FINAL REPORT **YEAR**: 2 of 2

Project Title: Refinement of practical fire blight control: Non-antibiotic and SAR

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Item	**2018-19**	**2019-20**
Salaries Faculty Res. Assist. 3.5 mo	17,339	17,686
Benefits OPE 61%	10,577	10,788
Undergraduate labor (&OPE 12%)	1,064	1,085
Equipment	0	0
Materials and Supplies	1,500	1,530
Local Travel	1,500	1,530
Plot Fees	2,000	2,040
Medford russet trials (plot fees and labor)	6,000	6,120
Total	**$39,980**	**$40,779**

OBJECTIVES:

1) In integrated programs with Blossom Protect + Buffer Protect, evaluate EPA-registered, NOP-approved materials with demonstrated anti-microbial activity (e.g., Previsto, Jet Ag, and lime sulfur) for their ability to suppress fire blight and to induce fruit russeting on apple and pear trees.

2) In integrated programs with Blossom Protect + Buffer Protect, evaluate the mineral material, alum ($KAl(SO_4)_2$) and an alum-containing stone powder for their ability to suppress fire blight and to induce fruit russeting on apple and pear trees.

3) Evaluate alternative yeasts for their ability to suppress fire blight.

4) Evaluate amount and placement of Actigard trunk paints for the purpose of protection from fire blight during primary bloom.

SIGNIFICANT FINDINGS:

- Blossom Protect (yeast), Previsto (copper) at full bloom and lime sulfur 4% at petal fall provided outstanding fire blight control.
- Lime sulfur 4% at petal fall did not increase fruit russeting of apple compared to a water treated control
- Alternative yeast strains (with Buffer Protect) suppressed fire blight but to a degree less than observed with Blossom Protect.
- Population size of *E. amylovora* on pear and apple flowers continued to increase during the post-petal fall period except when treated with lime sulfur at petal fall.
- Lime sulfur or Jet Ag (H_2O_2 in peracetic acid) sprayed near petal fall suppressed yeast populations on flowers.
- Alum (potassium aluminum sulfate, 8 lb/100 gal), applied after Blossom Protect, provides excellent fire blight control.
- Alum, an effective fire blight control material, reduces the pH of floral surfaces; lime sulfur increases pH of floral surfaces.
- Alum increased fruit russeting in both pear and apple.
- Concentrated Actigard treatments applied to the trunks of apple trees at 10% bloom provided partial suppression fire blight but not to the outstanding level observed in 2017.
- Kudos (prohexidione-CA) and Regalia (giant knotweed extract) provided partial fire blight suppression when sprayed onto flower clusters at 10% bloom.

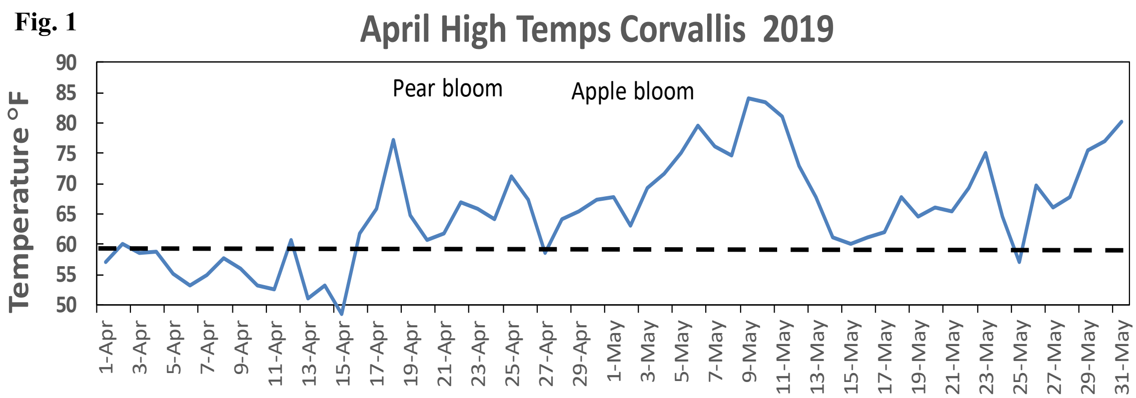
RESULTS

Experimental design. Objectives 1-3 were addressed in experimental orchards located at Oregon State University's Botany & Plant Pathology Field Laboratory near Corvallis (pathogen-inoculated), and at the OSU Southern Oregon Research and Extension Center near Medford, OR (fruit finish only). Experiments were arranged in a randomized complete block designs with 3 to 4 replications. Treatment suspensions were sprayed to near runoff with backpack sprayers during early morning hours. To enumerate pathogen and yeast populations on flowers, five flower clusters were sampled from each replicate tree at full bloom, petal fall, and one-week post-petal fall, which was followed by washing the flowers, recording the pH of the wash, and dilution plating the wash on a selective culture media. In Corvallis trials, incidence of fire blight was determined by counting and removing the blighted flower clusters on each tree at 2- to 4-weeks after bloom. In Medford, in late August, the percent of the fruit surface with symptoms of russeting was scored with a modified Horsfall-Barratt rating scale.

Weather in spring 2018: See continuing report from last year for spring 2018 weather information. *Weather in spring 2019*: Fire blight risk as determined by the heat unit model, COUGARBLIGHT, was moderate through the bloom periods of pear and apple. Epiphytic pathogen populations built up quickly on inoculated control trees and remained high (> 1 million cells per flower) through bloom. Nonetheless, in pear, fire blight incidence was lower than typical for an inoculated trials because of a light bloom (following heavy bloom in 2018). In apple, weather conditions during and after petal fall were very warm and dry, and therefore, all apple trees were misted with water near petal fall to promote infection.

For objectives 1-3, the water-controls averaged 25 infections per tree in Bartlett pear (9% of total clusters) and 93 infections per tree in Golden Delicious apple (38% of total clusters). For objective 4, water-treated control trees in the 6-yr-old Gala apple block averaged 22 strikes per tree (27% of total clusters); on non-treated trees averaged 11 strikes per tree (14% of total clusters).

Fig. 1



Objectives 1 and 2. Refer to the 2018 continuing report for data concerned with microbial populations on flowers and floral pH in that season. In this report, fire blight infection and fruit russeting data are shown for both 2018 and 2019 but the text mostly discusses refers to data from 2019. Also for 2019, non-antibiotic control focused on somewhat more complex spray programs to evaluate series of materials that could best meet these combined these attributes: i) outstanding infection suppression, ii) outstanding suppression of pathogen populations, iii) significant suppression of yeast populations at petal fall, and iv) negligible induction of fruit russeting. In general, these programs were initiated with the biological material Blossom Protect (+ Buffer Protect), followed by two different non-antibiotic chemical(s) (Table 1).

Infection suppression. In pear in 2019, outstanding suppression (> 70%) was observed with FireWall 50 (streptomycin sulfate), and Blossom Protect plus Buffer Protect (twice), Blossom Protect plus Buffer Protect (once) followed by alum (twice), and Blossom Protect plus Buffer Protect (once) then Previsto (once at full bloom) then 4% lime sulfur (once at petal fall) (Table 1). Other programs that included a treatment with a *Bacillus*-based material after Blossom Protect/Buffer Protect (e.g., Serenade Opti or Stargus) were less effective.

Pathogen populations in flowers. Population size of *E. amylovora* on pear flowers continued to increase during the post-petal fall period with the exception of integrated programs that included 4% lime sulfur at petal fall (Fig. 2). Treatment programs that included the *Bacillus*-based material, Serenade Opti, showed less suppression of pathogen populations than treatment programs that included Previsto copper or alum.

Yeast populations in flowers. Trees that received a non-antibiotic chemical(s) material after Blossom Protect had lower floral yeast populations than flowers from trees treated with Blossom Protect only (Fig. 3). Trees that received Previsto at full bloom and 4% lime sulfur at petal fall had the lowest yeast populations in the post-petal fall period.

*Floral pH***.** Relative to other treatments, lower floral pH measurements were associated with the treatment program that included alum, and higher floral pH measurements were associated with treatment programs that included lime sulfur (Fig. 4).

Table 1. Evaluluation of non-antibiotic materials for fire blight control in Bartlett pear and Golden Delicious apple, Corvallis, 2018 and 2019.

Treatment	Rate per 100 gallons water	70% bloom	Full bloom	Petal Fall	**PEAR 2018** % blighted floral clusters **		**APPLE 2018** % blighted floral clusters**		**PEAR 2019** % blighted floral clusters**	
Water		**---**[§]	**X**	**X**	29.7	**a**	35.9	**a**	9.0	**a**
FireWall	8 oz.	---	X	---	4.5	ef	0.8	d	1.7	cd
Serenade	20 oz.	---	X	X	-		-		5.1	abc
Alum 1%	133.5 oz.	---	X	X	16.9	abc	1.7	cd	-	
VP20	144 oz.	---	X	X	16.2	bc	1.1	cd	-	
Blossom Protect	21.4 oz.	X	X	---	5.8	def	2.2	bcd	2.7	bcd
Buffer Protect	150 oz.	X	X	---						
Blossom Protect	21.4 oz.	X	---	---	9.9	cde	**0.5**	d	2.4	cd
Buffer Protect	150 oz.	X	---	---						
then Alum 1%	133.5 oz.	---	X	X						
Blossom Protect	21.4 oz.	X	---	---	11.1	cd	3.1	bcd	-	
Buffer Protect	150 oz.	X	---	---						
then VP20	144 oz.	---	X	X						
Blossom Protect	21.4 oz.	X	---	---	**4.2**	ef	**0.9**	d	-	
Buffer Protect	150 oz.	X	---	---						
then Previsto 1%	96 fl. oz.	---	X	X						
Blossom Protect	21.4 oz.	X	---	---	-		-		**1.5**	d
Buffer Protect	150 oz.	X	---	---						
then Previsto 1%	96 fl. oz.	---	X	---						
then Lime sulfur 4%	512 fl. oz	---	---	X						
Blossom Protect	21.4 oz.	X	---	---	22.6	ab	1.0	cd	3.6	bcd
Buffer Protect	150 oz.	X	---	---						
then Serenade Opti	20 oz.	---	X	---						
then Lime sulfur 4%	512 fl. oz	---	---	X						
Blossom Protect	21.4 oz.	X	---	---	-		-		2.9	bcd
Buffer Protect	150 oz.	X	---	---						
then Previsto 1%	96 fl. oz.	---	X	---						
then Serenade Opti	20 oz.	---	---	X						
Blossom Protect	21.4 oz.	X	---	---	13.0	bcd	2.5	bcd	-	
Buffer Protect	150 oz.	X	---	---						
then Serenade Opti	20 oz.	---	X	---						
then Jet Ag	167 fl. oz		---	X						
Blossom Protect	21.4 oz.	X	---	---	14.6	bc	-		-	
Buffer Protect	150 oz.	X	---	---						
then Serenade Opti	20 oz.	---	X	X						
Regalia (popcorn)	64 fl. oz. **X**	---	---	---	-		-		5.5	abc
Blossom Protect	21.4 oz.	X	---	---						
Buffer Protect	150 oz.	X	---	---						
then Stargus	64 fl. oz.	---	X	---						
then Regalia	64 fl. oz.	---	---	X						

*** Trees inoculated with *Erwinia amylovora* strain Ea153N (streptomycin-sensitive) at 1×10^6 CFU/ml on 12 April (pear 2018), 25 April (apple 2018), and 18 April (pear 2019). ** Trees used in the experiments averaged 841, 256, and 266 flower clusters per tree for pear 2018, apple 2018, and pear 2019, respectively. For each treatment, percent blighted flower clusters was transformed arcsine($\sqrt{x}$) prior to analysis of variance; non-transformed means are shown. [§] X indicates material was sprayed at that bloom stage date; --- indicates material was not applied at that bloom stage. Means within a column followed by the same letter are not significantly different according to Fischer's protected least significance difference at $P = 0.05$.**

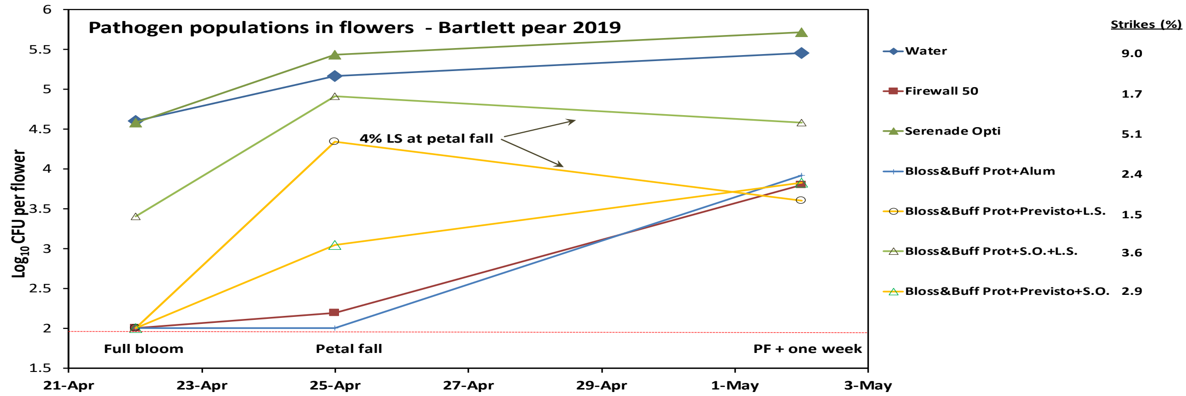


Fig. 2. Effect of treatments applied to A) Bartlett pear trees to suppress fire blight on the population size of *E. amylovora* strain 153N on flowers during April and May 2019. Pathogen populations were determined by washing five flower clusters (~25 flowers, bulked) from each replicate tree, and plating the wash onto a selective culture medium. $Log_{10} = 2.0$ was the detection limit of the assay. Data depict mean of each treatment program on each sampling date.

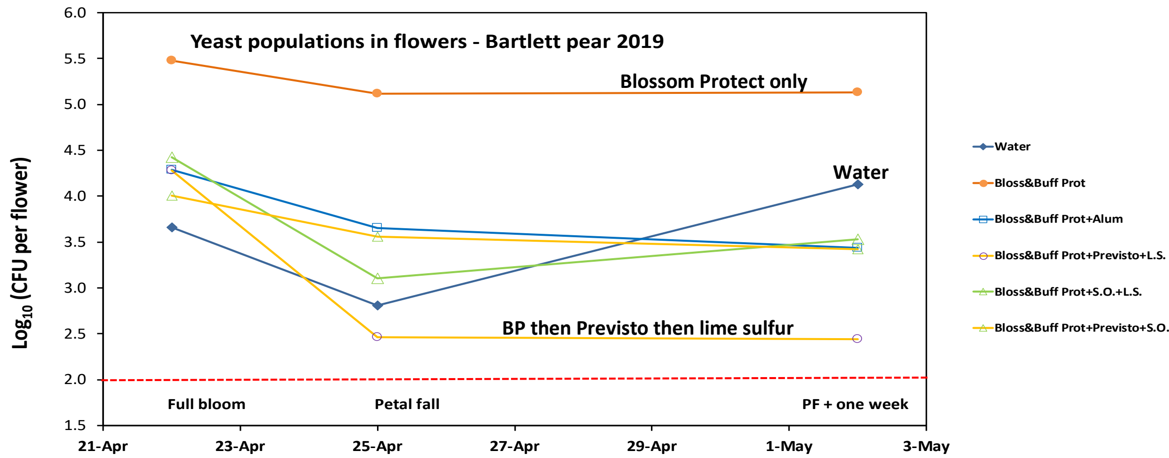


Fig. 3. Effect of treatments applied to Bartlett pear trees to suppress fire blight on the population size of *Aureobasidium pullulans* (the yeast in Blossom Protect) on flowers during April and May 2019. *A. pullulans* populations were determined by washing five flower clusters (~25 flowers, bulked) from each replicate tree and plating the wash onto a selective culture medium. Data depict mean of each treatment program on each sampling date.

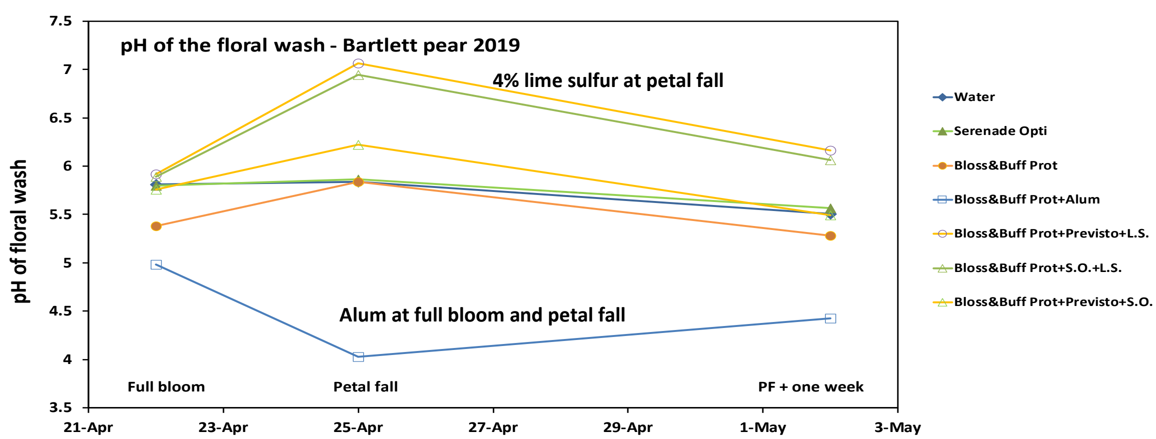


Fig. 4. Effect of treatments applied to Bartlett pear trees to suppress fire blight on the pH of floral surfaces during April and May 2019. A hand-held pH-probe was placed in a deionized-water wash of five flower clusters (~25 flowers, bulked in 25 ml of water) from each replicate tree. Data depict mean of each treatment program on each sampling date.

Fruit russeting. As in 2018, application of non-antibiotic fire blight materials to Comice pear and Granny Smith apple in 2019 resulted in significant differences ($P \leq 0.05$) in fruit russeting severity, although the specific material that caused the most russeting differed among crop species. For example, Serenade Opti or Previsto (full bloom) and then 4% lime sulfur (petal fall) was the most injurious treatment applied to Comice pear, but these same treatments resulted in the least amount of russeting on Granny Smith apple (less than the water control). Conversely, Blossom Protect and Buffer Protect was the least injurious to pear but the most injurious to apple. Noteworthy, and in contrast to 2018 results, integrated spray programs applied to apple in 2019 that contained Previsto copper (in 2019 this material was applied at full bloom only) did not increase fruit russeting relative to the water control. Also in 2019, as in the previous season, alum elevated russeting severity in both pear and apple compared to the water control.

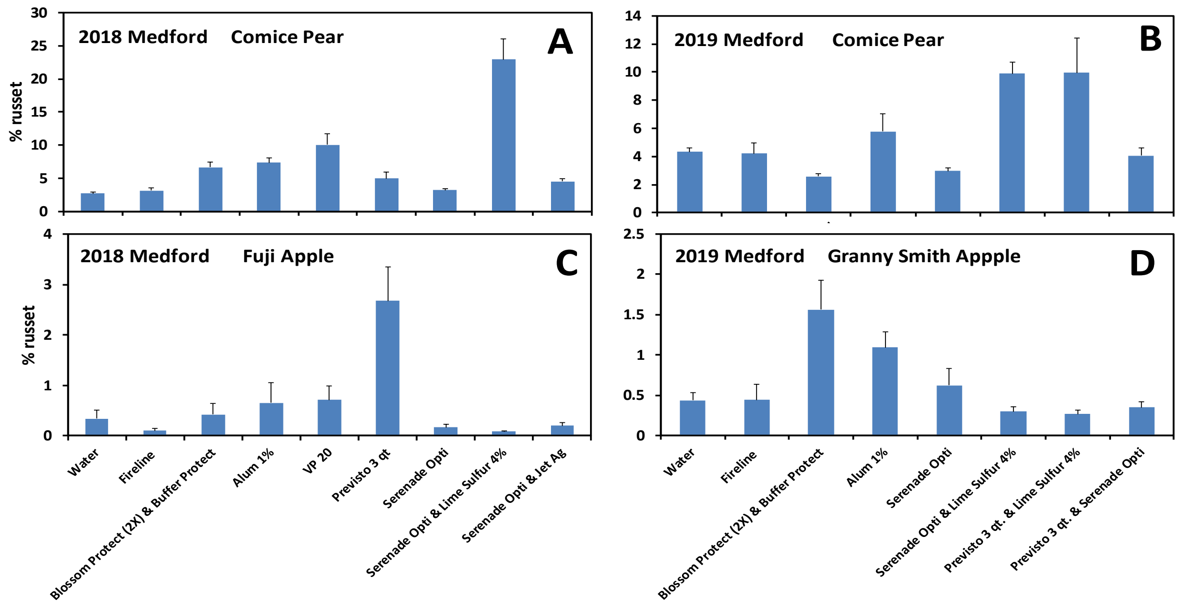


Fig. 5. Effect of non-antibiotic fire blight control materials applied to A,B) Comice pear and C) Fuji or D) Granny Smith apple trees on severity of russeting injury (%) of the fruit surface in the 2018 (A,C) and 2019 (B,D) seasons. Orchards were located near Medford, OR. Treatments were applied to trees at full bloom and at petal fall (April). In late August, 30 fruit from each replicate tree were rated for russeting severity. Data depict mean and standard error from four replicate trees that received each treatment.

Objective 3. Trials were established in Bartlett pear and Golden Delicious apple to evaluate the effectiveness of Blossom Protect for fire blight suppression relative to other yeasts. Treatments were Blossom Protect (1.6 g/liter, for which the titer was 1×10^7 CFU/ml of *A. pullulans*), a water-treated control and lab-grown yeast isolates: a postharvest biocontrol strain of *Cystofilobasidium infirmominiatum* strain YY6 (Spotts et al. 2009), two local field isolates of *A. pullulans* (strains AP3 (used in pear only in 2018) and AP6 (used in 2019)), and two local field isolates of *Cryptococcus neoformans* (strains C16 (used in 2018) and C9 (used in 2019)). Local strains of *A. pullulan*s and *C. neoformans* were isolated from flower washes during a 2016 experiment and identified by sequencing PCR-amplicons from primers ITS and ELO2. Yeast cultures were grown on PDA for 4 to 6 days (20°C), and then scraped from the media surface. Resulting cell suspensions were sprayed onto trees at 1×10^7 CFU/ml. Prior to spraying, Buffer Protect (11.2 g/liter) was added to each yeast isolate suspension. Experimental trees were inoculated with *Erwinia amylovora* strain 153N at 80% bloom.

Yeasts were readily recovered from flowers sampled from yeast-treated trees (Fig. 4) with the species that was applied to the trees being the dominant species recovered. Moreover, regardless of isolate, each yeast generally attained population sizes exceeding 1×10^4 CFU/ flower and these populations were significantly larger ($P \leq 0.05$) than the total yeast population size on the water-treated controls. Significant differences in yeast populations were observed among yeast treatments. For example, in both pear and apple in 2018, trees treated with C. *infirmominiatum* strain YY6 had significantly larger populations ($P \leq 0.05$) of this yeast on flowers compared to the *A. pullulans* populations measured on flowers treated with Blossom Protect (Fig. 4 A, C). For all trials, *A. pullulans* populations on Blossom Protect-treated trees were either statistically similar to or smaller than population sizes measured for the other yeast treatments (the exception was apple in 2019 where the *A. pullulans* population measured on Blossom Protect-treated trees was significantly greater ($P <$ 0.05) than the *A. pullulans* population on trees treated with local isolate AP6 of this fungus (Fig. 4D).

With regard to fire blight, all yeast treatments had a smaller incidence of infection than the water-treated control (Fig. 5). Fire blight suppression by Blossom Protect was always numerically superior to suppression by other yeast isolates and significantly superior ($P \leq 0.05$) to the other yeast isolates in two of the trials (Fig. 5A, C). Averaged over trials, Blossom Protect (plus Buffer Protect) applied twice provided 81 ± (s. e.) 5% control of fire blight. In contrast, the next most suppressive treatment, C. *infirmominiatum* strain YY6 (plus Buffer Protect) provided 58 ± 13% control.

Fig. 4. Log$_{10}$ (population size) of yeast isolates on A,B) Bartlett pear or C, D) Golden Delicious apple flowers sprayed at 70% and full bloom during spring seasons of 2018 (left) and 2019 (right) in orchards near Corvallis, OR. Treatments were water (black diamond), Blossom Protect (open circle), *C. infirmominiatum* YY6 (black square), two local field isolates of *A. pullulans*, AP3 (2018) and AP6 (2019) (open triangle), and two local field isolates of *C. neoformans*, C16 (2018) and C9 (2019) (black triangle); all yeast treatments were amended with Buffer Protect. Data depict mean and standard error of four treatment replicates on each sampling date.

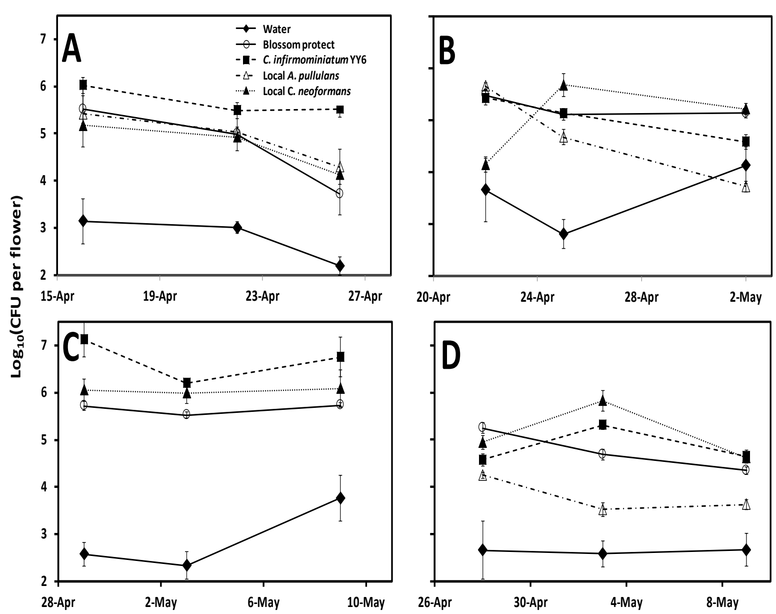
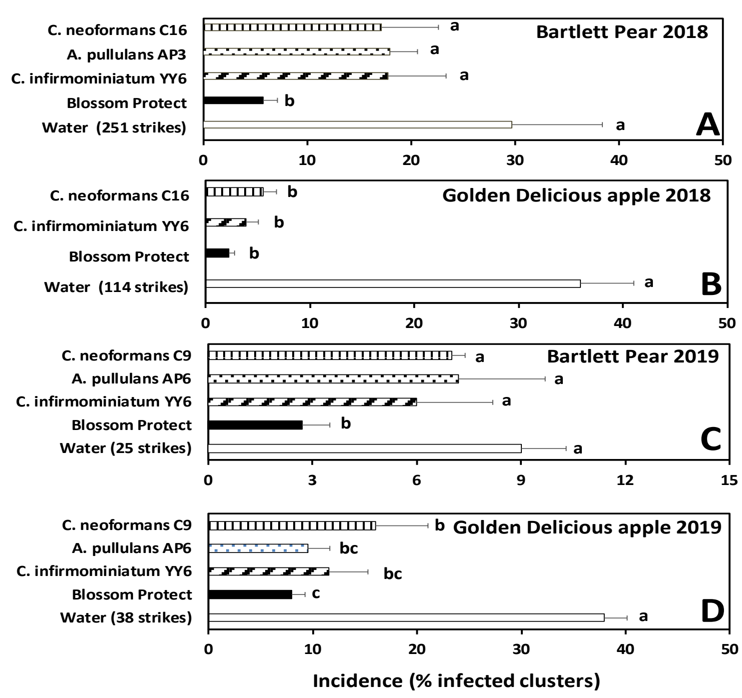


Fig. 5. Effect of yeast plus Buffer Protect treatments on incidence of fire blight infection in 'Bartlett' pear and 'Golden Delicious' apple orchards located near Corvallis, OR in April-May, 2018 and 2019. Yeast (or water) treatments were arranged in a RCB design with four replications and sprayed at 80% and full bloom. *E. amylovora* was inoculated onto trees on an evening between the two treatment dates. Incidence of infection = infected flower clusters/total flower clusters per tree. Absolute number of infections per tree (strikes) for the water controls are shown in parentheses. Within panel, bars labeled with a different small-case letter indicate a significant difference in disease incidence at *P* = 0.05; error bars represent one standard error of the mean.



Objective 4. Refer to the 2018 continuing report for fire blight incidence data concerned with prebloom application of SAR inducers to apple tree trunks.

In 2019, eight resistance inducing materials were evaluated: Actigard 50WG (acibenzolar-S-methyl, Syngenta Crop Protection, Greensboro, NC), Kudos (prohexidione calcium, Fine Americas, Walnut Creek, CA), Blush (prohydrojasmon, Fine Americas), Regalia (extract of *Reynoutria sachalinensis,* Marrone Bio, Davis, CA), Employ (harpin-αß protein, SymAgro, Visalia, CA), Ecoswing (extract of *Swinglea glutinosa,* Gowan, Yuma, AZ), Romeo (cell walls of *Saccharomyces cerevisiae* LAS117, Agrauxine, Beaucouzé, France), and proprietary material 'A'.

The experiment had 19 treatments with six replications but for analysis, treatments were categorized by how and when materials were placed onto each tree: a) concentrated material paints applied once to the tree trunk only, b) materials sprayed onto floral clusters once at 10% bloom, and c) materials sprayed three times onto flower clusters at 10% bloom, full bloom and petal fall. Control treatments were a non-treated control, and a water-treated control (sprayed with water at 10% bloom, full bloom and petal fall).

Concentrated materials were applied by spraying the tree's central leader with the material in a 1-liter, hand held pump sprayer (model 418, Solo Inc., Newport News, VA). The sprayer was equipped with a cone-shielded nozzle, and during application, the nozzle tip was positioned a distance of 1-cm from the trunk surface. All treatments except one were applied as a 'full' treatment, which meant spraying a 100-cm length of the central leader on two opposing sides of trunk; this treatment applied ~60 ml of spray suspension onto the tree. The exception, an Actigard '½' treatment, was applied to a 100-cm length of trunk to one side only. All materials were applied in combination with a surfactant to aid material absorption: 1% Break-Thru S 240 (polyether-modified polysiloxane, Evonik Corp., Richmond, VA) for Actigard and Blush, and 1% BioLink Spreader Sticker (organic soapbark spreader, Westbridge Agricultural Products, Vista, CA) for Regalia. For trees that received floral treatments, materials were sprayed to near runoff with 12-L backpack sprayers equipped with hand wands (0.5 liter/tree); amended surfactants are listed as footnotes to the data table.

Symptoms of fire blight were first observed on 15 May. Incidence of fire blight was determined by counting the number of blighted flower clusters (i.e. strikes) on each tree during three inspections on 20 and 30 May. Blighted clusters were removed immediately after counting. Incidence of disease (total strikes/total cluster number) was subjected to analysis of variance (Analyze-It Software v. 3.0, Leeds, UK).

Trees used in the study averaged 88 flower clusters per tree. For a pathogen-inoculated trial, disease intensity was moderate with fire blight infections on water-treated trees averaging 22 strikes per tree (27% of total clusters) and on non-treated trees averaging 11 strikes per tree (14% of total clusters). For concentrated material paints applied to the tree trunk only, Actigard (full trunk treatment) had an average infection incidence of 7%, which was 50% less infection than the non-treated control (14%) but this difference was not significant ($P = 0.08$). Other materials paints applied to the trunk only showed responses intermediate to Actigard (full trunk) and the non-treated control.

For materials sprayed onto floral clusters once at 10% bloom, infection incidence on trees that received Regalia and material A averaged 5 and 7%, respectively, which was significantly less ($P \leq 0.05$) than the nontreated control (14%). Similarly, for trees sprayed three times during the bloom period, incidences of infection (%) on trees treated with Actigard (4%), Regalia (4%), Employ (8%), material A (13%), and Regalia plus material A (16%) were significantly less ($P \leq 0.05$) than the water-treated control (27%).

Table 2. Evaluluation of SAR-inducer materials for fire blight control in Gala apple, Corvallis, 2019

Treatment	Rate	Date treatment applied* 23 Apr 10% bloom	26 Apr Full bloom	1 May Petal fall	Number of blighted clusters per tree**	Percent blighted floral clusters**
Trunk paint 1X	**per quart**					
Non-treated	-	**---**[§]	---	---	**11**	**14 a**[#]
Actigard - full	1 oz.	X	---	---	6	7 a
Actigard – ½ one side	1 oz.	X	---	---	10	10 a
Regalia	16 fl. oz.	X	---	---	7	9 a
Blush	16 fl. oz.	X	---	---	7	11 a
Flower clusters 1X	**per 100 gal**					
Water-treated	-	**X**	**X**	**X**	**22**	**27** a
Non-treated	-	**---**	---	---	**11**	**14** bc
Kudos [x,y]	3 oz.	X	---	---	15	17 ab
Kudos [x,y]	6 oz.	X	---	---	7	10 bcd
Actigard [y]	6 oz.	X	---	---	12	12 bcd
Kudos [x,y] Actigard	2 oz. 3.2 oz.	X	---	---	10	11 bcd
Regalia [z]	256 fl. oz.	X	---	---	3	5 d
Material A [z]	128 fl. oz.	X	---	---	7	7 cd
Bloom sprays 3X	**per 100 gal**					
Water-Treated	-	X	X	X	22	27 a
Actigard [z]	2 oz.	X	X	X	4	5 c
Employ [z]	2 oz.	X	X	X	8	10 bc
Regalia [z]	64 fl. oz	X	X	X	4	6 c
Material A [z]	16 fl. oz.	X	X	X	13	16 b
Material A [z] plus Regalia	16 fl. oz. 32 fl. oz.	X	X	X	11	13 bc
Ecoswing [z]	32 fl. oz.	X	X	X	14	17 ab
Romeo [z]	14.6 oz.	X	X	X	21	19 ab

* Trees mist inoculated on 24 April with 1 x 10^6 CFU/ml *Erwinia amylovora* strain Ea153N (streptomycin- and oxytetracycline-sensitive fire blight pathogen strain).

** 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.

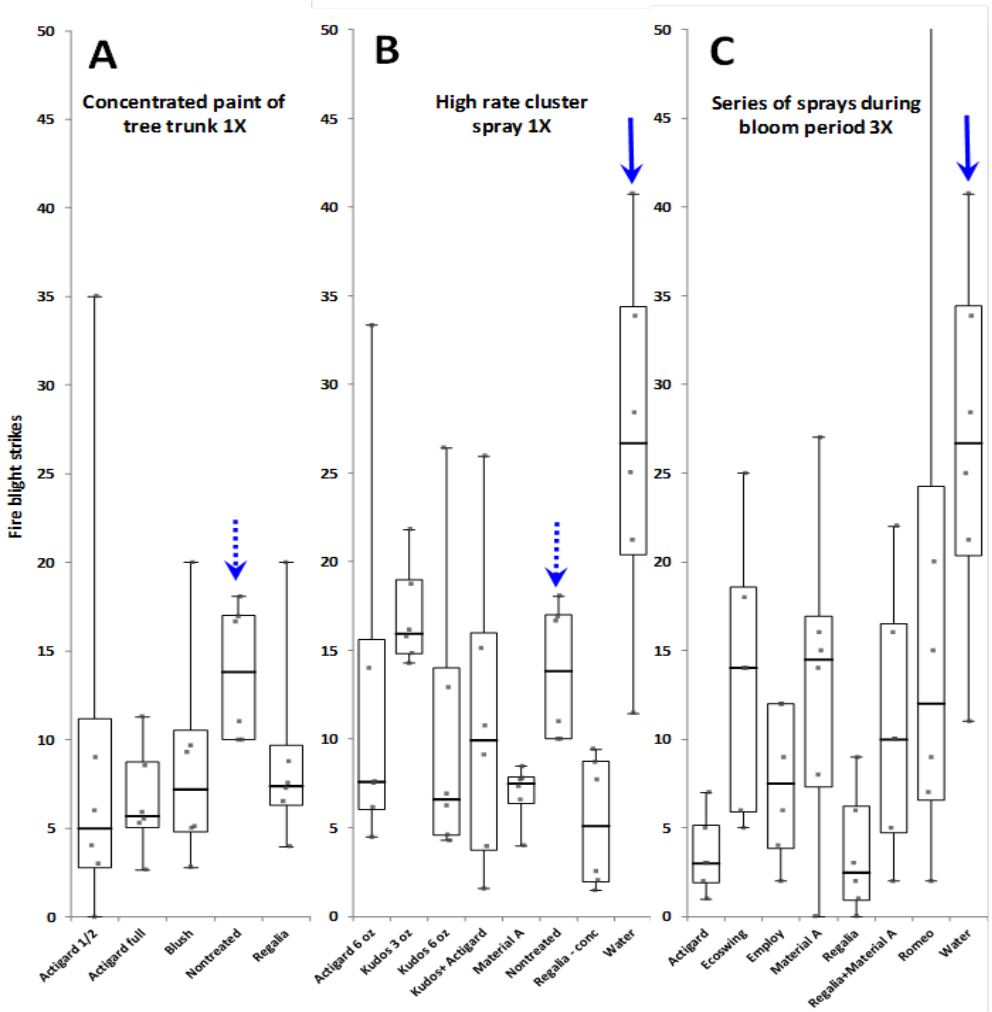
[#] Means within a column and within a section followed by same letter do not differ significantly ($P = 0.05$) based on Fischer's protected least significance difference.

[x] Amended 1:1 with ammonium sulfate.

[y] Amended with Regulaid: 16 fl. oz. per 100 gallons.

[z] Amended with BioLink Spreader-Sticker: 4 fl. oz. per 100 gallons.

Fig 5. Box plots of strike counts in individual trees that received an SAR-inducer treatment. Panel A: concentrated paint treatment of tree trunk once; panel B: high-rate treatment of flower clusters once; and panel C: three spray treatments during bloom period. Blue arrows mark control treatments: water-treated = solid line, nontreated = dashed line.



DISCUSSION

In the last decade, there has been an increase in the number of biopesticide materials available for non-antibiotic fire blight control. Many of these material achieved EPA-registration with only a limited number of field trials that demonstrated efficacy, and most are approved for organic production. Since 2010, in inoculated orchard experiments we have sought to understand on a comparative scale the value of these materials on fire blight suppression (as well as many more materials not listed in this report). In 2015, we added additional insight to material efficacy by measuring floral populations of the fire blight pathogen and yeasts at the growth stages of full bloom, petal fall and a week post-petal fall. And in 2017, we added measurements of floral pH and fruit russeting severity to provide a more complete understating of the antimicrobial impacts of the control programs as well as risk of inducing phytotoxic injury to developing fruit.

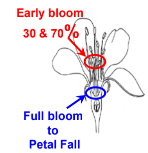
Overall, our data indicate strongly that integrated programs that begin with the biological material Blossom Protect (+ Buffer Protect), and are followed by a non-antibiotic chemical(s) can provide: i) outstanding infection suppression, ii) outstanding suppression of pathogen populations, iii) significant suppression of yeast populations at petal fall, and iv) negligible induction of fruit russeting. This control program is summarized in Fig. 6. Over all the years of effort, we have concluded that Blossom Protect (and Buffer Protect) is essential to organic fire blight control, and under higher infection risk conditions, the 'harsher' chemical materials (e.g., Previsto copper at full bloom and lime sulfur at petal fall) provide better suppression than comparatively softer materials such as Serenade Opti (and other *Bacillus*-based products). Nonetheless, our trial data on these more complex treatment programs (e.g., soluble copper then lime sulfur) is still relatively limited. Consequently, we will continue to evaluate these control programs in 2020. Current data also shows that a soluble copper (e.g., Previsto) at full bloom will suppresses/eradicates pathogen populations to a greater degree than a *Bacillus*-based material (e.g., Serenade Opti), and that among the harsher chemical materials, lime sulfur at petal fall poses the least risk of fruit russeting compared the soluble coppers or alum. Frustratingly, this project has revealed that alum, which provides excellent fire blight control when used at 8 lb/100 gallons (where it strongly reduces floral pH), poses a relatively high potential to induce fruit russeting.

Fig. 6. Current recommendation for non-antibiotic fire blight control.

Integrated, non-antibiotic fire blight control:

Example PNW non-antibiotic spray program with considerations for fruit safety

1) Prebloom (just prior to green tip):
   <u>Fixed copper</u> sanitation <u>if</u> fire blight was in orchard last year (5 to 6 lb/A)
2) Early bloom apple: (crop load thinning)
   <u>Lime sulfur</u> (plus oil) early bloom at 20 and 70% bloom
   Reapply biological if lime sulfur goes on after biological
3) 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 – cover every row
   In apple, Blossom Protect immediately after 2nd lime sulfur.
   In smooth-skinned pears in wetter areas, russet risk might be unacceptably high
4) Full bloom to petal fall, depending on cultivar russet risk/CougarBlight model risk:
   Low to moderate risk(negligible russeting risk):
   <u>Serenade Opti</u> every 2 to 4 days
   Improved control under high and extreme risk conditions (increased russeting risk):
   mix Serenade Opti with Cueva (<u>3 qts</u>/A)
   Previsto (3 qts/A) or <u>Cueva</u> (4 qts/A) every 3 to 6 days
5) Apples at petal fall: lime sulfur (2 to 4%) to clean up bacteria, yeast, mildew and rot fungi



On Objective 3 (understanding yeast biocontrol), our initial hypothesis was that *A. pullulans*-based biocontrol would not be strain specific. We adopted this hypothesis because *A. pullulans* is very common on pome flowers and because the *A. pullulans* strains in the Blossom Protect product were selected originally to suppress postharvest fruit rots of pome fruit and not fire blight. Somewhat surprisingly, some of the alternative yeasts attained higher populations on flowers than the Blossom Protect strains of *A. pullulans*. These yeast strains also suppressed fire blight, but not to the same degree as the strains in Blossom Protect. Results from Obj. 3 have been published in a journal article along with other data to create a relatively comprehensive guide to the use of this material for fire blight control in semi-arid orchard production systems (Temple et al. 2020, apsjournals.apsnet.org/doi/10.1094/PDIS-09-18-1512-RE).

Future research efforts on control of fire blight with yeasts should ask the question 'how do the Blossom Protect strains of *A. pullulans* provide superior suppression compared to other yeasts sprayed for this purpose?' Related to this, we have observed that pome flowers colonized by Blossom Protect strains of *A. pullulans* are not greatly suppressive of epiphytic populations of *E. amylovora*, and yet, fire blight is controlled. This may indicate that the apparent mechanism of biocontrol possessed the Blossom Protect strains is more complex than a simple explanation of superior competitive exclusion.

Regarding Objective 4, after very positive results with a concentrated trunk treatment of Actigard in 2017, we observed less infection with the full Actigard trunk paint (30 g/L) in 2018 and 2019; i.e., responses were partial/intermediate to the original observation. Given that the rate of Actigard we applied to trunks is very costly if every tree in an orchard receives the treatment, it is unlikely that an expensive approach yielding a partial response can be practical for preventative fire blight control. Spraying of flowers cluster at 10% bloom, however, is an approach that warrants further investigation (i.e., perhaps a partial response but material costs are less inexpensive). Most SAR materials applied to clusters at this early timing had less infection than the non-treated control; although, variability in the data meant not all differences were statistically significant. With Kudos (prohexidione-CA), the level of suppression we observed was less than has been reported by Cox at Cornell University. Nonetheless, the data argue for further evaluation; it also argues for a higher number of experimental replications and the utilization of the use of proper experimental controls.

EXECUTIVE SUMMARY

Project title: Refinement of practical fire blight control: Non-antibiotic and SAR

Key words: fire blight, non-antibiotic control

Abstract: Suppression of fire blight (caused by *Erwinia amylovora*) with non-antibiotic methods was investigated. Integrated organic programs beginning with Blossom Protect (70% bloom), followed by Previsto (fb) and then 4% lime sulfur (pf) provided outstanding control with negligible risk of fruit russeting. SAR materials Actigard, Kudos and Regalia provided partial suppression responses.

SIGNIFICANT FINDINGS:

- Blossom Protect (yeast), Previsto (copper) at full bloom and lime sulfur 4% at petal fall provided outstanding fire blight control.
- Lime sulfur 4% at petal fall did not increase fruit russeting of apple compared to a water treated control
- Alternative yeast strains (with Buffer Protect) suppressed fire blight but to a degree less than observed with Blossom Protect.
- Population size of *E. amylovora* on pear and apple flowers continued to increase during the post-petal fall period except when treated with lime sulfur at petal fall.
- Lime sulfur or Jet Ag (H_2O_2 in peracetic acid) sprayed near petal fall suppressed yeast populations on flowers.
- Alum (potassium aluminum sulfate, 8 lb/100 gal), applied after Blossom Protect, provides excellent fire blight control.
- Alum, an effective fire blight control material, reduces the pH of floral surfaces; lime sulfur increases pH of floral surfaces.
- Alum increased fruit russeting in both pear and apple.
- Concentrated Actigard treatments applied to the trunks of apple trees at 10% bloom provided partial suppression fire blight but not to the outstanding level observed in 2017.
- Kudos (prohexidione-CA) and Regalia (giant knotweed extract) provided partial fire blight suppression when sprayed onto flower clusters at 10% bloom.

FUTURE DIRECTIONS

- Multi-material, integrated programs (e.g., Blossom Protect then soluble copper then lime sulfur) require additional evaluation to better document expected efficacy and risk of fruit russeting.
- Future efforts on understanding control of fire blight with yeasts could be concerned with how Blossom Protect strains of *A. pullulans* achieve superior blight suppression compared to other yeasts.
- Pre- and early bloom resistance induction directed at treatment of flower clusters requires additional evaluation in trials with a high number of experimental replications and appropriately designed experimental controls.