cueva soluble copper or combinations of these materials showed enhanced suppression compared to Bloom Protect alone.
- In multi-location trials, Blossom Protect (comprised of two strains of *Aureobasidium pullulans*) showed a slight potential to increase fruit russeting at a wet location (Corvallis) and on sensitive cultivars (Comice and Golden Delicious), but did not induce russeting in Braeburn apple or Bartlett pear grown in semi-arid climates (Medford and Lakeport).
- Compared to Blossom Protect, the soluble copper Cueva showed a higher potential to russet fruit. Copper-induced russeting was observed on Bartlett pear fruit and Golden Delicious apple in a wet climate (Corvallis) and on Comice pear fruit in a semi-arid climate (Medford), but compared to treatment with water, was not increased significantly on Braeburn apple or Bartlett pear grown in a semi-arid climates (Medford and Lakeport).
- 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.
- Molecular methods to identify Blossom Protect strains of *A. pullulans* were verified and used to demonstrate that about half of *A. pullulans* isolates detected on trees treated with Blossom Protect were the strains from the biocontrol product, but that on trees not treated with Blossom Protect, the detected *A. pullulans* isolates were likely from a source within the orchard.
- Several additional materials – oxidizing agents (Oxidate and R42014), soluble copper (Previsto), alum (potassium aluminum sulfate), and E. amylovora-specific phage – showed potential to contribute to non-antibiotic fire blight control programs.

RESULTS & DISCUSSION

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

1.a. Fire blight control. Integrated fire blight control programs that began with Blossom Protect and followed by Serenade Opti, Cueva soluble copper or combinations of these materials were conducted during 2014 and 2015 in apple and pear orchards located in Corvallis, OR (**Fig. 1 & 2**).

Bartlett pear, 2014. 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 (Fig. 1A). 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 2014. 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 (**Fig. 1B**). 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).

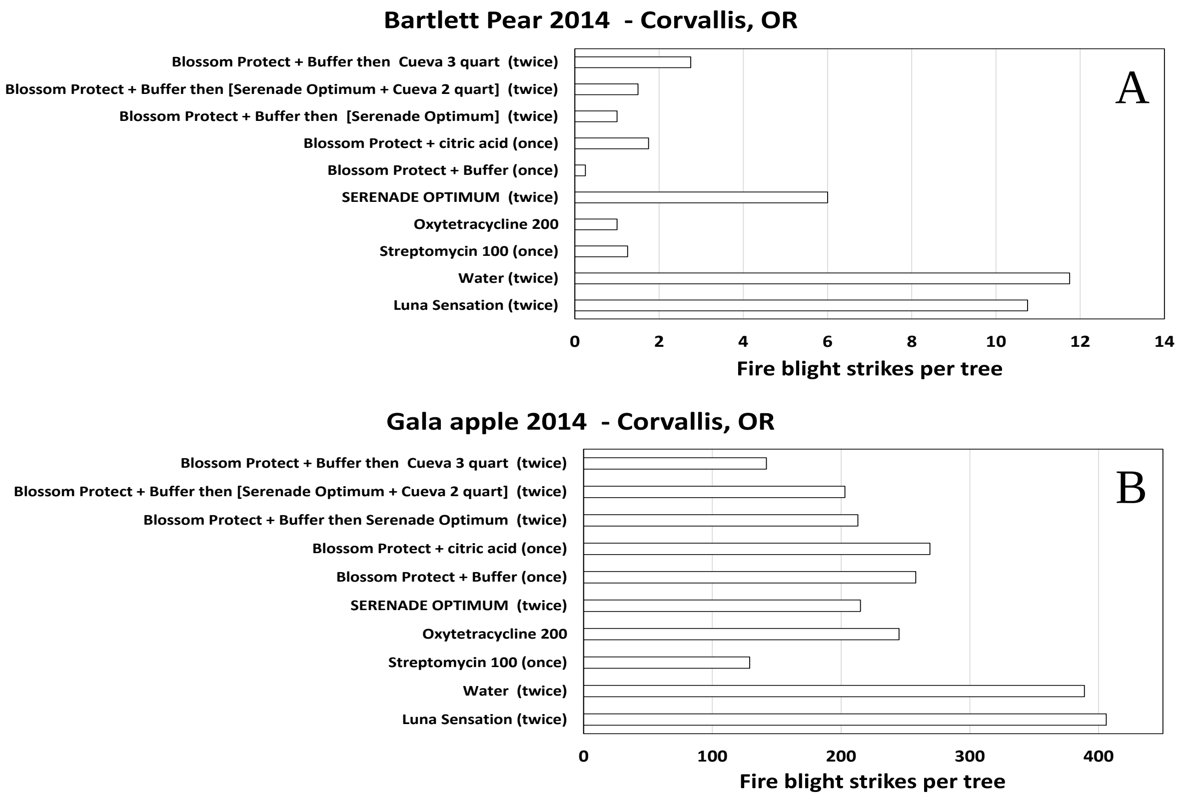


Fig. 1. Results of inoculated fire blight trials conducted near Corvallis, OR in 2014. Bars represent means of four replicate trees. Blossom Protect treatments were applied at 70% bloom; other materials were applied at full bloom and petal fall if applied twice. *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) was inoculated onto the trees on an evening 1 to 2 days before the full bloom treatment applications; inoculum concentration was 1 x 10^6 CFU/ml.

Bartlett pear, 2015. Trees used in the study averaged 319 flower clusters per tree. Fire blight risk as determined by the heat unit risk model, COUGARBLIGHT, was low during the bloom period. For a pathogen-inoculated trial, disease intensity was low with fire blight infections on water-treated trees averaging 12.5 strikes per tree (**Fig. 2A**). Compared to the water-treated control, all non-antibiotic programs that began with one treatment of Blossom Protect reduced significantly ($P < 0.05$) total strikes per tree and incidence of disease. Treatment programs where Cueva, R40214 (called 'Oxycom Ca' in the chart) and potassium aluminum phosphate (called 'Alum' in the chart) followed Blossom Protect resulted in significantly ($P < 0.05$) fewer blighted flower clusters than trees-treated with Blossom Protect only. Numerically, the fungicide Luna Sensation performed worse than the water treated control, which may be due to its ability to suppress secondary, non-target colonization of flowers by the yeast, *A. pullulans*. The antibiotic standards, streptomycin and oxytetracycline, did not provide significant fire blight suppression.

Golden Delicious apple, 2015. Trees used in the study averaged 288 flower clusters per tree. Fire blight risk as determined by the heat unit risk model, COUGARBLIGHT, was low in early bloom but high near petal fall. For a pathogen-inoculated trial, disease intensity was moderate with fire blight infections on water-treated trees averaging 78 strikes per tree (**Fig. 2B**). Compared to the water-treated control, all non-antibiotic programs that began with one treatment of Blossom Protect reduced significantly ($P < 0.05$) total strikes per tree and incidence of disease. Based on total number of blighted flower clusters per tree, the program where Previsto followed Blossom Protect resulted in significantly ($P < 0.05$) fewer blighted flower clusters than trees-treated with Blossom Protect only. The fungicide Luna Sensation performed similar to the water treated control.

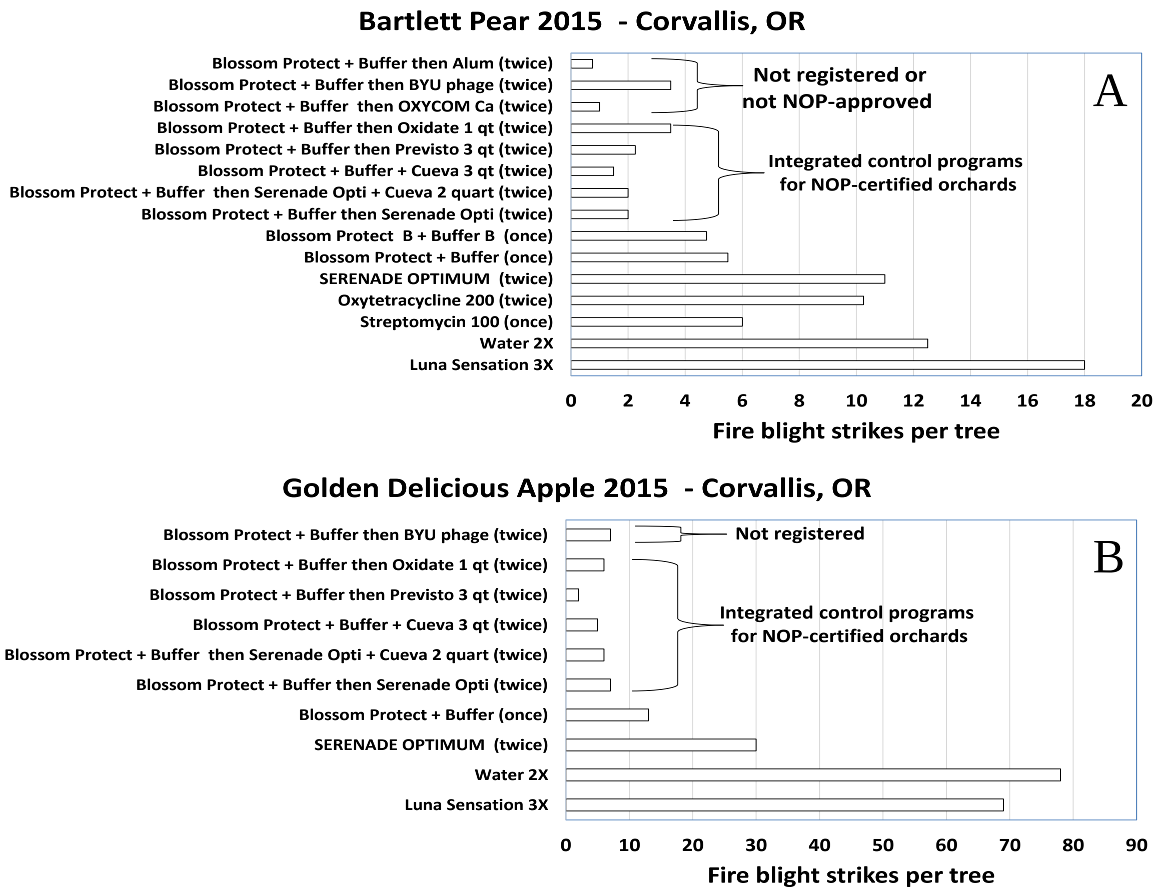


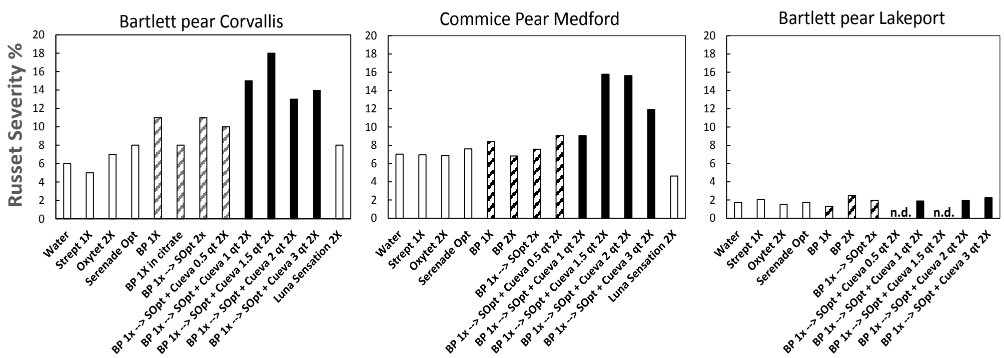
Fig. 2. Results of inoculated fire blight trials conducted near Corvallis, OR in 2015. Bars represent means of four replicate trees. Blossom Protect treatments were applied at 70% bloom; other materials were applied at full bloom and petal fall if applied twice. *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) on an evening 1 to 2 days before the full bloom treatment applications; inoculum concentration was 1 x 10^6 CFU/ml.

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.

In 2015, fire blight severity ranged from light (pear) to intermediate (Golden Delicious apple) but overall results were similar. Blossom Protect once provided 60 to 80% of the observed disease suppression. Following Blossom Protect with Serenade Opti, Cueva soluble copper or combinations of these materials showed enhanced suppression compared to Bloom Protect alone. In the pear trial, integrated non-antibiotic control was superior to the antibiotic treatments.

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

In 2014, fruit russet data was collected from pear trials: Bartlett-Corvallis, Bartlett-Lakeport, Comice-Medford. In 2015, russet trials were established in Corvallis (Bartlett pear and Golden Delicious apple), Medford (Braeburn apple) and Lakeport (Bartlett pear). Among locations, Corvallis showed the most fruit russet independent of treatment. At this location, in both years, trees that received soluble copper after Blossom Protect had russet severites that exceeded 10%; in contrast,



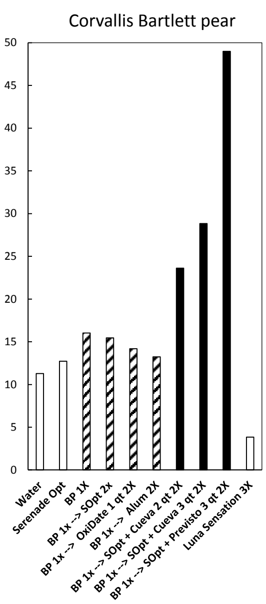
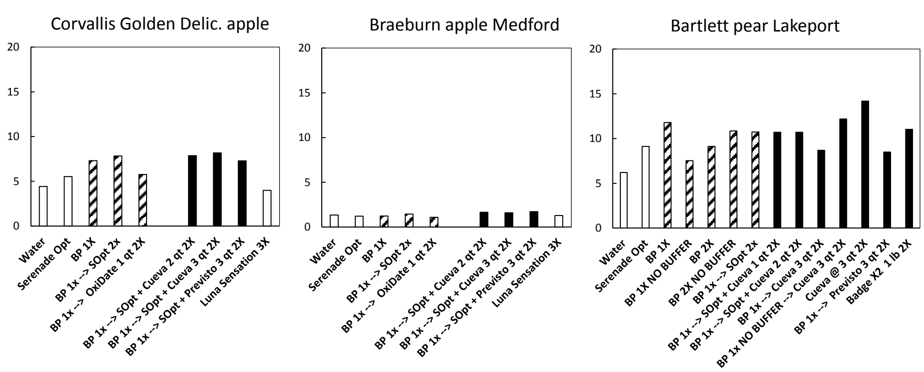


Fig. 3. Mean fruit russet severity (%) on pear and apple fruit treated with integrated non-antibiotic fire blight control programs. Open bar: fruit from trees that received a low russet potential treatment (water, Serenade Opti or oxytetracycline); hatched bar: fruit from trees treated with Blossom Protect only or Blossom Protect followed by non-russeting material; black bar: fruit from trees treated with Blossom Protect followed by soluble copper (Cueva or Previsto). Upper panel: 2014 trials; lower panel: 2015 trials.



trees that received Blossom Protect treatments (either alone or or followed by non-russeting material) showed intermdiate levels of fruit russuting compared to treatments that received water (low russeting) or copper (high russeting). Also at Corvallis, in 2015 the Golden Delicious apple fruit showed less rutteting than pear fruit but Blossom Protect or Blossom Protect then copper increased the level of fruit russting compared to the water treated control. Similary, at Medford in 2014, 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 russet severity in 2014 was low (< 2% severity) and not effected by any of the treatments, but russet was increased across all treatments in 2015. At several locations, the Luna Sensation treatment showed the lowest level of russet, which may indicate suppression of non-target spread of Blossom Protect strains of *A. pullulans* and/or suppression of indigenous russet-inducing yeast populations (see next section).

Discussion of A. pullulans populations and fruit russetting potential. Initially, pear cultivars 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 2015, we also added apple cultivars: Golden Delicious (russet sensitive) in Corvallis and Braeburn (russet tolerant) in Medford. 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 and Golden Delicious apple 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 Braeburn apple at the same location or Bartlett pear in Lakeport in the same season. In the semi-arid climates, Blossom Protect showed very little potential to enhance russet. In contrast, Cueva showed more potential to induce russetting; although based on Bartlett pear at Lakeport in 2014 and Braeburn apple at Medford in 2015, 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).

Implementation of non-antibiotic fire blight control is now 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 <u>Blossom Protect</u>:
  - 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:
  - <u>Serenade Optimum</u> every 2 to 5 days (<u>most fruit safe</u>)
  - Improved control: Mix Serenade Opt with Cueva (2 to 3 qt./A)
  - <u>Cueva</u> every 3 to 6 days (3 to 4 qt./A) (good blight control but <u>least fruit safe</u>)

1.c. 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 one to two sampling dates between full bloom and petal fall. Trials included those inoculated with the pathogen (Corvallis) and fruit russet evaluation trials in southern Oregon (Braeburn apple, Medford) and northern California (Bartlett pear, Lakeport). Over all trials, *A. pullulans* was detected on nearly every flower (> 99%) from trees treated with Blossom Protect, and was detected on a majority of flowers (> 50%) sampled from trees not treated with this material (**Fig. 4**). In general, the measured population sizes of *A. pullulans* on non-treated trees was 0.5 to 2 log units smaller than the population size of this organism on trees treated with Blossom Protect only. In 2015, oversprays of Serenade Optimum and soluble coppers 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. This result was in contrast to 2014 results where mixing Serenade Optimum with 2 or 3 quarts of Cueva significantly suppressed *A. pullulans* populations ($P \leq 0.05$) compared to Blossom Protect only (see 2014 progress report).

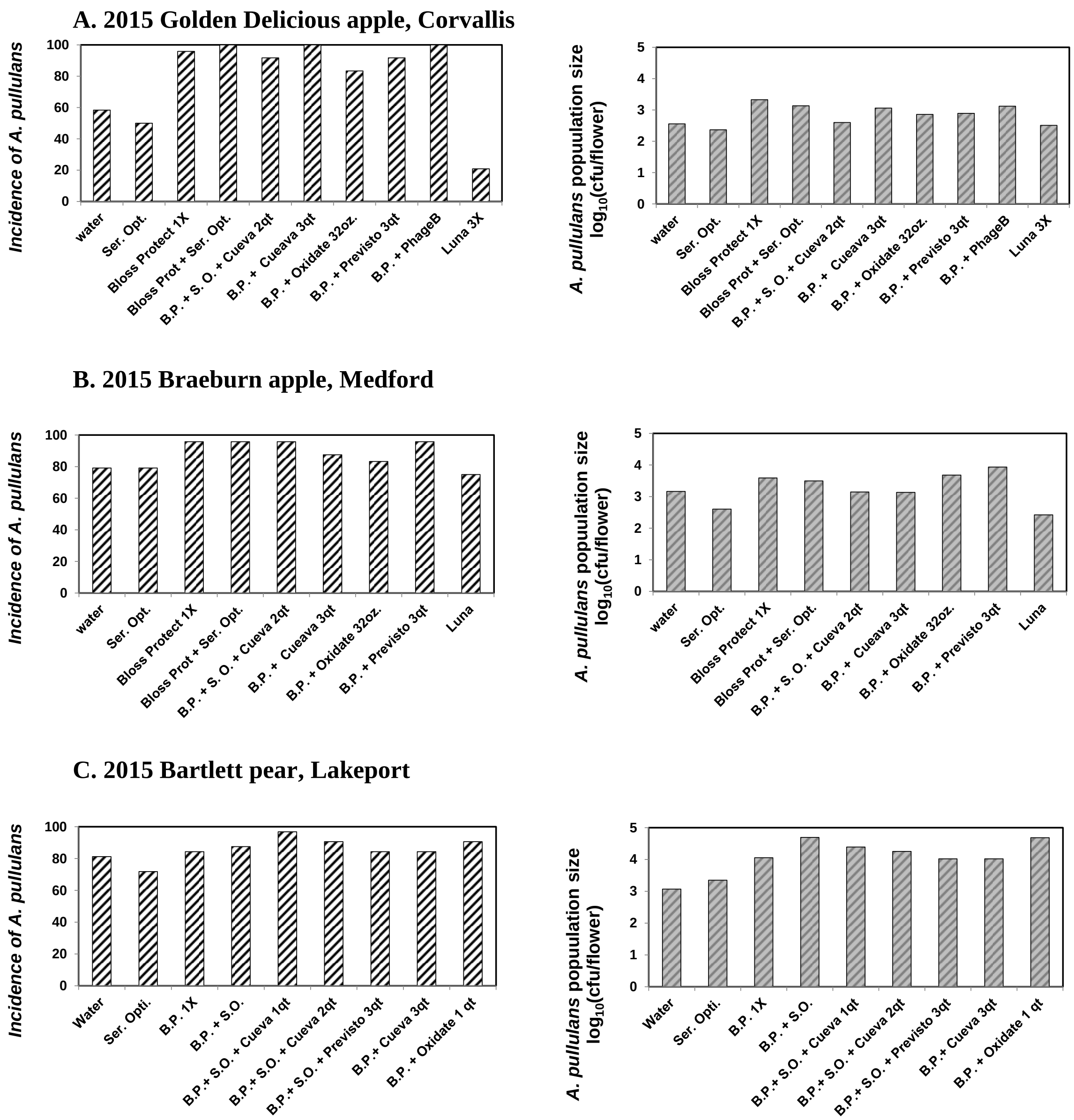


Fig. 4. Incidence (left panels) and population size (right panels) on *Aureobasidium pullulans* on pome fruit flower treated with integrated non-antibiotic fire blight control programs.

d. Molecular identification of *Aureobasidium pullulans* isolated from pome fruit flowers.

The fire blight biocontrol product, Blossom Protect, consists of strains CF10 and CF40 of *A. pullulans*, which are produced separately then mixed together in the bag. 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. With a PCR analyses that utilized general *A. pullulans* and strain-specific primers, we confirmed *A. pullulans* but also found the *A. pullulans* isolates were not Blossom Protect strains (see 2014 report).

These observations on *A. pullulans* led us to further investigate published molecular PCR protocols for specific identification of the Blossom Protect strains of *A. pullulans*. DNA extracted from CF10 and CF40 (positive controls) yielded their respective PCR products (**Table 1**).

Table 1. PCR products from *Aureobasidium pullulans*

Isolate:	Source:	PCR Primer set: AP ITS	SCAR6	SCH3RAPD
CF10	Blossom protect	100 bp	307 bp	-
CF40	Blossom protect	100 bp	-	962 bp
Non-Blossom Protect isolates of *A. pullulans*	orchards & fruit lots	100 bp	-	-

From each of three bags of Blossom Protect, we made 12 single spore isolations of *A. pullulans* (36 isolates in total). To verify the stability of the PCR markers in each isolate, we made ten sequential transfers of the single spore isolates (approximately 50 days to complete the 10 sequential transfers). We subjected the single spore isolates to PCR analysis after the 1st, 5th and 10th transfers. The general *A. pullulans* primer set (AP ITS) identified all isolates and stability of the strain-specific *A. pullulans* PCR markers was confirmed (**Fig. 5**).

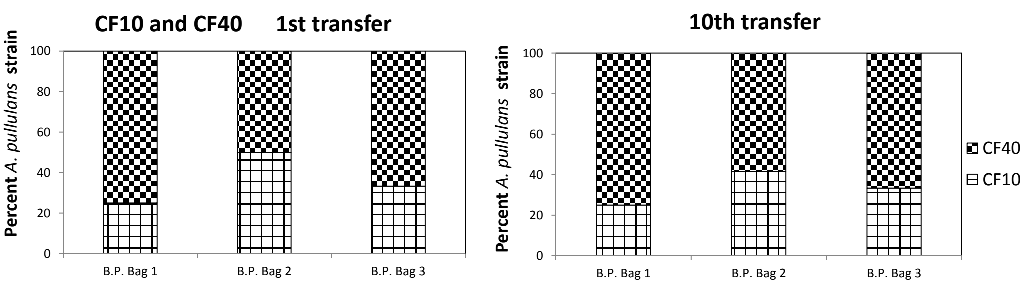
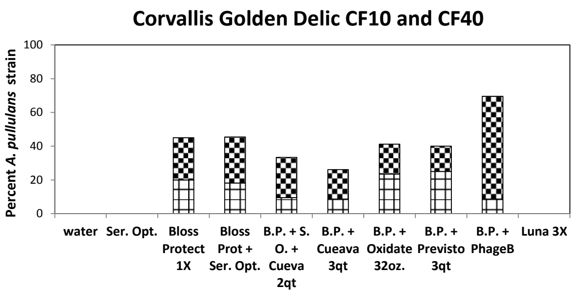


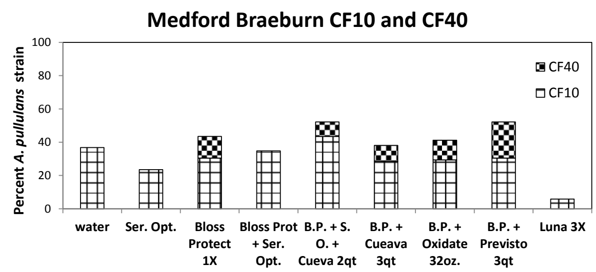
Fig. 5. Molecular identification of 36 *Aureobasidium pullulans* isolates from 3 bags of Blossom Protect (12 isolates per bag). After 1 and 10 sequential transfers of each isolate, all isolates were subjected to PCR analysis with primers specific to *A. pullulans* biocontrol strains CF10 and CF40.

From the fire blight and fruit russet trials in Corvallis, Medford and Lakeport, the individual floral wash samples used to measure *A. pullulans* population size by dilution plating (see above) were saved and stored frozen. Beginning in late summer, *A. pullulans* was re-isolated from each floral wash and subject to PCR analysis with the *A. pullulans* primer sets described in Table 1.

A. pullulans was readily re-isolated from the frozen floral wash samples, and colonies that we had visually identified as this organism were verified with the general *A. pullulans* primer set (AP ITS). In contrast, the primer sets specific for Blossom Protect strains of *A. pullulans* (CF10 and CF40) provided results with variation that could be attributed to the treatment that each tree received. In general, if a flower was from a tree treated with Blossom Protect, then ~50% of the floral washes yielded a positive reaction with primer sets specific to strains CF10 and CF40 (**Fig. 6**). If a tree did not receive Blossom Protect as a treatment (e.g., water or Serenade Opti), then the proportion of *A. pullulans* isolates identified as Blossom Protect strains was only 8%.

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. We used these tools to investigate strain identity of *A. pullulans* isolates from pome fruit flower treated with integrated non-antibiotic fire blight control programs. Based on isolation onto dilution plates and the general AP-ITS primer set, we conclude ate *A. pullulans* is a very common organism on pear and apple flowers. Surprisingly, however, Blossom Protect strains of *A. pullulans* were not as large of proportion of the total population as we expected. Blossom Protect strains of *A. pullulans* were only about half of the strains detected on flowers from trees treated with this product, and < 10% of strains on trees not-treated with this product. We still consider these results preliminary and will investigate further in 2016. If these observations remain consistent, it means we don't completely understand why Blossom Protect is highly effective for fire blight control. Perhaps the low pH buffer (Buffer protect) is playing a role larger than solely aiding the establishment of Blossom Protect strains of *A. pullulans* on floral surfaces. If so, then perhaps other materials used for fire blight could be enhanced with pH adjustment.





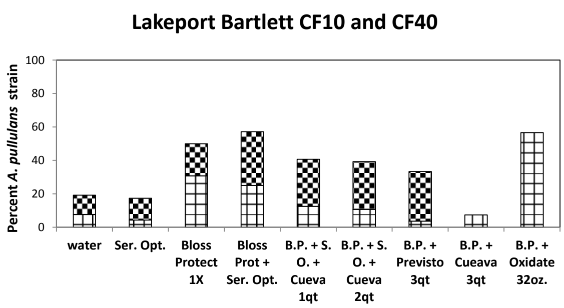


Fig. 6. Molecular identification of *Aureobasidium pullulans* isolates from pome fruit flowers treated with integrated non-antibiotic fire blight control programs.

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 16 -yr-old 'Gala' orchard near Corvallis, OR. COUGARBLIGHT, was low in early bloom but high near petal fall. For an inoculated trial, disease intensity was moderate with fire blight infections on water-treated trees averaging 42 strikes per tree (Table 1). Compared to the water-treated control, several materials by themselves reduced significantly ($P \leq 0.05$) incidence (%) of blighted flower clusters: the mineral potassium aluminum sulfate, the soluble coppers Cueva and Previsto, OxiDate (with HOLDit sticker), FireWall (streptomycin) and FireLine (oxytetracycline). Serenade Opti (with BioLink spreader) and FireQuencher A by themselves provided an insignificant level of suppression (23 to 28%); in contrast, the combination of Serenade Opti (with BioLink) and FireQuencher A provided very good control (70%), which was significantly ($P \leq 0.05$) better than either material alone. The combination of Double Nickel and Cueva also provided good control (58%), which was significantly ($P \leq 0.05$) better the Double Nickel alone. *P. agglomerans* strain C9-1 and the mixture of this bacterium with Fire Quencher both provided intermediate but insignificant levels of control (29 to 34%, respectively).

Discussion. Blossom Protect was excluded from this trial to determine how some of the materials we have been recommending for integrated, non-antibiotic control would perform without the pretreatment of the yeast. In this regard, Serenade, Cueva, and Oxidate were all intermediate performers, which we believe indicates the importance of Blossom Protect in the control program. From the perspective of certified organic production, Previsto is expected to be registered in 2016 and

Oxidate is expected to have a label modification to clarify that it can be used at the rate shown above. The mineral material, potassium aluminum sulfate, is being used for fire blight control in Europe, and a petition has been made to the National Organic Standards Board to allow for its use in organic agriculture (as a treatment for animal waste). More research on alum is needed to better understand its properties and to determine optimal rates. Fire Quencher A and B are experimental *E. amylovora*-specific phages (bacterial viruses) from the Dept. of Microbiology, Brigham Young University. Results of most phage treatments for fire blight control (and for plant disease control, in general) have been disappointing. Nonetheless, in the above table, the treatment of Fire Quencher A mixed with Serenade Opti and an ultraviolet protectant (sunscreen) provided outstanding control. We have proposed to look more closely at phages (two sources) and alum over the 2016-17 seasons.

Table 1. Gala apple, alternative materials fire blight trial, Corvallis, 2015.

Treatment	Rate per 100 gallons water	Date treatment applied* 9 Apr 70% bloom	15 Apr Full bloom	20 Apr Petal Fall	Number of blighted clusters per tree**		Percent blighted floral clusters***	
Water	-	**X**[§]	**X**	---	**42**	**a**[#]	**10.3**	**a**[#]
Double Nickel	64 fl. oz.	X	X	---	46	a	12.8	a
Fire Quencher B	16 fl. oz.	X	X	---	37	ab	10.7	ab
Fire Quencher A	16 fl. oz.	X	X	---	37	ab	7.9	ab
Serenade Opti and BioLink spreader	20 oz. 4 fl. oz.	X X	X X	--- ---	23	abc	7.4	abc
Pantoea vagans C9-1	10^8 cfu/ml	X	X	---	27	abc	7.4	abc
Pantoea vagans C9-1 plus Fire Quencher A	10^8 cfu/ml 16 fl. oz.	X X	X X	--- ---	23	abc	6.8	abc
Cueva (3 quarts)	96 fl. oz.	X	X	---	20	abc	6.2	abcd
Cueva (2 quarts)	64 fl. oz.	X	X	---	22	abc	5.8	bcd
OxiDate 2.0 plus HOLDit sticker	128 fl. oz. 32 fl. oz	--- ---	X X	X X	25	abc	5.8	bcd
FireLine 200 ppm	16 oz.	X	X	---	21	abc	5.8	bcd
Double Nickel plus Cueva (2 quarts)	32 fl. oz. 64 fl. oz.	X X	X X	--- ---	15	bc	4.3	cde
GWN 10073 (Previsto)	96 fl. oz.	X	X	---	14	c	3.4	de
Serenade Opti and BioLink spreader plus Fire Quencher A	20 oz. 4 fl. oz. 16 fl. oz.	X X X	X X X	--- --- ---	5	c	3.2	de
FireWall	8 oz.	X	X	---	9	c	2.7	de
Alum 2%	16.6 lb.	---	X	X	7	c	1.6	e

* Trees inoculated on 12 April with 1 x 10^6 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin-sensitive and nalidixic acid resistant fire blight pathogen strain).
** 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 same letter do not differ significantly (P = 0.05) based on Fischer's protected least significance difference.

EXECUTIVE SUMMARY

Significant findings:

- Blossom Protect applied once at 70% bloom continued to provide significant fire blight control in apple and pear.
- In 2015, integrated fire blight control programs that began with Blossom Protect and followed by Serenade Opti, Cueva soluble copper or combinations of these materials showed enhanced suppression compared to Bloom Protect alone.
- In multi-location trials, Blossom Protect (comprised of two strains of Aureobasidium pullulans) showed a slight potential to increase fruit russeting at a wet location (Corvallis) and on sensitive cultivars (Comice and Golden Delicious), but did not induce russeting in Braeburn apple or Bartlett pear grown in semi-arid climates (Medford and Lakeport).
- Compared to Blossom Protect, the soluble copper, Cueva, showed a higher potential to russet fruit. Copper-induced russeting was observed on Bartlett pear fruit and Golden Delicious apple in a wet climate (Corvallis) and on Comice pear fruit in a semi-arid climate (Medford), but compared to treatment with water, was not increased significantly on Braeburn apple or Bartlett pear grown in a semi-arid climates (Medford and Lakeport).
- After full bloom, *Aureobasidium pullulans* was detected on nearly 100% of flowers sampled from trees treated with Blossom Protect, and on most flowers sampled from non-treated trees.
- Molecular methods to identify Blossom Protect strains of *A. pullulans* were verified and used to demonstrate that about half of *A. pullulans* isolates detected on trees treated with Blossom Protect were the strains from the biocontrol product, but that on trees not treated with Blossom Protect, the detected *A. pullulans* isolates were likely from a source within the orchard.
- Several additional materials – oxidizing agents (Oxidate and R42014), soluble copper (Previsto), alum (potassium aluminum sulfate), and E. amylovora-specific phage – showed potential to contribute to non-antibiotic fire blight control programs.

Industry implications: Owing to the antibiotic phase-out in National Organic Program-certified production, the fire blight control programs developed from this research project have been implemented by the organic tree fruit producers in Washington State. Compared to antibiotic programs, the non-antibiotic spray programs are effective for fire blight suppression but carry somewhat higher risks of fruit russeting. These risks are influenced by orchard climate and cultivar. For example, a russet-tolerant apple cultivar grown in an arid environment is much less russet-prone than a sensitive pear cultivar produced in a wet climate. We view the yeast material Blossom Protect (*Aureobasidum pullulans*) as an essential component to non-antibiotic fire blight control programs. Blossom Protect is produced to an excellent quality standard, and in most of central Washington, the risk of fruit russeting from this material is negligible. Soluble coppers (Cueva and Previsto) are also effective for disease suppression but carry a risk of fruit russet that is higher than observed for Blossom Protect. Consequently, their use during the bloom period needs to be limited to tolerant cultivars in dry environments, and for russet sensitive cultivars, to periods of fruit development when risk of russet has diminished (summer). Results of this project lead to three recommendations for further research: i) continued search for effective materials that can substitute for copper in late bloom treatments, and ii) obtain a better understanding of the effects of the Buffer Protect companion to Blossom Protect on the floral microbiome, and iii) investigate the ecology of *A. pullulans* strains resident in orchards, which could perhaps lead to cultural manipulation of their populations.